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Protective and regenerative anti-pollution efficacy of a plant oil-based day and night cream: investigated by a novel approach to reveal the impact of pollutants on epidermal barrier integrity and lipid matrix

Dorothee Dähnhardt [1*], Stephan Dähnhardt-Pfeiffer [1], Isabel Simon [2], Dana Ditgen [3], Inge Holland [1], Dörte Segger [3], Jürgen Blaak [2] and Peter Staib [2*]

(Affiliation):
1 Microscopy Services Dähnhardt GmbH, 24220 Flintbek, Germany; sd@msd.sh (S.D.-P.)
2 Research & Development and Regulatory Affairs, Kneipp GmbH, 97084 Wuerzburg, Germany; isabel.simon@kneipp.de (I.S.); inge.holland@kneipp.de (I.H.); juergen.blaak@kneipp.de (J.B.) ***Present address...*
3 SGS INSTITUT FRESENIUS GmbH, 20354 Hamburg, Germany; Dana.Ditgen@sgs.com (D.D.); Doerte.Segger@sgs.com (D.S.)
* Correspondence: ddaehnhardt@microscopy-consulting.com (D.D.); peter.staib@kneipp.de (P.S.)

Dr. Peter Staib
Kneipp GmbH
Research & Development and Regulatory Affairs
Winterhäuser Str. 85
97084 Würzburg
Germany
Phone: +49 (0)931 8002 299
Fax: +49 (0)931 8002 275
Email: peter.staib@kneipp.de

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Abstract

In the wake of ever-increasing environmental pollution, human skin in the modern urban world is exposed to increased levels of harmful environmental pollutants. Many studies have shown that these pollutants can weaken the epidermal skin barrier and thus facilitate the penetration of these substances into the skin. An important goal of modern skin care against harmful environmental influences should therefore be to protect and strengthen the epidermal barrier and to repair occurring damage quickly and efficiently. With this in mind, in the present study we investigated what damage cigarette smoke causes to the epidermal barrier and (i) whether the regular application of a O/W emulsion (Day Cream) can protectively strengthen the epidermal barrier against environmental damage and (ii) whether a cigarette smoke-induced disruption of the epidermal barrier is restored faster and better by the regular application of a another O/W emulsion (Night Cream) than in product-untreated skin. The two products are slightly different in plant-oil, active ingredient composition and texture. Firstly, the study has shown that the Lipbarvis® method is suitable for measuring the effect of cigarette smoke, in contrast to conventional biophysical measurement methods (transepidermal water loss, skin hydration). Secondly, both products were able to improve skin barrier function in the corresponding test scenario. Thereby, the protective effect of the studied Day Cream on the epidermal skin barrier could be shown as well as the regenerative property of the tested Night Cream.

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Keywords

skin care, anti-pollution, skin barrier, intercellular lipid lamellae, cigarette smoke

1. Introduction

The skin is the largest human organ and forms the first and most important barrier and protective layer between the human body and its surrounding environment: the stratum corneum (SC) [1].

In the wake of increasing air pollution, human body and in particular the skin is exposed to increased stress in our modern urban world [2]. It is exposed outdoors to harmful substances such as polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs), oxides, particulate matter (PM), ozone, and heavy metals, in addition to UV radiation [3,4]. Indoors, cigarette smoke is an additional important dermal air pollutant [5,6]. In its 2022 report, WHO estimates that 91% of the world's population are exposed to air levels that exceed WHO guideline limits [7].

Increasing air pollution shows various negative effects on human skin. Very commonly described is premature aging of the skin and increased pigmentation [6,8,9], but also other skin diseases such as psoriasis [10,11] and acne [12], as well as inflammatory and allergic skin conditions like/such as atopic dermatitis and further eczematous lesions [5,13] and even skin cancer [9] can result from harmful environmental pollutants.

The pathways and sites of action of pollutants within the skin can vary greatly depending on the composition and structure of the respective components. Valacchi and coworkers [14] were able to show that air pollutants foster alterations in the skin microbiome and increase the colonization of the SC with pathogenic bacteria [12]. Every day we are exposed to pollution, indoors and outdoors, and because of this daily exposure, an intact skin barrier is so important. The epidermal permeability barrier (EPB), and in particular the SC, protects the human body from massive water loss (inside-outside barrier), but also prevents the penetration of harmful substances into the skin (outside-inside barrier). The epidermal barrier, formed at the transition from stratum granulosum to SC, represents a complex structure of proteins (involucrin, loricrin, profilaggrin, filaggrin) and lipids (ceramides, free fatty acids, and cholesterol) [15]. When the skin is exposed to increased environmental stress, pollutants entering the skin leading to increased formation of reactive oxygen species (ROS) and decreased levels of antioxidants in cells such as catalase, superoxide dismutase, and glutathione peroxidase [16,17]. The increased ROS activate matrix metallo-proteinases that degrade collagens, increase the number of melanocytes, and cause lipid peroxidation, all of which damages the EPB, compromising the skin`s natural protective and defensive function. Pollutants can thus penetrate the skin more easily and trigger immunological and inflammatory reactions [2,18].

The direct relationship between harmful environmental factors and epidermal skin barrier disruption was demonstrated by Kim et al [19]. Subjects exposed to elevated PM 2.5 particulate matter were deficient in filaggrin and had impaired EPB function. These results also confirm the data of Lee et al. [20]. Filaggrin expression was inhibited by urban particulate matter and increased pro-inflammatory cytokines, which may negatively affect EPB function. Percoco and colleagues [21] demonstrated in *ex vivo* skin that lipid oxidation induced by cigarette smoke enhances the interaction of

lipids with polar products and consequently alters lipid structure and EPB physiology. Furthermore, more ordered lipid chains and a disturbance of the homeostasis of barrier proteins and lipids could be demonstrated in the epidermis of smokers [22].

Based on these findings, a reasonable approach to protect the skin from harmful environmental influences seems to be the development of skin care products that can neutralize free radicals and/or protect and repair the EPB structure and function [23]. There are a variety of studies using *in-vitro* and *ex-vivo* methods to show the effects of anti-pollution products or their ingredients (e.g., antioxidants). What remains open in these studies is whether the effects are also transferable to the *in vivo* situation and give consumers real protection against harmful environmental effects [2]. To bridge the gap between *in-vitro*, *ex-vivo* and *in vivo* studies on human subjects and make product use studies ethically feasible, Bielfeldt and coworkers developed the smoke chamber model [24]. It uses cigarette smoke as a model substance. Cigarette smoke contains all the major key components of environmental pollution and offers the advantage of being a method with high reproducibility and sensitivity that can be used to measure the impact of pollutants with a small number of subjects [24].

In the *in-vivo* studies conducted to date, the squalene monohydroperoxide (SQOOH) and malondialdehyde (MDA) content, the erythema, melanin, and sebum index, as well as the content of other markers of skin inflammation and oxidative stress (gluthatione, carbonyl protein) were determined to assess the protective properties of a skin care product [24-27]. The protective effects of anti-pollution products on EPB integrity were also assessed by transepidermal water loss (TEWL) determination [25,27], but data are rare concerning the impact on the SC lipid bilayer structure.

In addition to the previously performed methods, in this study, we took a new approach and investigated the influence of cigarette smoke on the skin barrier using the Lipbarvis® technique. We investigated the a) protective and b) regenerative efficacy of two marketed plant oil-based skin care products (Day Cream, Night Cream) on the EPB under damage by cigarette smoke as a model for air pollution.

2. Methods and materials

2.1. Subjects, test products and study schedule

The present skin care study was conducted in line with the European Community Good Clinical Practice (EC-GCP, Commission Directive 2005/28/EC; Exception: no ethics vote) standards as a randomized, intra-individual single-centre study in accordance with the Revised Declaration of Helsinki, local laws, and regulations. A total of 24 qualified subjects with healthy skin were included. The subjects were all female and aged between 23.3 and 61.0 years (on average 44.1 ±12.0 years). The study was carried out between May and June in 2020 (SGS INSTITUT FRESENIUS GmbH, Hamburg, Germany). Two plant oil-based formulations (O/W emulsion), a day and night cream, were used to introduce this novel study approach (Table 1A/B).

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

(Day Cream, Internal No. CTC012)	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 Sinensis Peel Oil Expressed, Linalool, Benzyl Alcohol, 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)

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

(Night Cream, Internal No. CNC013)	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 Sinensis Peel Oil Expressed, Linalool, Benzyl Alcohol, 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)

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The **protective properties** of the test product (Day Cream) were investigated by the following study design (Table 2A): after the inclusion of the subjects in the study, biophysical measurements (baseline, t0, day 2) and Lipbarvis® sampling were performed. Therefore, three different test sites were available on the volar forearms. After baseline measurements and samplings, one test site was product-treated with the Day Cream twice daily (morning/evening) for two weeks, while the other two remained product-untreated. After two weeks of regular product application (t1, day 16), biophysical measurements were performed again on all three test sites. Then, the treated test site (product-treated) and one of the two product-untreated test sites were exposed to cigarette smoke (product-untreated), while the second product-untreated test site was neither treated with the product nor exposed to cigarette smoke (control). After another 24 hours, the biophysical measurements were performed at all three test sites and Lipbarvis® samples were taken from the product-treated and product-untreated smoke exposed test sites (t2, day 17).

The **regenerative properties** of the test product (Night Cream) were examined as follows (Table 2B): After the inclusion of the subjects in the study, biophysical measurements were performed (baseline, t0, day 1) on two test sites (product-treated/product-untreated). Subsequently, both test sites were treated with cigarette smoke (air pollution model) while a further test site was neither to be treated with the product nor exposed to cigarette smoke (control). To determine the effect of cigarette smoke on the skin, biophysical data were collected again after 24 hours (t1, day 2), and Lipbarvis® samples were taken. Afterwards, the skin care treatment was started on one of the two test sites exposed to cigarette smoke. The test product was applied twice daily (morning/evening) by the subjects at home. Biophysical data as well as Lipbarvis® samples were obtained from both test sites (product-untreated and product-treated) after 14 days of test product treatment (t2, day 16).

Table 2A. Schedule of study procedure "protective"

	Day Cream (study design „protective")			
	t0 (day 2)	**t0-t1**	**t1 (day 16)**	**t2 (day 17)**
Preparation				
Informed consent	X			
In-/Exclusion criteria	X			
Parameter				
Biophysical measurements (TEWL, SC hydration, ss-pH)	X		X	X
SC lipid matrix analysis by Lipbarvis® (nICCL)	X			X
Test site 1: test product application (twice daily, 14 days)	X*	X	X	
Test site 2: not product-treated	X	X	X	
Cigarette smoke exposure on both test sites (30 min)			X	

* The first application of test materials was performed after the instrumental measurements and visual evaluation at the Study Site, under supervision of a technician; TEWL = Transepidermal water loss;
SCH = Stratum Corneum Hydration; ss-pH = Skin surface pH; nICCL = normalized intercellular lipid lamellae

Table 2B. Schedule of study procedure "regenerative"

	Night Cream (study design „regenerative")			
	t0 (day 1)	**t1 (day 2)**	**t1-t2**	**t2 (day 16)**
Preparation				
Informed consent	X			
In-/Exclusion criteria	X			
Parameter				
Biophysical measurements (TEWL, SC hydration, ss-pH)	X	X		X
SC lipid matrix analysis by Lipbarvis® (nICCL)		X		X
Cigarette smoke exposure on both test sites (30 min)	X			
Test site 1: test product application (twice daily, 14 days)		X*	X	X
Test site 2: not product-treated		X	X	X

* The first application of test materials was performed after the instrumental measurements and visual evaluation at the Study Site, under supervision of a technician; TEWL = Transepidermal water loss;
SCH hydration = Stratum Corneum Hydration; ss-pH = Skin surface pH; nICCL = normalized intercellular lipid lamellae

2.2. Cigarette smoke exposure

Cigarette smoke was generated using a self-devised smoking apparatus. For each voluntary participant, three Mohawk Classic Red cigarettes (Grand River Enterprises GmbH, Rietz / Kloster Lehnin, Germany) were utilized. The smoke from each cigarette was conveyed through bespoke plexiglass chambers (the same chambers utilized for

the creation of suction blisters, minus the bottom layer including the apertures), affixed to the designated area, within an approximate time span of five minutes. This was achieved by employing a Rotilabo®-membrane vacuum pump CR-MV100 (Carl Roth GmbH + Co. KG, Karlsruhe, Germany) operating at a steady airflow to pull the smoke from the filtered Mohawk cigarettes through the plexiglass chambers onto the specified skin region. The pump was switched off after each single cigarette, and the smoke from the particular cigarette was allowed to linger on the respective area for additional five minutes. Two regions, each with a diameter of 3.5 cm, were exposed to cigarette smoke for an overall duration of 30 minutes [28]. The use of cigarette smoke is justified as it is widely accepted and documented as an experimental model for examining the effects of airborne pollution exposure on the skin [24,29].

2.3. Biophysical measurements

Biophysical measurements of TEWL and SC hydration (SCH) were performed on the volar forearms after the acclimatisation phase for at least 20 minutes at 21 ± 1°C and 50 ±10% relative humidity.

TEWL measurements were performed with the DermaLab® system (Cortex Tech-nology ApS, Hardsund, Denmark) and the evaluation of SCH corneometry (skin capacitance) was performed with the Corneometer® MDD4 (CM 825 probe, Courage+Khazaka electronic GmbH, Köln, Germany) both according to the corresponding EEMCO guidelines under appropriate ambient conditions of 21 ±1°C and relative humidity of 50 ±10% [30,31]. The predefined TEWL measurement sequence covered a period of 45 seconds. Mean values and the corresponding standard deviations of the last eight determined TEWL data of a measurement sequence were calculated. Three single measurements per test site were performed. SCH was determined five times per test sites and mean values were calculated. Subjects were acclimatised to the measurement environment with the skin test areas left uncovered for at least 20 min. Measurements at each skin test area were performed in a horizontal position with a constant pressure of 1,5 N (by device).

2.4. Lipbarvis® sampling and Transmission electron microscopy

The normalized length of lipid lamellae in the intercellular space (nICLL) as a marker for the integrity of the epidermal barrier was determined by the noninvasive skin sampling technique Lipbarvis® (Microscopy Services Dähnhardt GmbH, Flintbek, Germany). The Lipbarvis® procedure has been described several times in the literature and has been applied in quite a few studies [32-34]. A special glue-carrier system is used to remove the corneocytes from the skin surface. This is followed by fixation and embedding of the samples and subsequent analysis of the intercellular lipid lamellae (ICLL) organization in the SC by transmission electron microscopy (TEM CM 10, FEI, Eindhoven, Netherlands), calculated as normalized ICLL (nICLL). The values and the data were used for statistical analysis. All Lipbarvis® samples were obtained from an area of the test sites that was not used for instrumental measurements. Before treating the test fields, a Lipbarvis® baseline value (t0) was taken for the study of both the regenerative and protective properties of the Night and Day cream, respectively. A baseline value was not taken for the Day and Night cream individually, as it is known from unpublished data that there are no differences in terms of Lipbarvis® values on the forearm in the test areas.

Concerning the evaluation of the protective properties of the Day Cream, Lipbarvis® samples were taken from 12 out of the 24 subjects. The different number of subjects for Lipbarvis® (n=12) versus TEWL and SCH (n=24) were defined based on previously described approaches and publications [33,34]. To investigate the protective

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properties of the Day Cream, Lipbarvis® samples were taken at baseline (t0) and time t2 (day 17, Table 2A). At time t2, samples were taken from two out of the three possible test sites – the product-untreated, cigarette-smoke-exposed, and the product-treated, cigarette smoke exposed test site.

Regarding the evaluation of the regenerative properties of the Night Cream, Lipbarvis® samples were taken from the corresponding test sites at baseline and 24 h after cigarette smoke exposure at t1 (day 2). After 14 days, samples were taken at time t2 (day 16) from two of the three possible test sites (product-untreated, cigarette smoke exposed, and product-treated, cigarette smoke exposed test site).

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2.45. Statistical analysis

The analysis of the study objective was performed by SGS INSTITUT FRESENIUS GmbH using the computer software Microsoft EXCEL (Office 365) and STATISTICA® (version 13.3). Microsoft EXCEL was used for the calculation of the relative data, the means, and the standard deviations. STATISTICA® was used for analysing the distribution of the data (Kolmogorov-Smirnov-Test) and for analysing the significance of differences between the test regimens or points in time (ANOVA, analysis of variance). The hypothesis of a normal distribution was accepted when there was a p-value > 0.05. Concerning the differences between the treatment situations and the points in time, in the case of a p-value < 0.05 a difference was accepted as statistically significant.

3. Results

3.1. Protective study design

3.1.1. Non-invasive biophysical measurements

Different biophysical measurements were performed to investigate SC function after treatment with the Day Cream and subsequent cigarette smoke exposure (protective study design) After 2 weeks of product application with the Day Cream, a significant reduction ($p=0.0063$) in TEWL was shown compared to the product-untreated skin (Figure 1, t1). However, subsequent exposure of cigarette smoke did not show significant changes for the control, product-untreated and product-treated site (Figure 1, t2). SCH showed a significant increase ($p<0.0001$) on product-treated compared to product-untreated skin after 2 weeks of product application (Figure 2, t1). 24 hours after the skin was exposed to cigarette smoke, the SCH remained significantly higher ($p<0.0001$) at the test site treated with the product compared to the product-untreated test site (Figure 2, t2).

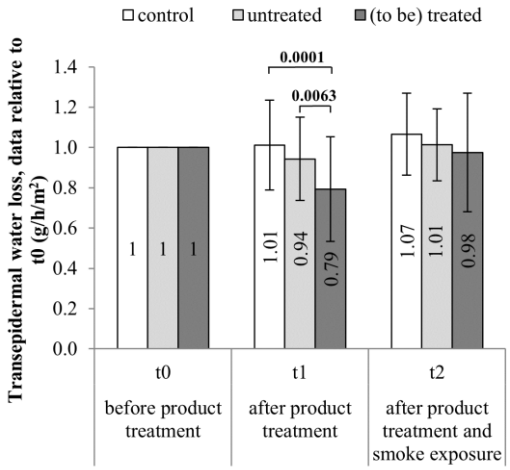

Figure 1. TEWL (data relative to t0) before (t0, day 2) and after two weeks of product treatment with Day Cream (protective study design) (t1, day 16) and after smoke exposure (t2, day 17). One test field served as a control area, one area was not product-treated, but exposed to cigarette smoke (product-untreated area) and one field was product-treated after the baseline measurement as well as exposed to cigarette smoke ((to be) product-treated).

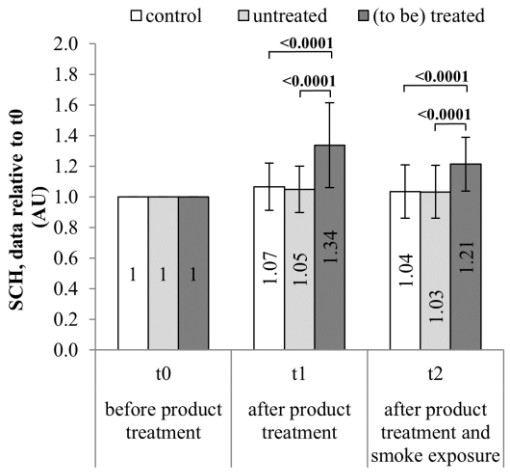

Figure 2. SCH (data relative to t0) before (t0, day 2) and after two weeks of product treatment with Day Cream (protective study design) (t1, day 16) and after smoke exposure (t2, day 17). One test field served as a control area, one area was not product-treated, but exposed to cigarette smoke (product-untreated area) and one field was product-treated after the baseline measurement as well as exposed to cigarette smoke ((to be) product-treated).

3.1.2. TEM investigation and morphometric analysis

In addition to the biophysical measurements, the influences of cigarette smoke and skin care treatment on the epidermal barrier were determined by morphometric measurement of the lipid lamellae in the intercellular space (nICLL) in TEM images. Concerning the determination of the protective study design, Lipbarvis® samples were taken at t0 (baseline, day 2) and at t2 (after 2 weeks of product application followed by cigarette smoke exposure, day 17). The product-untreated skin showed a significant decrease in nICLL values from 200 to 160 (nICLL/1000nm²) (p=0.0011), while the previous product-treated skin showed a significant increase in nICLL values from 200 to 224 (nICLL/1000nm²) after cigarette smoke exposure (p=0.0036) (Figure 3 & 4). The nICLL values at t2 were significantly higher for the product-treated than for the product-untreated skin (p<0.0003) (Figure 3, t2).

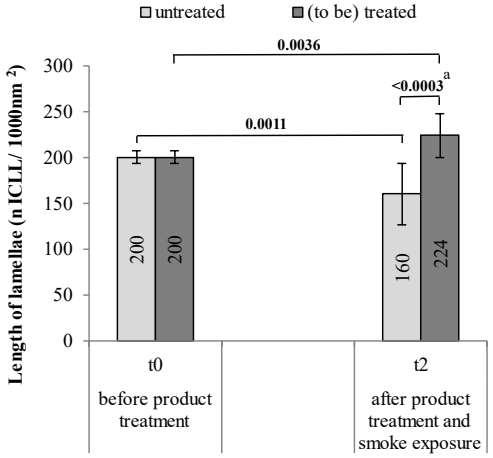

Figure 3. Normalised intercellular lipid lamellae (nICLL) before (t0, day 2) and after two weeks of product treatment with Day Cream (protective study design) and subsequent smoke exposure (t2, day 17). One test field was not product-treated but exposed to cigarette smoke (product-untreated area) and one field was product-treated after the baseline measurement, as well as exposed to cigarette smoke ((to be) product-treated).

A TEM image of the lipid lamellae in the intercellular space between the corneocytes in the middle of the Stratum corneum is shown in figure 4. For a better recognition and a faster assignment the TEM images were colored. The lipid lamellae in the intercellular space are colored orange~~light brown~~, while areas with little to no lipid lamellae are colored blue. The corneocytes adjacent to the intercellular space are stained dark brown.

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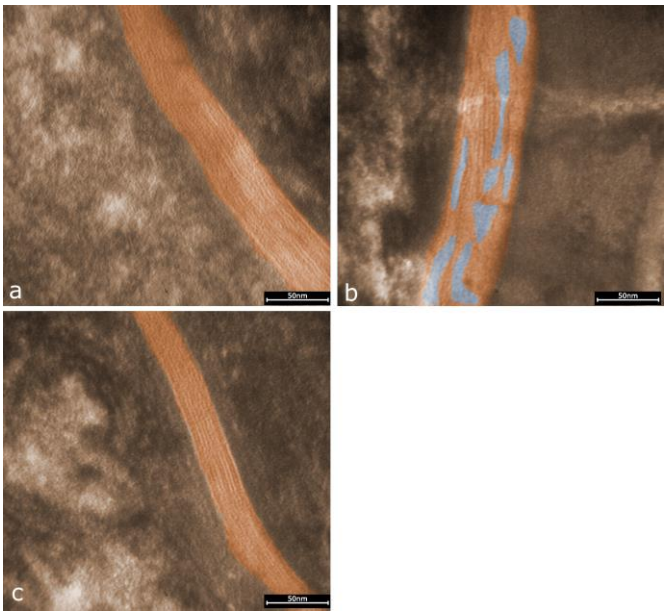

Figure 4. TEM images of the intercellular lipid lamellae in the intercellular space of the stratum corneum before (a) and after two weeks and a single smoke exposure of the product-untreated test site (b) and the Day Cream-treated test site (protective study design) (c). The lipid lamellae in the intercellular space are coloured ~~light brown~~orange, while areas with little to no lipid lamellae are coloured blue. The corneocytes adjacent to the intercellular space are stained dark brown.

3.2. Regenerative study design

3.2.1. Non-invasive biophysical measurements

Different biophysical measurements were performed to investigate SC function after previous smoke exposure and sub-sequent treatment with the Night Cream (regenerative study design). The exposure to cigarette smoke on untreated skin surface led virtually to no significant changes in TEWL values before (t0, day 1) and after smoke exposure (t1, day 2) (Figure 5). The subsequent 2 weeks of product application resulted in a significant decrease in TEWL values on the product-treated skin site compared to the product-untreated skin site ($p=0.0037$) (Figure 5, t2, day 16). There were no significant differences in SCH values for all three test sites determined between t0 and t1 (Figure 6). After the 2 weeks of treatment, SCH significantly increased on the product-treated test site compared to the untreated ($p<0.0001$) and control sites ($p=0.0001$) (Figure 6, t2).

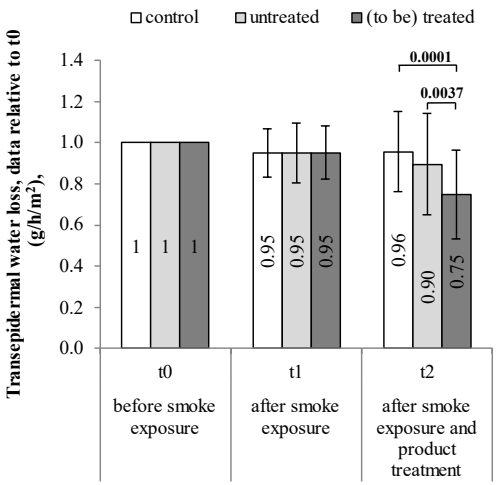

Figure 5. TEWL (data relative to t0) before (t0, day 1) and after smoke exposure (t1, day 2) followed by two weeks of product treatment with Night Cream (regenerative study design) (t2, day 16). One test field served as a control area, one area was not product-treated, but exposed to cigarette smoke (product-untreated) and one field was product-treated after the baseline measurement as well as exposed to cigarette smoke ((to be) product-treated).

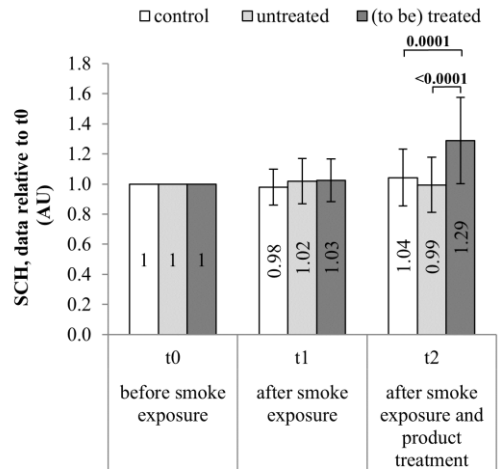

Figure 6. SCH (data relative to t0) before (t0, day 1) and after smoke exposure (t1, day 2) followed by two weeks of product treatment with Night Cream (regenerative study design) (t2, day 16). One test field served as a control area, one area was not product-treated, but exposed to cigarette smoke (product-untreated area) and one field was product-treated after the baseline measurement as well as exposed to cigarette smoke ((to be) product-treated).

3.2.2. TEM investigation and morphometric analysis

In addition to the biophysical measurements, the influences of cigarette smoke and skin care treatment on the epidermal barrier were determined by morphometric measurement of the lipid lamellae in the intercellular space (nICLL).
Under regenerative study design, Lipbarvis® samples were taken at baseline (t0, day 1), 24 h after cigarette smoke exposure (t1, day 2), and after 2 weeks with and without product application (t2, day 16). Cigarette smoke exposure resulted in a significantly perturbed structure of intercellular lipid lamellae in the intercellular space, shown by a decrease of nICLL values from 200 to 146 (nICLL/1000nm^2) compared to baseline on both test sites ($p<0.0001$) (Figure 7 & 8). Subsequent product application for 2 weeks resulted in a significant increase in nICLL values from 146 to 222 (nICLL/1000nm^2) ($p<0.0001$). The product-untreated test site also showed a significant increase in nICLL values from 146 to 202 (nICLL/1000nm^2) ($p<0.0001$). Nevertheless, the product-treated skin showed significantly higher nICLL values at t2 than the product-untreated skin ($p=0.0036$) (Figure 7).

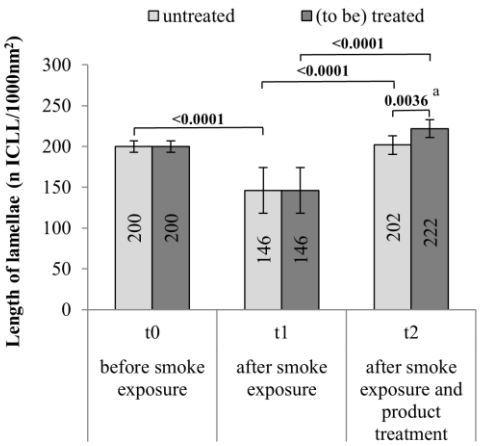

Figure 7. Normalised intercellular lipid lamellae (nICLL) before (t0, day 1) and after smoke exposure (t1, day 2) followed by two weeks of product treatment with Night Cream or no treatment (regenerative study design) (t2, day 16). One test field was not product-treated (product-untreated area) after smoke exposure and one field was product-treated after smoke exposure ((to be) product-treated). a: obtained from relative data.

A TEM image of the lipid lamellae in the intercellular space between the corneocytes in the middle of the Stratum corneum is shown in figure 8. For a better recognition and a faster assignment the TEM images were colored. 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.

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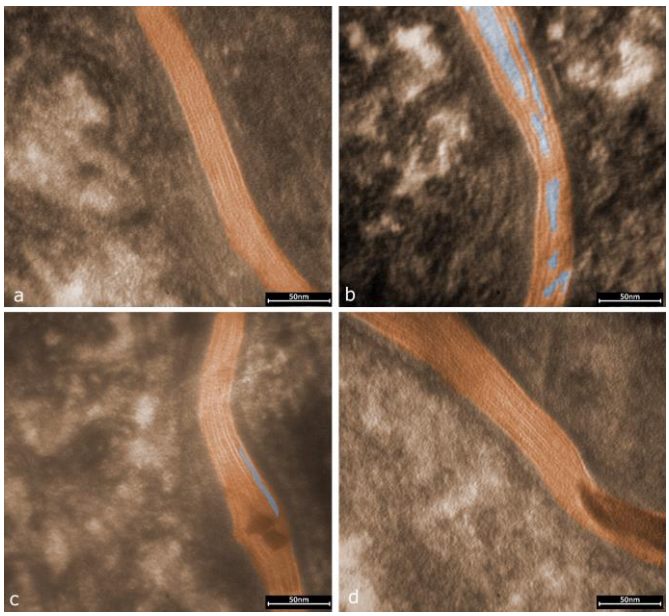

Figure 8. TEM images of the intercellular lipid lamellae in the intercellular space of the stratum corneum before (a) and after smoke exposure (b) followed by two product-untreated weeks (c) as well as two weeks of product treatment with Night Cream (regenerative study design) (d). The lipid lamellae in the intercellular space are coloured ~~light brown~~orange, while areas with little to no lipid lamellae are coloured blue. The corneocytes adjacent to the intercellular space are stained dark brown.

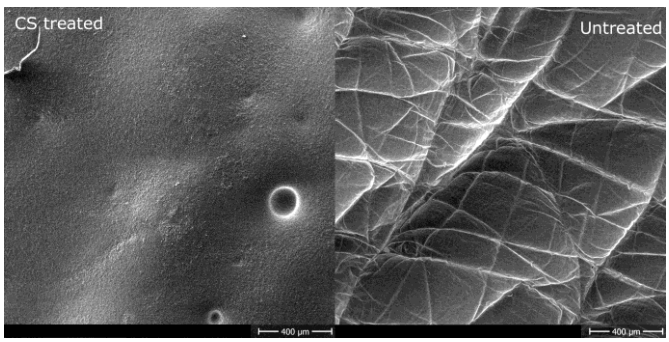

Figure 9. Scanning electron micrograph of a silicone print of the skin surface after exposure to smoke. Left image shows the tar film on the skin after smoke exposure, right image shows the skin surface without smoke exposure.

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4. Discussion

It has been proven in numerous studies that environmental pollutants can weaken the EPB [6,9,19-22,35,36]. A disturbed skin barrier allows them to penetrate the skin more easily and the development of a variety of skin diseases can be the result of increased exposure to environmental pollutants [23,24]. An important goal of modern skin care treatment against harmful environmental influences should therefore be to protect and strengthen the epidermal barrier and to regenerate as well as repair occurring damage quickly and efficiently. Taking this background into consideration, we investigated in the present study the harm cigarette smoke causes on the epidermal barrier and whether the regular use of a plant oil-based O/W emulsion (Day Cream) can protectively strengthen the epidermal barrier from environmental pollution. In the second part of the study, we investigated whether a cigarette smoke-induced disruption of the epidermal barrier is restored faster and better by regular application of a plant oil-based o/w emulsion (Night Cream) compared to product-untreated skin.

The results of the study showed no significant changes in TEWL and SCH 24 hours after the skin was exposed to cigarette smoke. In the chosen study design, these biophysical parameters cannot prove the influence of cigarette smoke on the skin. This is also confirmed by the data of Ditgen and coworkers [28].

A possible explanation for the non-significant changes in the TEWL values would be that the ingredients of cigarette smoke deposited on the skin surface, in this case above all the tar components, form a film (Figure 9). This film has an occlusive effect as long as it is not lost through the progressive exfoliation of the uppermost cell layers in the SC. The possible TEWL increase after smoke exposure caused by a damaged EPB was maybe not obvious because of the occlusive layer of pollutants on the uppermost layers of the SC prevents the increased water transport to the outside. This also explains the unchanged values for skin moisture. From this film deposited on the SC, chemical ingredients of cigarette smoke could penetrate the deeper layers of the epidermis and trigger a variety of reactions there [18]. A penetration of ingredients from cigarette smoke into the lowest layers of the epidermis is therefore also very likely in the *in-vivo* situation. Cigarette smoke ingredients also result in lipid peroxidation of ceramides, especially Ceramid EOS and free fatty acids, possibly leading to a reduction in intercellular lipid lamellae length in the SC [24,28]. This study also showed that lipid peroxidation of ceramides, especially Ceramid EOS and free fatty acids, already occurs in the middle layers of the SC and that the length of intercellular lipid lamellae in the SC is reduced.

The influence of cigarette smoke on the quality of the EPB, i.e., on the structure of lipid lamellae in the intercellular space in the SC, shown again in our study, is comparable to observations made by Ditgen and coworkers [28]. Aside EPB structure the physiology and function is also significantly affected by cigarette smoke [20-22]. The length of lipid lamellae is significantly reduced 24 hours after smoke exposure. This effect can be compensated by topical application of the tested skin care product. This suggests a faster repair of the EPB through increased production of lipids and the formation of lipid lamellae in the SC.

The changes in epidermal lipid lamellae lengths and their organisation resulting from the exposure to cigarette smoke are shown in this study. The focus was on an *in-vivo* measurement that does not focus on the lipids and proteins present on the skin surface, but explicitly focuses on the state of the lipid lamellae in the middle SC. For

classification of the determined nICLL data it is important to know that healthy skin shows values of 195 nICLL and higher, dry skin has values of 100 nICLL and lower, while atopic and very dry skin shows values of 50 nICLL and lower [37].

Subsequent smoke exposure led to a reduction in the length of lipid lamellae in the product-untreated test site, which demonstrates that the damage in the present cigarette smoke model works. In contrast, product-treated skin, which was exposed to cigarette smoke after a two-week treatment regimen showed a significantly better EPB than at baseline ($p=0.0036$) and compared to untreated skin exposed to cigarette smoke ($p<0.0003$) (Figure 4).

The identification of the exact mechanism of action was not the subject of the present study, as the focus was on the overall effect of the formulation. Nevertheless, the combination of tocopheryl acetate [29,38], panthenol [39], and various plant oils [40] was probably responsible for the shown effects because they are representing the primary active ingredients accompanied with the product given pH of 4.5 (Table 1). A protection of the EPB against damage by cigarette smoke through the upstream application of the care product for 14 days was demonstrated, as well as the rapid restoration of the epidermal barrier through the application of the care product after the smoke exposure. To keep the skin healthy, it is very important to maintain and support the EPB function permanently and/or situationally by skin care product usage. It was shown that two slightly different vegetable oil-based O/W emulsions were able to maintain barrier EPB function in a regenerative (Night Cream) and protective (Day Cream) way, respectively. Against this background topical cosmetic formulations can play a crucial role to overcome negative effects on the epidermal EPB function and structure and thereby keep skin healthiness. As described the tested marketed products are different in plant-oil composition, active ingredients, and texture/richness but both products contain plant oils, panthenol and antioxidants. Consequently, these three formula elements could be helpful and recommended to support skin integrity.

Commenté [MOU4]: pH 4.5 oder pH 5??

5. Conclusion

In summary, the data of this study show, that the effects of cigarette smoke on the EPB can be demonstrated using the Lipbarvis® method even when biophysical measurement methods such as TEWL and SCH reach their limits. Nevertheless, a limitation of this study could be the lack of data showing the "self-repair" of the EPB after damage without product application. Further future *in-vivo* studies could investigate the self-repair of the SC as a function of time, irritation model and intensity. The effect of the skin care product on the ability of cigarette smoke to accumulate on the skin surface as a preventive “occlusive layer” could also be a possible subject of further studies. These data in combination may provide further insights into the effect of cigarette smoke on the epidermis, regenerative mechanisms, protective measures by certain cosmetic active ingredients and finally provide data regarding skin aging due to environmental pollution.

In the present work, a protective and regenerative study design using two skin care products was investigated for the first time with respect to epidermal barrier integrity and structure in the context of cigarette smoke as a model of pollution induced skin barrier damage. Finally, the presented approach could be an innovative procedure to substantiate cosmetic product claims.

Author Contributions:

Conceptualization, D.D. (Dorothee Dähnhardt), S.D.-P., J.B., 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., J.B., I.S., I.H.; writing—review and editing, D.S., D.D. (Dana Ditgen) and P.S.; visualization,

J.B., I.S., D.D. (Dorothee Dähnhardt) and S.D.-P.; supervision, P.S.; project administration, J.B., I.H., I.S., D.S. and D.D. (Dorothee Dähnhardt). All authors have read and agreed to the published version of the manuscript.

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Informed Content Statement

Informed consent was obtained from all subjects.

Data Availability Statement

Not applicable

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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Conflict of Interest Statement

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., J.B. and P.S. are employees of Kneipp GmbH in Würzburg, Germany. D.S. and D.D. (Dana Ditgen) are employees of SGS INSTITUT FRESENIUS GmbH in Hamburg, Germany. The authors have no conflicts of interest to declare.

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