sofw journal
Home & Personal Care Ingredients & Formulations
powered by SOFW

# Blue Light and Premature Skin Aging – New *In Vivo* Test Method

D. Dähnhardt, A. v. Seebach, S. Dähnhardt-Pfeiffer, D. Segger

**SOFW Journal 1+2/22** | Volume 148 | Thannhausen, Germany, February 17, 2022

# Blue Light and Premature Skin Aging – New *In Vivo* Test Method

D. Dähnhardt, A. v. Seebach, S. Dähnhardt-Pfeiffer, D. Segger

**abstract**

The harmful effects of blue light rays on the skin are increasingly becoming the focus of the cosmetics industry and consumers. So far, only a few *in vivo* test methods for measuring the effects of blue light on the skin have been described in the literature. Direct detection methods that show the immediate effects of blue light on the collagen network of the skin are still missing. In the work presented here, a new *in vivo* test method for measuring the effect of blue light on the skin is presented.
The skin of volunteer subjects was irradiated with a light source with a narrow emission spectrum and low-dose blue light (6 $J/cm^2$). Before irradiation, and 1 hour respectively 24 hours after irradiation, we were able to detect the increase in MMP-1 concentration and the decrease in type 1 procollagen in the blister fluid of the suction blisters. We were able to visualize the collagen network with the help of scanning electron microscopy (SEM) on the underside of the suction blister roofs and quantitatively evaluate it with the help of an image analysis. Here, too, after blue light treatment, there was an increasing degradation of the collagen fibers as well as a decrease in the length of the collagen strands.
The presented study can be used as an effective *in vivo* test method for products that want to reduce the negative effects of blue light on the skin and reduce premature skin aging.

## 1. Introduction

The skin is the largest human organ, and it forms the first and one of the most important barriers and protective layers between the human body and its environment. In the course of increasing air pollution, human skin is exposed to increased stress in our modern urban world [1]. In addition to UV radiation, it is also exposed to a variety of pollutants, particulate matter and ozone outdoors. In recent years, the influence of blue light on the skin has increasingly moved into the consciousness of harmful external environmental influences.

The solar energy hitting the Earth consists of UV radiation (280-400 nm), visible light (400-760 nm) and infrared radiation (760 nm – 1 mm). For UV light, the damaging effect and the influence on skin physiology has been extensively proven [2,3,4].

In contrast, there are only a few investigations for the range of visible light beyond the UV spectrum, i.e. with wavelengths greater than 400 nm. When sunlight hits the skin, 4-7% is reflected by the stratum corneum (SC) [5]. The rest of the light is scattered on the fine structures of the skin, such as organelles and collagen fibers, or absorbed in the deeper layers of the skin by photoacceptors. While most of the UV-B radiation is absorbed in the epidermis, about 80% of the UV-A radiation penetrates to the dermal epidermal barrier and about 10% reaches the hypodermis [6]. Visible light, i.e. with wavelengths greater than 400 nm, penetrates much deeper into the skin, about 20% reach the hypodermis [6].

In order for biological activity to be triggered in the skin, the irradiated light must be absorbed by photoacceptors and thus be able to pass into an excited state. The photoacceptors excited in this way can then act on other molecules and convert the light energy into a molecular reaction [7]. In the blue wavelength range, flavoproteins, porphyrin-containing enzymes, bilirubins, retinol, carotenoids and melanin are classified as photoacceptors. These cause cellular reactions and clinical effects in the skin via various diverse biochemical processes [8].

It has often been described that the formation of reactive oxygen species (ROS) and matrix-degrading enzymes is induced by radiation [9]. DNA damage, cell shrinkage and premature cell death are also described to be consequences of high-energy blue light in the skin. They lead to a reduced proliferation of fibroblasts as well as a reduced production of procollagen 1 and ATP, so that the skin ages prematurely and fatigues as a result [10]. Blue light radiation as one of the possible causes of extrinsic skin aging is therefore becoming increasingly important in the cosmetics industry.

For the investigation of the effects of blue light on the skin, a look at the literature shows a variety of different methodological approaches. For example, different light sources were used, so that a clear assignment of biochemical and cellular reactions to a certain wavelength range, that of blue light (400-480 nm), is difficult. The light sources used range from fiber optic sources with filter discs (400-700 nm) to multi-LED lamps with eight

different LEDs in the wavelength range from 412 to 940nm to LEDs with a very narrow emission spectrum around 450nm [11,12]. Likewise, the selected illumination intensities are very different (8 J/cm$^2$ to 480 J/cm$^2$) [13]. Against this background, the challenge was to use a standardized blue light source with a narrow irradiation spectrum and a maximum intensity at 450 nm with the method presented here and thus to be able to make a clear assignment of the effects of blue light.

The further aim of this work was to develop a methodological approach that can directly map the effects of blue light treatment on the collagen network of the skin. For this purpose, we used a blue light source with a very narrow emission spectrum, which enables a reproducible blue light application depending on the distance to the skin surface and the duration of irradiation. After irradiation, suction blisters were generated, from which the interstitial fluid was taken for biochemical analysis. The suction blister roofs were used for microscopic examination to visualize and quantify the quality of the collagen and fiber network on the dermal side of the dermal/epidermal barrier. Between blue light application and sampling, two time windows (1 hour and 24 hours) were applied to detect possible time delays in the skin's response to blue light irradiation. The MMP-1 and procollagen peptide type 1 concentration in the interstitial fluid were analyzed; the morphology of the collagen and fiber network on the dermal side of the suction blister roofs using scanning electron microscopy (SEM).

## Material and methods

### Subjects and study design

In this preliminary study, epidermis and skin fluids were obtained from blue light exposed and non-exposed control skin of 3 healthy male and female Caucasian subjects (ages 50.7 ± 6.3 years; nonsmokers). Suction blisters were collected from unexposed skin and from blue-light exposed skin 1 and 24 hours after exposure. The entire viable epidermis with intact basal layer was used for SEM examination, and the interstitial fluid within the suction blisters were used for the analysis of MMP-1 and procollagen-1 expression levels.

The pilot study was conducted in the laboratories of SGS INSTITUT FRESENIUS GmbH in accordance with the principles of the Declaration of Helsinki and its amendments, and a written declaration of consent was obtained from all subjects.

### Blue light irradiation

The irradiation of the skin surface with blue light was carried out with an LED light source (MSD in-house development) with a narrow irradiation spectrum between 420-470 nm and the maximum intensity at 450 nm. The primary light intensity of this LED light source is 105 lm. Blue light was applied to the subjects' forearm, maintaining a distance of 15 cm between the skin and the light source to avoid thermal influences of the blue light source. The skin surface was irradiated with blue light (450 nm) for 15 minutes on an area of 9 cm². The measurement of the total applied light was carried out with a UV meter and the FS VIS D2 E110 sensor. This resulted in a total energy of 5.5 J/cm$^2$ after the 15 min, which was applied to the skin surface.
Suction blisters were taken before (t0), after 1 (t1) and 24 hours (t2) after blue light treatment.

### Generation of suction blisters

Suction blisters were created on the skin of each test area by using custom-made Plexiglas suction chambers with three circular openings of 7 mm in diameter. A vacuum of about 550 - 850 mbar was applied, and the blisters were created over a period of 1.5 - 2.5 hours. The blisters formed in the diameter of the suction openings of the Plexiglas chamber, whereby after about 2 hours the epidermis slowly detached from the underlying dermis. The blister fluid (interstitial fluid) was extracted with a sterile syringe and the roofs of the resulting suction blisters were then removed under sterile conditions. The resulting small wounds were treated with Hansaplast® Active Gel Strips. All wounds were completely healed and scar-free within 6-10 days.

One hour and 24 (± 2) hours after blue light exposure, the formation of suction blisters was performed on the exposed as well as on the non-exposed areas to analyze the expression level of MMP-1 and procollagen type 1 in the blister fluid. The suction blister roofs were transferred to a fixing solution for the subsequent examination in the SEM and stored at 4°C until further use.

### Determination of procollagen 1 and MMP-1

The suction blister fluid (interstitial liquid) was removed from each test area with a sterile syringe and stored at -80°C until further analysis. The procollagen 1 and MMP-1 content in the interstitial fluid of each sample was measured with ELISA kits. (RayBio® Human MMP-1 ELISA Kit, RayBiotech, Georgia, USA and Procollagen type IC-Peptide (PIP) EIA Kit, TaKaRa Japan).

### Scanning electron microscopy and image analysis

The suction blister roofs were re-fixed in osmium tetroxide, dehydrated in an ascending ethanol row and then critical point dried to avoid drying artifacts and thus optimally preserve the fine structures on the dermal side of the suction blister roofs. For the subsequent SEM examination of the suction blister roofs, they were sputtered with gold. The image acquisition was carried out digitally. The quantitative analysis of the collagen and fiber structures was carried out using a Plugin from ImageJ. Before the calculation of the proportion of collagen and fiber structures is carried out, a coloring of the image material is selected in order to better distinguish fiber structures from the background.

## Results

The blue light used leads to an increase in MMP-1 concentration in the subjects. The relative comparison between blue light exposed and untreated skin shows an increase in MMP-1 values after just one hour (t1=1.3, **Figure 1**). This trend becomes even clearer after 24 hours. The exposed skin shows a doubling of the MMP-1 values (t2=2.1) compared to the untreated skin.

The blue light treatment shows a clear effect with regard to procollagen type I only after 24 hours. The relative content to the untreated skin area shows a reduction in the procollagen type I values with a value of 0.58 **(Figure 2)**.

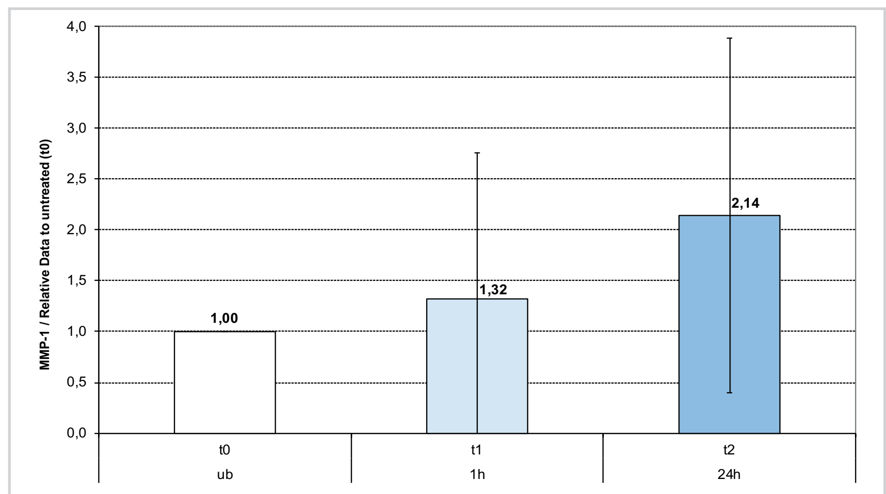


**Fig.1** Analysis of suction blister fluid before (t0), as well as one (t1) and 24 (t2) hours after blue light exposure to the skin. Shown are the relative data of the specific matrix metalloproteinase-1, i.e. the ratio between treated and untreated skin at the respective measurement times.

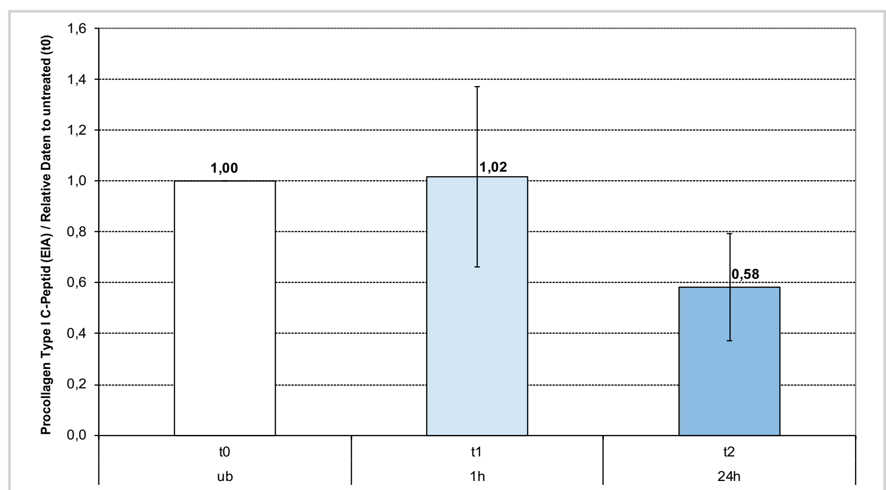


**Fig.2** Analysis of suction blister fluid before (t0), as well as one (t1) and 24 (t2) hours after exposure to blue light on the skin. Shown are the relative data of procollagen type 1, i.e. the ratio between treated and untreated skin at the respective measurement times.

The SEM examinations after blue light treatment show a significant change in the collagen and fiber structure on the dermal side of the suction blister roofs, while in the control sample the fiber structures of the basal collagen structures are clearly recognizable and clearly pronounced **(Figure 3, A)**, these become significantly less already 1h after blue light application **(Figure 3, B)**. Not only are there numerically fewer fiber structures, but the diameter and length of the individual fibers also change. 24h after the blue light application, these collagen and fiber structures are significantly reduced compared to the untreated control and the 1h value **(Figure 3, C)**. Significantly fewer fiber structures are recognizable in number as well as in length and diameter of the individual fibers. This is also shown by the quantitative determination of collagen and fiber structures at higher magnification in the SEM. The areas colored red correspond to the fiber structure or collagen content in the respective image. **Figure 4** shows the quantitative evaluation of collagen and fiber contents. Compared to the area not exposed to blue light, significantly lower values are already shown one hour after the blue light application, which are then reduced by another 37% 24 h after the blue light exposure compared to the non-exposed skin.

## Discussion

Skin aging induced by blue light is becoming increasingly important in the attention of the cosmetics industry and consumers. Against this background, we wanted to develop a new *in-vivo* test method that allows to show effects on the collagen network even at a low doses of blue light.

For this purpose, during the development of this method, we extensively examined the undersides of suction blister roofs in the SEM and found that they are 20-25% covered with the stratum lucidum of the basement membrane, to which collagens and the fiber network are anchored in many ways. By means of immune markings, we were able to clearly prove in the course of the method development that the fiber structures are different collagens.

Our data after blue light exposure of the skin show a decrease in collagen and fiber network as a function of time. Collagens, like many other structure giving fibers, are subject to constant up and down. It can therefore be assumed that over time, here after 1 and after 24 h, the biochemical framework conditions for the synthesis of collagens become worse and the parameters responsible for the degradation of collagen increase. This is shown in the

study presented here in the analysis of the biochemical parameters MMP1 and procollagen1. For the MMP1, it is clearly visible that there is an increase over time and thus could break down collagens. For procollagen type 1, a decrease in the concentration in the suction blister fluid can be seen, a build-up of collagen fibers is thus not promoted. However, if one compares the results found here with those in the literature, which are not always transferable as already described, there are some parallels. An increase in the concentrations of MMP1 in the medium of the investigated 3D-RHE models could be shown after an application of visible light with an intensity in between 65J/cm² and 180J/cm² by *Liebel et al* 2012 [9].

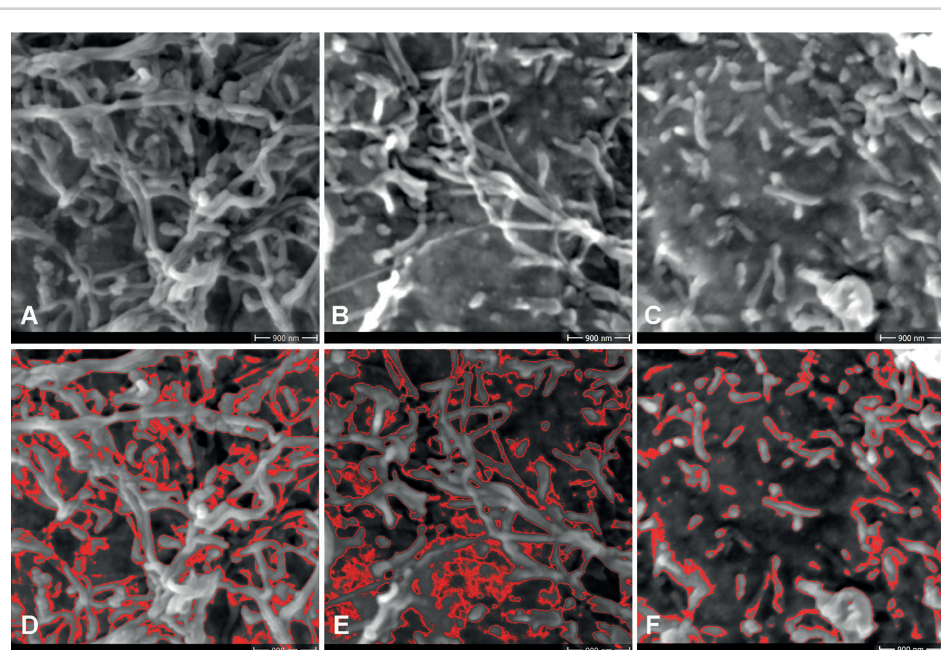


**Fig. 3** Scanning electron microscopy (SEM) images of the collagen network at the suction blister roof underside before blue light exposure (A, D), one hour after blue light exposure (B, E) and 24 hours after blue light exposure (C, F). Images A, B and C each show the original REM images, while images D, E and F show the collagen network outlined in red on the original images.

These effects were also induced by irradiation with UVA/B light. In 2010, *Liebmann and co-authors* were able to show that irradiation of cells with light of the wavelength 412-426 nm has an influence on the proliferation of keratinocytes [11], while *Mahmoud and colleagues* (2010) described an increasing pigmentation of the skin in an *in vivo* experiment with visible light without UVA content [13]. Similar results were also shown by *Campiche and co-authors* (2020) [12]. In an *in vivo* study, after blue light irradiation of the skin with a light source of a narrow emission spectrum, it was demonstrated that treatment with a microalgae extract reduces the pigmentation of the skin.

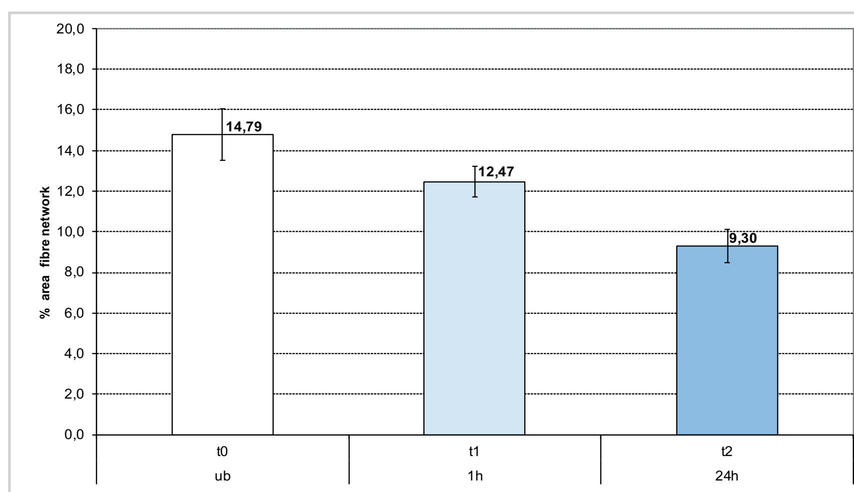


**Fig.4** Analysis of the areas covered with collagen and fiber network on the dermal side of the suction blister roofs before exposure to blue light (t0, untreated), as well as one (t1) and 24 hours (t2) after blue light treatment.

In addition to the similarities described here, the studies have also shown that lighting intensity and illumination spectrum play just as much a role in the investigation of the effects of blue light on the skin as the enclosed skin type [11,12,13]. Against this background, we were surprised that even with the low-threshold blue light application used here, clear effects were visible. Further studies with a larger number of subjects and product applications will further complement the methodology presented here and further elucidate the effect of blue light on the skin.

## Conclusion

With the pilot study presented here, we introduce a new *in-vivo* test method with the help of which the effects of blue light on the epidermis can be clearly demonstrated. The methodology allows to investigate the influences on the collagen network and the enzymes MMP-1 and procollagen type 1 even at a low blue light dosage. It is thus an effective *in vivo* test method for products that want to reduce the negative effects of blue light on the skin and reduce premature skin aging.
A decisive advantage of the method is that the microscopic images give a direct image of the collagen network of the skin, so that meaningful and reproducible results can be obtained even with a small number of cases.

## References:

[1] *Rembiesa J, Ruzgas T, Engblom J, Holefors A.* The impact of Pollution on Skin and Proper Efficacy Testing for Anti-Pollution-Claims. Cosmetics, 2018, 5.4.

[2] *Brash DE, Ziegler A, Jonason AS, Simon JA, Kunala S, Leffell DJ.* Sunlight and sunburn in human skin cancer: p53, apoptosis and tumor promotion. J Invest Dermatol Symp Proc (1996) 1:136-42

[3] Leffell DJ, Brash DE. Sunlight and skin cancer. Sci Am. (1996) 275:52-3 56-59

[4] *Suschek CV, Schroeder P, Aust O, Sies H, Mahotka C, Horstjann M.* The presence of nitrite during UVA irradiation protects from apoptosis. Faseb J. (2006) 17:2342-44

[5] *Diffey BL.* What is light? Photodermatol Photoimmunol Photomed (2002) 18(2):68-74

[6] *Svobodova A, Vostalova J.* Solar radiation induced skin damage: review of protective and preventive options. Int J Radiat Biol. (2010); 86 (12):999-1030

[7] *Karu T.* Photobiology of low-power laser effects. Health Phys (1989) 56:691-701

[8] *Schütz R.* Blue light and the skin. Challenges in sun protection. Curr Probl Dermatol. Basel Karger (2021) 55:345-373

[9] *Liebel F, Kaur S, Ruvolo E, Kollias N, Southhall MD.* Irradiation of Skin with Visible Light Induces Reactive Oxygen Species and Matrix Dagrading Enzymes. J Invest Dermatol (2012) 132, 1901-1907

[10] *Cho S, Lee MJ, Kim MS, et al*. Infrared pulsed vivible light and heat from natural sunlight participate in the expression of MMPs and type 1 procollagen as well as infiltration of inflammatory cell in human skin *in vivo*. J Invest Dermatol (2008) 50(2):123-33

[11] *Liebmann J, Born M, Kolb-Bachofen V.* Blue-Light Irradiation regulates Proliferation and Differentiation in Human Skin Cells. J Invest Dermatol (2010) 130, 259-269.

[12] *Campiche R, Curpen SJ, Lutchmanen-Kolanthan V, Gougeon S, Cherel M, Laurent G, Gempeler M, Schuetz R.* Pigmentation effects of blue light irradiation on skin and how to protect against them. International Journal of Cosmetic Science, 2020, 42, 399-406.

[13] *Mahmoud BH, Ruvolo E, Hexsel CL, Liu Y, Owen MR, Kollias N, Lim HW, Hamzavi IH.* Impact of Long-Wavelength UVA and Visible Light on Melanocompetent Skin. J Invest Dermatol (2010) 130, 2092-2097.

**authors**

D. Dähnhardt[1], A. v. Seebach[2], S. Dähnhardt-Pfeiffer[1], D. Segger[2]

[1] Microscopy Services Dähnhardt GmbH
Plambeckskamp 2 | 24220 Flintbek | Germany

[2] SGS Institut Fresenius GmbH
Dammtorwall 7a | 20354 Hamburg | Germany

Corresponding author: Dr. Dorothee Dähnhardt | dd@msd.sh
Microscopy Services Dähnhardt GmbH
Plambeckskamp 2 | 24220 Flintbek | Germany