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REVIEW

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Highlighting nuances of blue light phototherapy: Mechanisms and safety considerations

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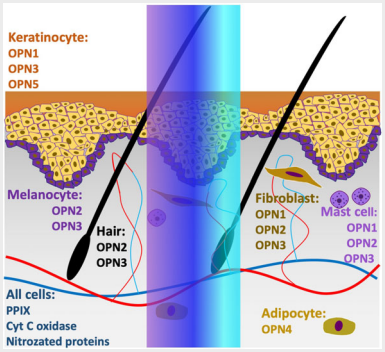
Abstract



The efficacy of blue light therapy in dermatology relies on numerous clinical studies. The safety remains a topic of controversy, where potentially deleterious effects were derived from in vitro rather than in vivo experiments. The objectives of this work were (1) to highlight the nuances behind "colors" of blue light, light propagation in tissue and the plurality of modes of action; and (2) to rigorously analyze studies on humans reporting both clinical and histological data from skin biopsies with focus on DNA damage, proliferation, apoptosis, oxidative stress, impact on collagen, elastin, immune cells, and pigmentation. We conclude that blue light therapy is safe for human skin. It induces intriguing skin pigmentation, in part mediated by photoreceptor Opsin-3, which might have a photoprotective effect against ultraviolet irradiation. Future research needs to unravel photochemical reactions and the most effective and safe parameters of blue light in dermatology.

KEYWORDS

dermatology, melanins, photobiology, photochemical processes, phototherapy, pigmentation, safety

1 | INTRODUCTION

1.1 | Light-tissue interactions and photochemical reactions

The terrestrial solar spectrum includes ultraviolet (UV), visible and infrared (IR) radiation. As early humans evolved under the influence of the sunlight, they developed mechanisms both to efficiently utilize it to fulfill key physiological functions and to protect the body against its excessive amount [1].

Advancements in the development of light sources fueled dramatic expansion of applications of light-based technologies in dermatology over recent decades. The

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clinical effects of light are based on photochemical, photothermal, photoablative, and photomechanical interactions of photons with cells and biological tissues, which triggers a plethora of responses at molecular, cellular, tissue, and organ levels [2-4]. Power density or irradiance in W/cm^2 and pulse duration (or exposure duration in case of continuous wave light source, cw) govern light interaction with biological tissue (see Figure 1). The group of photochemical reactions stems from empirical observations that light can induce chemical effects and reactions within molecules leading to photobiological effects. In contrast to all other interactions, photochemical reactions occur at very low power densities (typically 1 mW/cm^2 to few a few tens of mW/cm^2) and long exposure times (ranging from seconds to an hour) [4]. The operating window falls within the range of what one might expect from solar radiation (where for example irradiance integrated over the 400–495 nm band is about 13 mW/cm^2 [5], which would result in a dose of approx. 48 J/cm^2 for 1 h exposure time). An exemplary list of photochemical interactions induced by wavelengths across UV, visible and IR spectrum range is given in Table 1.

UV radiation (UVR) is the most widely studied spectral range, in terms of its impact on human health, with the classical examples including vitamin D production, melanogenesis (controlled by a complex regulatory mechanism, implicating pathways activated by receptor-dependent and independent mechanisms, in hormonal, auto, para, or intracrine fashion), dermatological applications [19] but also undesired effect of UVR including carcinogenesis [15, 20–23]. The UVR spectrum is divided into several bands (see Table 2), each with distinct optical penetration

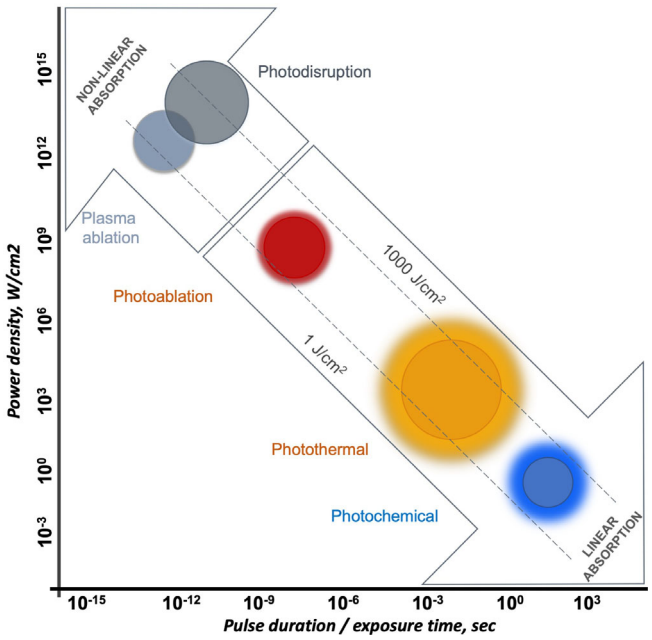

FIGURE 1 Laser-tissue interactions as a function of pulse duration in seconds (or illumination time for a continuous wave source) and irradiance in W/cm^2. Key laser-tissue interactions include photochemical, photothermal, photoablative and photomechanical (plasma ablation and photodisruption). While pulse duration and irradiance span 18 orders of magnitude, all these interactions are contained within a relatively narrow radiant exposure window between 1 and 1000 J/cm^2. Figure is adapted accordingly to Niemz and Uzunbajakava [2, 4]

TABLE 1 Major photochemical reactions and corresponding examples induced by UV, visible and NIR light

Photochemical reaction	Example
Linear addition to an unsaturated molecule	UV-induced crosslinking of DNA and proteins (the thymine in DNA is linked with the cysteine residue in proteins) [6]
Cycloaddition of unsaturated molecules	UV-induced formation of ring product, thymine dimer in DNA from two thymines [6]
Photofragmentation	UV-induced degradation of vitamin B, formation of lumiflavin by splitting off of the side chain of riboflavin [7] Blue light (420–450 nm) dependent nonenzymatic generation of nitric oxide from S-nitroso albumin in human skin [8, 9]
Photooxidation and photoreduction	UV-induced addition of peroxy group by the ring structure of cholesterol [10] Blue light (450 nm) induced photoreduction of FADox in cryptochrome [11] Blue, red, and IR-light induced photo-oxidation and photo-reduction of cytochrome c oxidase [12, 13]
Photohydration	UV-induced addition of water molecule to uracil at 5–6 double bond [14]
Cis-trans isomerization	Visible light-induced conversion of all-trans retinal to 11-cis retinal during light reception via opsins in human eye [15]
Photorearrangement	UV-mediated conversion of 7-dehydrocholesterol to vitamin D_3 [16–18]
Energy transfer	All photosensitized reactions with exogeneous and endogenous photosensitizers [6] Blue light (400–490 nm) and red light induced photosensitization of protoporphyrin IX [15, 19]

Abbreviations: NIR, near-infrared; UV, ultraviolet.

TABLE 2 Spectral regions of the terrestrial solar radiation

Spectral band name	Wavelength	Comment
UVR	280–400 nm	Extreme UV (1–100 nm) and UVC (100–280 nm) are completely absorbed by atmosphere
UVB	280–315 nm	The intensity at short wavelength cutoff of solar radiation at about 290 nm is strongly influenced by atmospheric ozone [24]
UVA	315–380 nm	ISO 20473:2007 definition of UVA [25] It serves for as International Standard for application in the field of optics and photonics
	315–400 nm	CIE definition of UVA, based on bioactinic effects of radiation, which intentionally allows for overlap between the UV-A and visible light in the deep violet range, 360–400 nm [26]
Visible	380–780 nm	ISO 20473:2007 definition of visible light [25]
	400–780 nm	CIE definition [26]
Blue	380–495 nm	
Violet blue Blue Cyan	380–420 nm 420–450 nm 450–495 nm	
Infrared	780 nm to 50 μm	
NIR	780 nm to 3 μm	
Mid-IR	3–50 μm	

Abbreviations: CIE, Commission Internationale de l'Éclairage; NIR, near-infrared; UVR, ultraviolet radiation.

depths and different consequential *physiological impacts* across various human organs and cells (such as the eye and skin) [25, 26]. Yet, the different standards variably define UVA spectral band, for example, Commission Internationale de l'Éclairage (CIE) allows for a blurred boarder between UVA and visible light around 360–400 nm [25, 26], the band often referred to as "actinic," while the latest ISO 20473:2007 definition of UVA is 315–380 nm [25].

It has long been appreciated that UVR can result in skin photodamage, manifesting as induction of apoptosis, arrest of cell proliferation or epidermal hyperplasia, DNA damage, oxidative stress, and appearance of "sunburn" dyskeratotic cells [27–29], and photoaging. Other major hallmarks include damage of collagen, elastin, fibrillin, activation of matrix metalloproteinases (MMPs) as well as altered equilibrium of immune cell populations [27, 28, 30, 31]. Moreover, UVR triggers photoprotection via the stimulation of melanogenesis [22, 23, 32–34]. Despite these well-appreciated potentially undesired effects, UV-based phototherapy continues to serve as an important and proven tool in the dermatological treatment of a range of skin diseases such as psoriasis vulgaris, vitiligo, atopic dermatitis (AD), eczema, and many more. The associated patient benefits are considered to outweigh the risks, and indeed has paved the way towards home-use of UVR and light-based devices [35–37].

The terrestrial photon flux of the Sun is greatest in the visible range [15]. Intriguingly, as the sun rises, reaches its peak at noon, and further decreases towards the dawn, the spectrum it emits not only has the highest intensity but also its highest variability in the violet-blue region (380–500 nm) [15,38] (see Figure 2). And so, we might expect that this part of the solar spectrum likely impacted disproportionately and significantly on terrestrial organisms via multiple molecular targets and associated interaction mechanisms [8, 39–47].

In an attempt to find a "safer" yet effective therapy, the last decade has witnessed a significant increase in the use of the blue light (400–495 nm) within the parameter window (irradiance and exposure time), which induces photobiological effects [8, 39, 48, 49]. Applications include its use as an exogenous photosynthesizer in the treatment of actinic keratosis (AK) during photodynamic therapy (PDT) [19] and on endogenous molecular targets in neonatal jaundice [50], acne [19, 49], psoriasis vulgaris [48], eczema [48] alopecia [51], blood pressure reduction [52], stimulation of endorphin release [53], pain relief [54], chronic wounds [55–57], and so forth.

As for the photochemical reactions underlying the therapeutic effects of blue light (see Table 1), the energy transfer reaction between protoporphyrin IX and oxygen mediates blue light PDT during acne treatment (endogenous photosensitizers) and during AK treatment with aminolevulinic acid (ALA), as the exogeneous photosensitizer [49, 58–65]. Photofragmentation under blue light, leading to nonenzymatic nitric oxide release from nitrozated proteins [8, 66], has been proven to be a mechanism underlying blue light therapies for psoriasis [67–70], eczema [71], endorphin release [52, 53], and blood pressure reduction [53]. Photooxidation and photoreduction of cytochrome c oxidase under blue light play a role in photobiomodulation [12, 13]. Whether it is a photoreduction of FADox in the circadian clock protein, cryptochrome, that occurs under blue light irradiation remains to be investigated. Yet, it was shown experimentally that 450 nm light impacts human

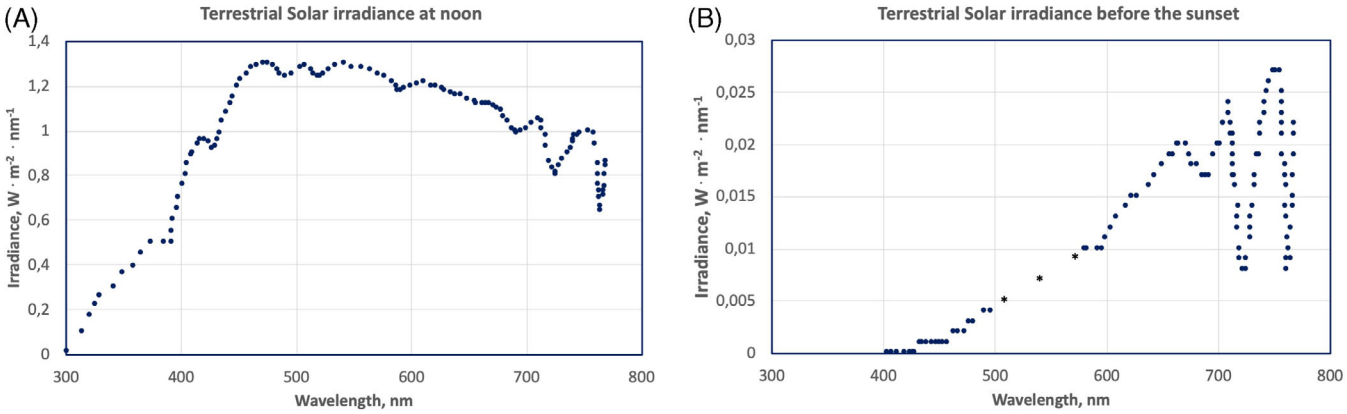

FIGURE 2 Example of terrestrial solar irradiance variability during the day. Irradiance at noon (A) and just before sunset (B) from above on a horizontal plane in Lund (South Sweden, 55.5°N 13.4° E on 15 July 2002, the ozone column was assumed to be 300 Dobson units and the ground albedo 0.2, aerosol 0 and air pressure 1000 millibar). Adapted by permission from the Springer Nature: Springer, Photobiology: The science of life and light by Lars Olof Björn, Ch. Terrestrial Daylight. COPYRIGHT 2008, https://link.springer.com/book/10.1007/978-0-387-72655-7. The graph is digitized using WebPlotDigitizer v4.5 based on the original data published by Björn [15] where the graphs were computed using an algorithm by Bird and Riordan [38]. Missing data in plot B are marked with asterisk. The data for the vertical plane pointed in the compass direction of the sun can be found in the original book by Björn [15]

cryptochrome and also exerts a positive impact on hair growth [44]. Cis–trans isomerization of retinal in the light-sensitive opsin mediates human vision and there is a growing evidence that opsin-family proteins are also involved in a response of human skin cells to UV and blue light [1, 13, 41, 42, 45–47, 72–77]. For example, it was shown that UVA can induce opsin-based phototransduction in normal human dermal fibroblasts [75] and UV-blue light (280–400 nm) can induce retinal-dependent phototransduction via opsin 5 in human epidermal melanocytes [76]. It has not been confirmed yet, however, that these opsin-based reactions in human skin occur *in vivo* via cis–trans isomerization of retinal as is the case in human vision.

These few examples accentuate a complexity of a multitude of photochemical reactions involved in interaction of blue light with human skin cells and accentuate a need for gaining a greater understanding of the operational parameter windows (wavelength, irradiance, dose, pulsing, treatment regime, etc.) of each of the reactions in relation to desired therapeutic effect. Several nomenclatures have emerged that relate the wavelength of light to its visually perceived color [78]. However, no agreed subdivision of the blue light spectrum has been proposed that connects the particular wavelength with a specific physiological response(s) in the skin. For clarity of communication in this review, we divide blue light into three bands: violet blue (380–420 nm); blue (420–450 nm); and cyan (450–495 nm; see Table 2).

1.2 | Controversies between in vivo and in vitro studies

While the efficacy of blue light therapies is supported by numerous clinical studies across a wide range of skin conditions, safety remains a topic of discussion. A source of much of this controversy likely emanates from a difficulty in accurately interpreting the scientific literature that is largely based on data from growing human skin cell types in isolated 2D monocultures (e.g., fibroblasts, keratinocytes, melanocytes, etc.) rather than as intact and multicellular 3D skin tissues. Thus, much caution is needed when reviewing data purporting to link blue light irradiation effects on photodamage of DNA, fibrillin, elastin, collagen, induction of apoptosis, alterations in proteins, including MMPs, and so forth in human skin cells [79]. While in vitro cell culture data may identify fundamental mechanisms of light interaction with cutaneous cells [8, 80–82] and clearly are attractive for researchers eager to avoid the complexity of running clinical studies on living human subjects, there is growing concern in biomedicine as to whether cell culture models can ever accurately reflect multilayered processes in real living humans [83]. Thus, great care is needed when interpreting blue light data obtained from in vitro cell models as well as extrapolating those readouts to physiologically relevant (i.e., real-world) clinical settings for example, skin aging and other visual manifestations.

In the first instance, the design of research studies involving light interaction with cells and tissues requires an in-depth understanding of relevant parameters (physical, optical, biological, and chemical conditions), which need to be fulfilled to enable photobiological effects to be accurately interpreted [82]. It may surprise many readers, but an overarching systematic approach and well-defined protocols are often lacking for many of the published in vitro studies of human skin cells [39, 82]. Second, the interpretation and extrapolation of in vitro cell-based data at an in vivo organ and whole body level require

very careful consideration. Light propagation characteristics in (nearly transparent) cell cultures differ considerably compared with opaque human skin of variable and considerable thickness [44, 84]. Moreover there is a lack of deep knowledge about the differing optical properties of different human skin layers, as well as the impact of skin appendages (hair follicles, sweat glands, etc.) and vasculature, and so forth [84]. An example of a workflow required to link optical parameters such as irradiance during in vitro studies to those applicable for in vivo situation, as well as quantitative results of photon density attenuation by the skin layers at 450 nm as a function of depth is shown in Figure 3. A schematic comparison between the in vitro case, where photon density of the incoming beam experiences virtually no attenuation, while the optical beam traversing turbid media such as human skin can experience drastic attenuation, which can be about 0.6 times for the papillary dermis but 10 times or more for the reticular dermis is shown in Figure 4 [84]. This in vitro to in vivo extrapolation is further complicated by the myriad physiologically relevant interactions between cells of different histological types and between these cells and extracellular matrix (ECM) in complex multicellular tissues like skin, as well as contributions from feedback control mechanisms, including the skin's antioxidant replenishing capacity, blood perfusion, oxygen gradients, and so forth. The now established neuroendocrine function of the skin, (orchestrated by interaction between dermal cellular populations such as fibroblasts, immune cells, endothelial cells, and sensory fibers (to name a few) [85]) further accentuates a need in a rational approach towards interpretation and validation of blue light data obtained from in vitro models, so that optimal clinical studies on human subjects can be designed and undertaken. As the number of applications where blue light could be of clinical value is rapidly expanding, we take the opportunity here to stand back to assess the current state-of-the-art in this domain.

Therefore, the goal of this study was to identify and review the published clinical study literature using blue light, specifically those studies where safety aspects were investigated using histological and immunohistochemical analysis of human skin biopsies. While doing this, we addressed the prevailing fragmented and contradictory views relating to the safety aspects of blue light therapy, to clarify the benefits to risks of exposure to visible light of 400–495 nm. Last, we propose new research directions that should help shape a more integrated view of blue light safety and modes of action that mediate cellular and tissue responses.

2 | METHODOLOGY

An extensive literature search was performed using PubMed (accessing articles from 1966 till Q1 2022) to

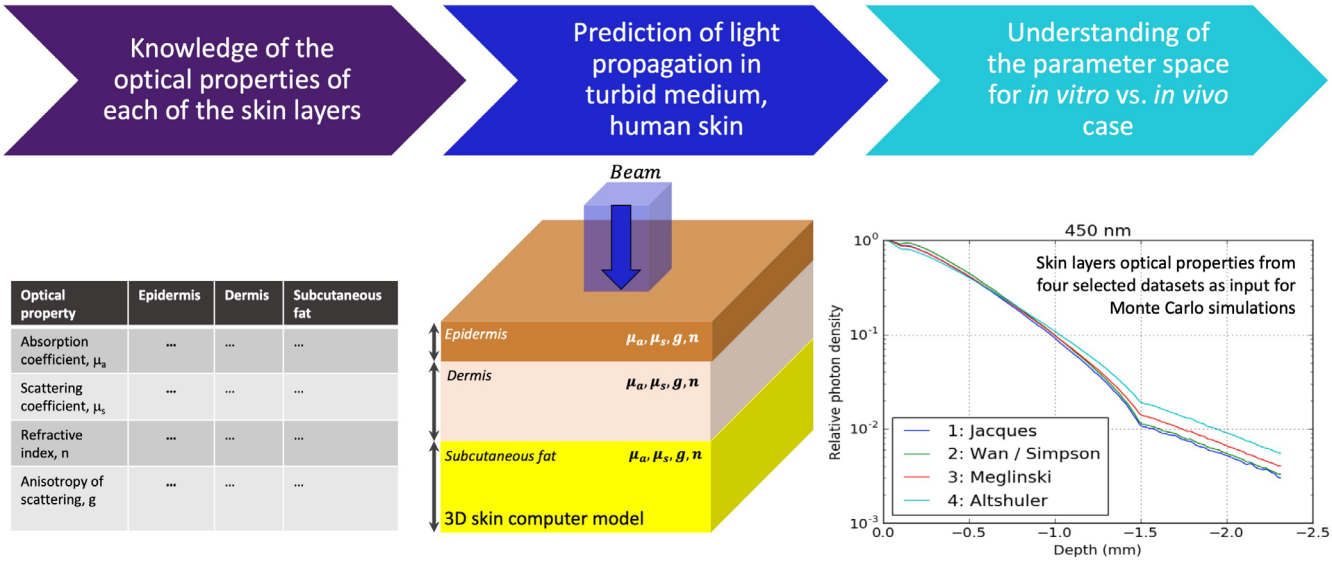

FIGURE 3 A schematic workflow showing the steps needed in order to link optical parameters such as irradiance during in vitro studies to those applicable for in vivo situation. This includes obtaining a validated and trustworthy set of the optical properties for each individual skin layer, performing Monte Carlo simulation of light propagation in turbid media such as human skin and finally interpreting a map of light attenuation in each of the skin layer in order to link irradiance during in vivo studies and the equivalent irradiance for in vitro cell studies. Quantitative results of photon density as a function of depth in the skin for 450 nm light is given accordingly to the results of Mignon et al. [84]

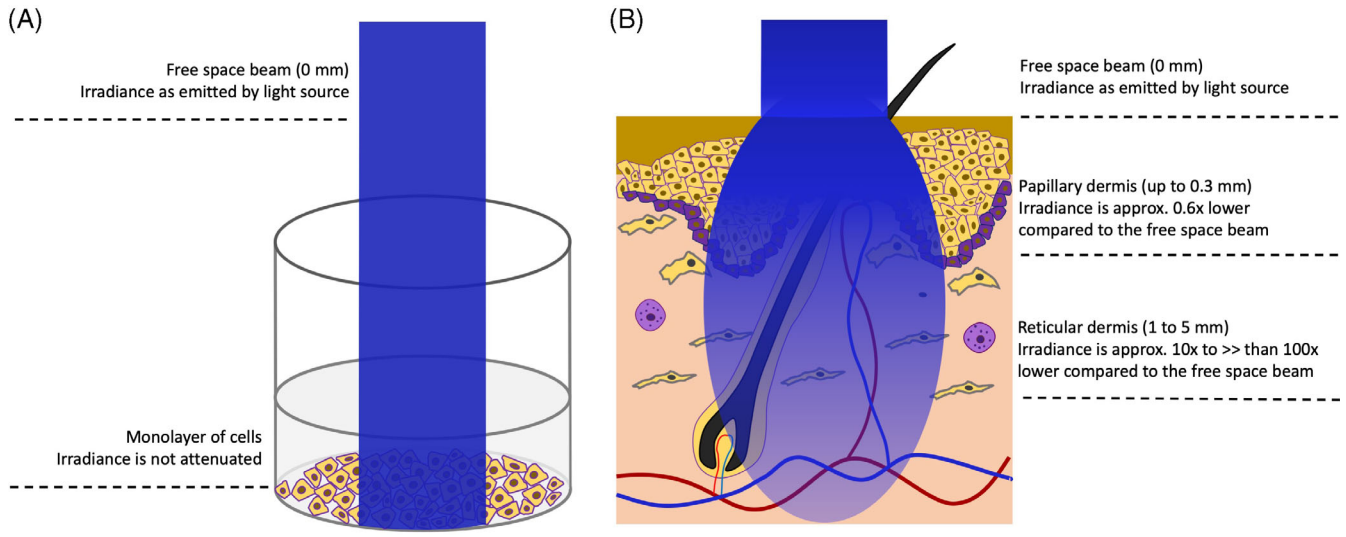

FIGURE 4 A schematic representation of light propagation for in vitro (A) versus in vivo (B) case. During in vitro studies on cell cultures the optical beam traverses through translucent and weekly absorbing (if at all) culture medium and hence it experiences virtually no attenuation compared with the free space beam. During in vivo studies optical beam traverses though turbid medium, which is absorbing (due to melanin, blood and water) and also scattering. As a result, the photon density is attenuated compared with the free medium. The attenuation for 450 nm can be, for example, 0.6 times at the level of papillary dermis and 10 times and up to more than 100 times at a level of reticular dermis

specifically identify and to review published reports of clinical studies using blue light on human subjects, where histological and immunohistochemical analysis of skin biopsies was performed. Our particular attention was drawn to those publications, where both clinical data and data from skin biopsies were available. Only these can provide the key missing links between molecular and cellular effects and likely clinical results—valuable information that is not available from in vitro studies alone. In particular, we assessed evidence on the impact of blue light on DNA damage, induction of apoptosis, oxidative stress, impact on the major components of the dermal ECM (collagen and elastin), inflammation, and pigmentation. Our search strategy included primary literature with blue light data on (i) key potential risks; (ii) key relevant therapeutic areas; (iii) coverage of the full range of blue light that is, 400–500 nm; and (iv) studies where both clinical and histological data were available.

We excluded: review articles, studies on neonates, articles reporting only in vitro data using human cells and animal studies. Included were therapeutic applications of blue light for the treatment of, for example, psoriasis vulgaris, eczema, hair loss (alopecia), acne, vitiligo, melasma, jaundice and inflammation in general. The details of the literature queries employed are presented in Table 3. A query was formulated by combining keywords about a light source, risks, clinical applications, and filtering by the exclusion criteria. Abstracts were screened by two independent experts, and duplications were eliminated. The search resulted in 57 unique articles; full-text versions of which were read and used as input. An additional filter was applied to identify publications containing both histopathological and clinical data, which resulted in seven original articles.

3 | RESULTS

In what follows, we present the data on the impact of blue light at clinical and histological levels in the order of increasing the wavelength of light and increasing the dose of treatment. We purposefully did that in an attempt to identify potential trends in the reported data, such as for example, wavelength-dependency of the obse effects.

3.1 | Assessing risk of blue light in relation to photodamage

Induction of apoptosis, arrest of cell proliferation, DNA damage, oxidative stress, and appearance of "sunburn" or dyskeratotic cells are hallmarks of UVR-induced photodamage [27, 28]. Summary information on impact of blue light on photodamage is given in Table 4. Ramaswamy et al. [86] performed a clinical study using blue light

TABLE 3 Keyword clusters used to construct a literature query

Risk	Application	Light	Study type
Aging	Skin	Blue light	Clinical
Skin damage	Cutaneous	Blue-violet light	Human (subjects)
Photodamage	Psoriasis	Violet blue light	
Cell damage	Dermatitis	Cyan light	
Pigmentation	Eczema	415 nm	
Oxidative stress	Acne	450 nm	
ROS	Hair	500 nm	
DNA damage	Alopecia		
	Jaundice		
	Vitiligo		
	Melasma		
	Inflammation		
	Hypertension		
	Blood pressure		
	Wound		

Abbreviation: ROS, reactive oxygen species.

source emitting at 417 ± 5 nm at 10 J/cm^2 ($n = 10$, patients with facial AK), where these lesions were treated with following a short incubation with ALA for PDT. Standardized facial biopsies were taken from non-AK skin before, 24 h and 1 month after therapy, and used to assess changes in proliferation (Ki67), cell cycle arrest/DNA damage (p53), and oxidative DNA damage (8-oxoguanine). PDT did not cause any changes in epidermal proliferation, at 24 h or 1 month after PDT compared with baseline, and so contrasts with cell cycle arrest seen after even a modest UVR exposure. In contrast, numerous p53-positive keratinocytes were detected immediately before PDT in all AK patients, which was interpreted as reflecting the presence of p53 mutations, which are found in ~50% of AKs [90]. Importantly, the incidence of p53-positive keratinocytes at 24 h and at 1-month post-PDT were variably higher or lower than the baseline count, presumably reflecting sampling differences and patient variability, but there was no consistent or statistically significant change at either time point. This study therefore also suggests that blue light PDT does not cause DNA damage, while in contrast, p53 is markedly induced even by a suberythemogenic UV dose [91, 92]. Furthermore, no 8-hydroxy-2-deoxyguanosine (8-OHdG), a marker of ROS-induced DNA damage, was detected in any biopsy 24 h after blue light PDT in marked contrast to positive control skin that was irradiated with UVA at 10 J/cm^2.

Duteil et al. [87] compared the effects of narrow-band blue light of 415 ± 5 nm (irradiance 30 mW/cm^2 at a range of doses up to 90 J/cm^2) with that of UVB exposure (at 1.5 minimal erythema dose [MED] at 89 ± 24 and 137 ± 44 mJ/cm^2, respectively) and 630 nm red light exposure (150 J/cm^2). The study was performed on healthy male subjects ($n = 12$, phototype III and IV, irradiation on the mid back). The results showed that cellular damage such as apoptosis, necrosis, DNA damage, oxidative stress (detected as mild-to-moderate 8-OHdG expression in mitochondria of basal keratinocytes and perivascular fibroblasts) were mainly induced by UVB exposure and not by blue light.

Kleinpenning et al. [88] investigated the impact of blue light with a peak emission at 420 nm (390–460 nm) at a daily dose of 20 J/cm^2 delivered over 5 consecutive days (healthy human subjects, $n = 8$, irradiated on the buttock area, skin types I–III), resulting in cumulative dose of 100 J/cm^2. Such dosimetry was carefully selected to be able to scrutinize the safety aspect of blue light at a dose typically applied during the clinical treatment of acne and AK. Punch biopsies were taken at 3 h after the first irradiation on Day 1 (control), and then on Days 2, 3, 5 and lastly 14 days following irradiation. Photodamage was accessed in the excised skin biopsies by evaluating the expression of p53, as an apoptotic marker, keratinocyte vacuolization (H&E, hematoxylin and eosin stain) and presence of "sunburn cells" [31], as characterized by dyskeratotic, vacuolated, and pyknotic keratinocytes with a strongly eosinophilic cytoplasm.

Importantly, there was no significant change in p53 expression seen over the whole treatment period. In fact, a temporary and statistically significant *decrease* in p53 expression was detected after the initial 24 h. p53 is upregulated in healthy skin to repair UV-induced DNA damage, while chronic sun exposure results in the loss or

TABLE 4 Impact of blue light on apoptosis, proliferation, cell damage and DNA damage based on histological and immunohistochemical analysis

Histological sign	Marker	Central wavelength and range	Dose and irradiance	Results	References
Apoptosis	p53	417 ± 5 nm	10 J/cm^2 ALA PDT	p53-positive cell counts at 24 h and 1 month were variably higher or lower vs. baseline, no consistent or statistically significant change	[86]
Apoptosis	p53	415 ± 5 nm	75.5 J/cm^2 37.7 J/cm^2 30 mW/cm^2	p53 expression less important than due to UVB p53 expression almost absent compared with UVB	[87]
Apoptosis	p53	420 nm 390–460 nm	20 J/cm^2 100 J/cm^2 over 5 days	No significant change over treatment period Temporary statistically significant decrease 24 h treatment ($p = 0.01$)	[88]
Apoptosis	p53	400–700 nm 400–495 nm	480 J/cm^2 100 J/cm^2 200 mW/cm^2	No difference with respect to control site	[89]
Proliferation	Ki67	417 ± 5 nm	10 J/cm^2 ALA PDT	No consistent or statistically significant change at 24 h or 1 month after PDT	[86]
Proliferation	Ki67	415 ± 5 nm	75.5 J/cm^2 30 mW/cm^2	No difference vs. control	[87]
DNA damage	8-OHdG	417 ± 5 nm	10 J/cm^2 + ALA PDT	No DNA damage in contrast to 10 J/cm^2 of UVA	[86]
DNA damage	8-OHdG	415 nm ± 5 nm	75.5 J/cm^2 30 mW/cm^2	No positive staining	[87]
Cell damage	Vacuolization (H&E)	420 nm 390–460 nm	20 J/cm^2 100 J/cm^2 over 5 days	Increase in perinuclear vacuolization of keratinocytes ($p = 0.02$)	[88]
Cell damage	Sun burn cells (H&E)	420 nm 390–460 nm	20 J/cm^2 100 J/cm^2 over 5 days	No sunburn cells visible	[88]
Cell damage	Necrosis (H&E)	415± 5 nm	75.5 J/cm^2 37.7 J/cm^2 30 mW/cm^2	Necrosis less important than due to UVB Necrosis almost absent compared with UVB	[87]
Cell damage	Morphology (H&E)	400–700 nm 400–495 nm	480 J/cm^2 100 J/cm^2	No difference with respect to control site	[89]

Abbreviations: ALA, aminolevulinic acid; 8-OHdG; 8-hydroxy-2-deoxyguanosinePDT, photodynamic therapy; UV, ultraviolet. Irradiance is given, when clearly reported in the original article.

mutation of p53, facilitating carcinogenesis. Therefore, an increase in p53 levels may reflect direct DNA damage or an ability of the cell to detect DNA damage, or both. The study by Kleinpenning et al. [88] demonstrated that p53 expression did not change during and after blue light (390–460 nm) irradiation. Moreover, no "sunburn" (dying) cells were detected at any stage before during or after blue light irradiation, although there was some evidence, albeit transitory, of keratinocyte vacuolation. This study concluded that blue light with a 420 nm central wavelength administered at 20 J/cm^2 over 5 days does not cause detectable DNA damage or early photodamage and that its short-term application in dermatological practice can be deemed to be safe.

Meanwhile, Mahmoud et al. [89] applied a light source emitting 98.3% visible light (400–700 nm) including 26%* of blue light (400–495 nm), 46%* of green light (500–595 nm), 1.5% IR (700–1800 nm), and 0.19% UVA1 (340–400 nm), (*author's estimate based on digitization of the published power spectral density). The study was

performed on healthy subjects ($n = 20$, skin types IV–VI, $n = 2$ skin type II, irradiation on the lower back). The highest dose for the visible light used in this study was 480 J/cm^2 and the highest estimated dose for blue light (400–495 nm) was 100 J/cm^2. Importantly, there was no difference in skin and cell morphology between the irradiated and control skin biopsies after 24 h, and p53 expression was not elevated in the exposed skin. The authors therefore conclude that the single irradiation as described did not cause any thermal and actinic DNA damage in healthy back skin.

3.2 | Assessing risk of blue light on ECM and immune cells

The major hallmarks of dermal photoaging are damage to collagen, elastin, fibrillin, activation of MMPs, as well as alterations in immune cell populations [27, 28, 30, 31]. Summary information on impact of blue light on ECM and immune response is given in Table 5. A study by Ramaswamy et al. [86] analyzed the impact of blue light PDT with 417 ± 5 nm wavelength at 10 J/cm^2 on Langerhans cell (LC) number in patients with facial AK ($n = 10$), as the number of these important immunosurveillance cells has been shown to decrease (leading to immunosuppression) after ALA or MAL-PDT using red light at the standard dose of 37 J/cm^2 [34, 94, 95]. Indeed, epidermal LC number decreases markedly in human skin after UV irradiation, and by 75% at 1 h after standard red light PDT [96, 97]. Examining non-AK facial biopsies before, 24 h and 1 month after blue light therapy, these researchers reported however, no consistent changes in the distribution, dendricity or number of LC after blue light PDT.

Kwon et al. [65] performed a clinical trial using 420 nm blue light and 660 nm red light. Treatment at fluence of 0.91 and 1.22 J/cm^2 for blue and red light accordingly (the irradiance 6.1 mW/cm^2 for the blue light and 8.1 mW/cm^2 for the red light) was performed twice a day for 4 weeks ($n = 35$ patients with mild-to-moderate acne, skin phototypes III-V). Histological examination of biopsies taken from the treated skin revealed a significant decrease in inflammatory and noninflammatory acne lesions (by 77% and 54%, respectively) at 12 weeks posttreatment compared with baseline. Other changes in the treatment group included sebum output reduction, attenuated inflammatory cell infiltrations and a decreased size of the sebaceous gland, changes that were associated with a decreased expression of interleukin (IL)-8, IL-1a, MMP-9, toll-like receptor (TLR)-2, nuclear factor (NF)-kB, insulin-like growth factor (IGF)-1 receptor, and sterol response element binding protein (SREBP)-1.

Kleinpenning et al. [88] evaluated the impact of blue light irradiation, over consecutive 5 days, on healthy buttock skin of eight subjects with skin types I to III (peak emission 420 nm, 390–460 nm range, single daily dose of 20 J/cm^2) on the manifestation of photoaging. Examining punch biopsies, taken daily and on Day 14 posttreatment, these researchers reported no disorganization of collagen and elastin fibers, and no significant changes to dermal MMP-1 expression or inflammatory cells infiltration. Thus, blue light exposure did not exert any detectable deleterious dermal effects.

In a study by Becker et al. [93], a broad-spectrum (315–1050 nm) light source with a total fluence of 43.7 J/cm^2, where 66% of the emission spectrum was blue light between 400 and 500 nm (28.9 J/cm^2) was applied on patients with severe chronic AD. Biopsies from $n = 9$ patients were examined after exposure to light. No decrease in the number of skin-infiltrating CD4+ T cells, or evidence of their apoptosis, was observed, in striking contrast to reported UVR-mediated effects. Similarly, unlike known depletion effects of UVR, this study also reported a relative increase in CD1a + epidermal LCs and HLA-DR positive dermal dendritic cells—both known antigen-presenting cells (APC; $p = 0.07$). Moreover, there was no observed alteration in either mast cell numbers or their degranulation (H&E and Giemsa staining) status upon irradiation with blue light.

Finally, application of a broadband light 400–700 nm at a maximal dose of 480 J/cm^2, containing up to 100 J/cm^2 of blue light (400–495 nm) and 220 J/cm^2 of green light (500–595 nm) in study by Mahmoud et al. [89] did not reveal any thermal or actinic damage effects in the dermis (including no change in p53 expression) 24 h after irradiation.

3.3 | Other histological findings

One professional system (ClearLight, Lumenis, Santa Clara, CA, USA) with the highest power spectral density at 410–420 nm but emitting also in 580–650 nm was used by Omi et al. [58] in 28 patients with facial acne (see Table 5), where excised biopsies were also examined. This study reported almost 65% improvement in acne lesions with associated changes in the distribution of dermal mast cells and fibroblasts around affected sebaceous glands after four sessions of therapy.

3.4 | Impact of blue light on pigmentation

Increased skin pigmentation is one of the only consistently reported and unintentionally induced effects of

TABLE 5 Impact of blue light on extracellular matrix and immune response based on histological and immunohistochemical examination of skin biopsies

Histological sign	Marker	Central wavelength and range	Dose and irradiance	Results	References
Collagen morphology	Von Giesen staining	420 nm 390–460 nm	20 J/cm^2 100 J/cm^2 over 5 days	No disorganization of collagen fibers during and after the irradiation period	[88]
Collagen damage	MMP-1	420 nm 390–460 nm	20 J/cm^2 100 J/cm^2 over 5 days	No change in dermal MMP-1 expression between treated and control	[88]
Elastin morphology	Von Giesen staining	420 nm 390–460 nm	20 J/cm^2 100 J/cm^2 over 5 days	No disorganization of elastin fibers during and after the irradiation period.	[88]
Elastin morphology	Antibodies	420 nm 390–460 nm	20 J/cm^2 100 J/cm^2 over 5 days	Elastin fibers showed regular-shaped candelabra-like structures and no disorganization	[88]
Elastin morphology	H&E, acid orcein	400–700 nm 400–495 nm	480 J/cm^2 124 J/cm^2 200 mW/cm^2	No thermal and actinic DNA damage	[89]
Inflammation	LC, CD1+	417 ± 5 nm	10 J/cm^2 + ALA PDT	Cell distribution, dendricity, number did not show any consistent change	[86]
Inflammation	MC (H&E, toluidine blue)	420 and 660 nm	0.9 and 1.22 J/cm^2 6.1 and 8.1 mW/cm^2 2×/day, 4 wks	Reductions in inflammation based on H&E and mast cell count	[65]
Inflammation	IL-8	420 and 660 nm	0.9 and 1.22 J/cm^2 6.1 and 8.1 mW/cm^2 2×/day, 4 wks	Statistically significant decrease	[65]
Inflammation	MMP-9	420 and 660 nm	0.9 and 1.22 J/cm^2 6.1 and 8.1 mW/cm^2 2×/day, 4 wks	Statistically significant decrease	[65]
Inflammation	TLR-2	420 and 660 nm	0.9 and 1.22 J/cm^2 6.1 and 8.1 mW/cm^2 2×/day, 4 wks	Statistically significant decrease	[65]
Inflammation	NF-kB	420 and 660 nm	0.9 and 1.22 J/cm^2 6.1 and 8.1 mW/cm^2 2×/day, 4 wks	Statistically significant decrease	[65]
Inflammation	IL-1a	420 and 660 nm	0.9 and 1.22 J/cm^2 6.1 and 8.1 mW/cm^2 2×/day, 4 wks	Statistically significant decrease	[65]
Inflammation	IGF-1 receptor	420 and 660 nm	0.9 and 1.22 J/cm^2 6.1 and 8.1 mW/cm^2 2×/day, 4 wks	Statistically significant decrease	[65]
Inflammation	Inflammatory cells, H&E	420 nm 390–460 nm	20 J/cm^2 100 J/cm^2 over 5 days	No inflammatory cells present	[88]

TABLE 5 (Continued)

Histological sign	Marker	Central wavelength and range	Dose and irradiance	Results	References
Inflammation	MC (H&E, Giemsma)	400–500 nm	28.9 J/cm^2 5 Tx 144.5 J/cm^2 15 Tx 433.5 J/cm^2	No alteration of the MC numbers or their degranulation	[93]
Inflammation	LC (CD1a +)	400–500 nm	28.9 J/cm^2 5 Tx 144.5 J/cm^2 15 Tx 433.5 J/cm^2	No increase in antigen presenting cells, CD1a + epidermal Langerhans cells and HLA-DR dermal DC	[93]
Inflammation	APC (HLA-DR+)	400–500 nm	28.9 J/cm2 5 Tx 144.5 J/cm^2 15 Tx 433.5 J/cm^2	No increase in antigen presenting cells, CD1a + epidermal Langerhans cells and HLA-DR dermal DC	[93]
Inflammation	anti-CD4 (for CD4+ T cells)	400–500 nm	28.9 J/cm^2 5 Tx 144.5 J/cm^2 15 Tx 433.5 J/cm^2	No decrease in the number of T cells, no signs of lymphocyte apoptosis	[93]
Lipogenesis	SREBP-1; mRNA of srebp-1	420 and 660 nm	0.9 and 1.22 J/cm^2 6.1 and 8.1 mW/cm^2 2×/day, 4 wks	Decreased in sterol response element binding protein	[65]

Abbreviations: ALA, aminolevulinic acid; APC, antigen-presenting cells; IGF, insulin-like growth factor; IL, interlukin; LC, langerhans cell; MC, Mast cells; MMP, matrix metalloproteinases; NF, nuclear factor; PDT, photodynamic therapy; SREBP-1, sterol response element binding protein-1; TLR, toll-like receptor. Irradiance is given when clearly reported in the original article.

blue light therapy in skin, which for some individuals may be considered undesirable.

Duteil et al. [87] compared the minimal pigmentation dose (MPD) of 415 nm blue light versus 630 nm red light as well as the MED for UVB ($n = 12$ healthy male subjects, mid-back skin irradiated, skin phototypes III–VI). MPD was evaluated 7 days after irradiation. This was defined as the lowest dose that induced a coloration difference (L^* [Lightness] defined by CIE; using measuring device Konica-Minolta CR400 chromameter) of $\Delta L^* = -2$ compared with the nonexposed skin. The MED for UVB light was 113 ± 4 2 mJ/cm^2 and the MPD for 415 nm light was 58 ± 20 J/cm^2. Interestingly, while photometric assessment clearly showed a decrease in skin lightness and an increased clinical pigmentation score (already starting at 10 J/cm^2 of 415 nm light), melanocyte number was not affected by any of the treatments. Individuals with darker skin phototype showed a higher propigmentary response. There was, however, no evidence of any effect on epidermal cell proliferation, as assessed by Ki67 immunolabeling. Similarly, dermal melanophage (cells that phagocytose melanin when the latter is moved into dermis) number did not change, while melanophage numbers increased after exposure to 1.5 MED UVB light or 150 J/cm^2 red light (630 nm). Blue light induced higher levels of pigmentation than UVB (assessed visually and using Konica-Minolta) at 24 h after exposure, as expected, as "immediate tanning" is mainly associated with UVA-associated wavelength. 415 nm light induced a dose-dependent and skin type-dependent hyperpigmentation, that was still highly pronounced even 3 months after exposure to the blue-violet light.

Kleinpenning et al. [88] assessed the impact of blue light (420 nm, 20 J/cm^2 daily dose over 5 consecutive days) on skin pigmentation ($n = 8$ healthy individuals, buttock area illuminated, skin types I–III, see Table 6). Minimal clinically detectable hyperpigmentation was reported in just one of the eight irradiated subjects. This was confirmed immunohistologically as an increase in Melan-A positive cells (presumably melanocytes) already 3 h after the first irradiation with subsequent increase over 4 days of treatments and a decrease after cessation of irradiations.

Mahmoud et al. [89] studied the differential impact of visible light (including 400–495 nm blue light, ∼ 40 mW/cm^2 irradiance, and 500–595 nm green light, ∼90 mW/cm^2 irradiance) versus that of the 340–400 nm UVA on melano-competent skin ($n = 12$ healthy individuals, lower back irradiation, Fitzpatrick skin phototypes IV–VI). Authors also investigated dose-dependent behavior of light in melano-competent skin and benchmarked this against the response of two individuals with skin phototype II. As for the time dynamics, immediate tanning, as determined by diffuse reflectance spectroscopy was

TABLE 6 Impact of blue light on pigmentation based on histological and immunohistochemical examination of skin biopsies

Skin type	Marker/method	Central wavelength and range	Dose and irradiance	Results	Ref
III and IV	Masson-Fontana, histochemistry	415 ± 5 nm	75.5 J/cm^2 37.7 J/cm^2 30 mW/cm^2	No increase vs. control	[87]
III and IV	MITF immunohistochemistry	415 ± 5 nm	75.5 J/cm^2 37.7 J/cm^2 30 mW/cm^2	No increase vs. control	[87]
III and IV	Chromametry, L^*	415 ± 5 nm	60 ± 19 J/cm^2 55 ± 23 J/cm^2 30 mW/cm^2	MPD 7 days postirradiation for skin type III MPD 7 days post irradiation for skin type IV	[87]
I–III	Mela-A, histochemistry	420 nm 390–460 nm	20 J/cm^2 100 J/cm^2 over 5 days	In $n = 1$ (out of $n = 8$) subjects, min. Clinical hyperpigmentation and an increase in Melan-A-positive cells over the hours (from 0 h to 3, 24, and 48, 96 h)	[88]
IV–VI	Diffuse reflectance spectroscopy	400–700 nm incl. 400–495 nm	8–480 J/cm^2 2–100 J/cm^2 200 mW/cm^2	Immediate pigmentation after 30 min postirradiation at 40 J/cm^2 broadband light (10 J/cm^2 blue light); dose-dependent pigmentation; no pigmentation in skin type II	[89]
IV–VI	Reflectance confocal microscopy	400–700 nm incl. 400–495 nm	8–480 J/cm^2 2–100 J/cm^2 200 mW/cm^2	2 and 24 h postirradiation migration of melanin from basal cells to the upper epidermal cell	[89]
IV–VI	Mason-Fontana histochemistry	400–700 nm incl. 400–495 nm	8–480 J/cm^2 2–100 J/cm^2 200 mW/cm^2	24 h postirradiation redistribution of melanin from the basal layer into the keratinocytes in the upper spinous cell layers	[89]

Abbreviation: MITF, microphthalmia-associated transcription factor. Irradiance is given when clearly reported in the source.

already evident in skin types IV–VI 30 min after exposure to the lowest dose (40 J/cm^2 dose) of visible light (400–700 nm) irradiation, of which 10 J/cm^2 derived from the component 400–495 nm blue light and 18 J/cm^2 from green light. This pigmentation was characteristically brown, in contrast to initially grayish color induced by UVA light (which later turned brown after 24 h) and was sustained during the 2-week period of the study. Induced pigmentation was even stronger in skin phototype V individuals. In contrast, no pigmentation was induced in individuals with skin phototype II. Confocal microscopy and Fontana Masson staining revealed redistribution of melanin pigment from basal cells to the upper epidermal cell layers as early as 2 and 24 h after irradiation compared with control.

Clinical data (without examination of biopsies) reporting on the potency of blue light in inducing skin hyperpigmentation in patients with different skin diseases and in healthy subjects have been published by several research groups (see Table 7). The absence of skin biopsy assessment makes it impossible to conclude any effect of blue light on melanocyte and keratinocyte proliferation and viability as well as understanding the mechanism of pigmentation induction. To maximize scientific

TABLE 7 Impact of blue light on hyperpigmentation as observed in clinical studies based on noninvasive measurements without histological confirmation

Skin type	Group/method	Central wavelength and range	Dose and irradiance	Results	Ref
IV	Patients with acne; Mexameter (Courage and Khazaka)	415 ± 5 nm 633 ± 6 nm	48 J/cm^2, 40 mW/cm^2 96 J/cm^2 80 mW/cm^2 20 min 2×/wk Tx for 4 wks	Melanin level increased by 6.7 after 1× Tx after 415 nm, $p = 0.31$ Melanin level decreased by 15.5 after 1× 633 nm, $p = 0.002$	[63]
N/A	Patients with AGA; clinical observation	417 ± 10 nm	120 J/cm^2, 60 mW/cm^2 2 Tx/wk; 10 wks	Darkening of the hair shaft in 30% of patients	[51]
Not reported	Patients with PV; clinical	420 nm	120 J/cm^2 100 mW/cm^2 20 min	The severity of the hyperpigmentation increased from minimal to mild according to the number of treatments. An 80% of the treated plaques showed surrounding hyperpigmentation	[67]
I–IV	Patients with PV; patient VAS; physician VAS	420 nm 453 nm	90 J/cm^2 100 mW/cm^2 15 min 7 Tx/wk; 4 wks	Hyperpigmentation in 59% of patients treated with 420 nm Hyperpigmentation in 50% of patients treated with 453 nm Long-existing >2 wks after the end of treatment with both wavelength	[68]
I–IV	Patients with PV; Mexameter (Courage and Khazaka)	450 nm	Dose: 90 J/cm^2 cw: 50 mW/cm^2 Peak: 200 mW/cm^2 Peak: 100 mW/cm^2	Self-reported hyperpigmentation in 50% of patients. Mexameter values for the treated site are above the control over Tx period (not statistically significant)	[70]
III and IV	Healthy subjects; Hyperspectral imaging, chromameter measurements and photographs	420–500 nm, 450 nm central	60 J/cm^2, 4 daily Tx; 240 J/cm^2 cumulative dose	Continuous increase in melanin after each Tx, reaching statistical significance after 4Tx. Melanin content remained constant until Day 28 after that. A significant decrease in ITA° value immediately after the irradiation phase at Day 3, suggesting visible hyperpigmentation. Skin darkening based on measured L^* values (using photographic measurements)	[98]

(Continues)

TABLE 7 (Continued)

Skin type	Group/method	Central wavelength and range	Dose and irradiance	Results	Ref
I–III	Healthy skin	453 nm	18 J/cm^2 Cw 10 mW/cm^2 Pulsed 200 mW/cm^2	Increase in b^* and decrease in L^* and ITA immediately after exposure to light were attributed to immediate pigment darkening	[99]
III and IV	Healthy subjects; visual evaluation, Mexameter	456 nm light (FWHM 20 nm)	45–270 J/cm^2 in a single Tx	MPPD determined 2–4 h after irradiation by visual examination varied between 135 and 180 J/cm^2 (average was 180 J/cm^2) and 135–225 J/cm^2	[100]

Abbreviations: AGA, androgenetic alopecia; FWHM, full width at half maximum; ITA, individual topology angle; MPPD, minimal persistent pigment darkening; PV, psoriasis vulgaris; VAS, visual-analogue scale.

learnings and to create value for wider and future patient's groups we attempted to extract from these studies (i) wavelength-dependent, (ii) dose-dependent (iii) skin phototype-dependent behaviors, and (iv) time dynamics, if these were indeed reported.

Lee et al. [63] published that during acne treatment in patients with skin phototype IV in response to 415 nm light at 48 J/cm^2, melanin level increased already after one treatment (though this data did not reach statistical significance). In a study conducted by Lodi et al. [51] using 415 nm light at 20 J/cm^2 on patients with androgenetic alopecia, 90% of patients treated biweekly for 10 weeks showed a statistically significant increase in hair density and hair diameter with remarkable and unexpected darkening of the hair reported in 30% of patients. Kleinpenning et al. [67] showed that treatment of psoriatic plaques with 420 nm light at 120 J/cm^2 leads to significant pigmentation in 80% of the treated lesions and that pigmentation increases from minimal to mild according to the number of treatments (skin phototype not reported), highlighting dose dependency. Weinstable et al. [68] compared the effects of 420 nm light at 90 J/cm^2 versus 453 nm light at the during clinical studies on patients with psoriasis vulgaris (skin phototypes I–IV). Strikingly, hyperpigmentation occurred in 59% of patients treated with 420 nm and only in 50% of patients treated with 453 nm light, at the same dose. The reported hyperpigmentation was long-existing, lasting more than 2 weeks after the end of treatment with both wavelengths. This possibly reflected the cellular turnover time of the epidermis to "clear-out" these hypermelanized keratinocytes. To decrease the promelanizing impact of blue light Pfaff et al. [70] conducted a clinical study on patients with psoriasis vulgaris using a pulsed blue 453 nm light source that was developed to reduce the thermal impact, by allowing relaxation of temperature build-up due to absorption by melanin. Perilesional hyperpigmentation was still reported by 50% of the patients, although difference from controls did not reach statistical significance.

As for the studies conducted on healthy subjects, Campiche et al. [98] applied hyperspectral imaging, chromameter measurements and photographs to assess clinically visible hyperpigmentation after irradiations of volar forearm of healthy subjects with 420–500 nm light of 60 J/cm^2, cumulative dose over several treatments of 240 J/cm^2. Continuous increase in melanin following each blue light irradiation was reported based on hyperspectral images, reaching statistical significance after the blue light irradiation protocol ended. Melanin content remained constant until Day 28 after that, again possibly reflect the cellular turnover time of the epidermis. Interestingly, they reported that hyperpigmentation was partially mitigated by both a topical formulation containing 3% of a microalgal product and a formulation containing 3% niacinamide (Vit-B3). Falcone et al. [99] investigated the impact of 453 nm narrow band light on skin color ($L^*a^*b^*$ values, ITA, and absorbance) at 18 J/cm^2 (both using cw irradiance of 10 mW/cm^2 and pulsed irradiance 200 mW/cm^2) in skin types I–II (healthy skin, without and with acute perturbation such as tape stripping and histamine iontophoresis). An increase in b^* and a decrease in L^* and ITA immediately after exposure to light were attributed to immediate pigment darkening. Jo et al. [100] determined minimal persistent pigment darkening dose (MPPD) in response to 456 nm light ($n = 10$, healthy female subjects, back irradiated, skin types III and IV). Light dose varied from 45 to 270 J/cm^2 in a single treatment. MPPD 2–4 h after irradiation (based on visual evaluation and Mexameter measurements) on

unprotected and protected skin with TiO_2-containing cream varied between 135–180 and 135–225 J/cm^2, respectively.

4 | DISCUSSION

As the quest for a light therapy offering more attractive risk-benefit ratio continues, the lower energy blue light photons, spanning the 400–495 nm range, being the "nearest neighbor" of UVR (i.e., UVA) has become the focus of attention. By now, a significant increase in the clinical use of the blue light phototherapy has been reported [8, 39, 48, 49] including PDT of AK [19], treatment of neonatal jaundice [50], acne [19, 49, 58–65], psoriasis vulgaris [48, 67–70, 101], eczema [48, 71, 93], alopecia [51], blood pressure reduction [52], stimulation of endorphin release [53], chronic wound healing [55–57], and many more, where the efficacy and thus the benefits of blue light therapies is evidenced from numerous clinical studies.

As for the risks, the increasing number of in vitro studies using 2D human cell cultures that purport to show DNA damage, photodamage of fibrillin, elastin, collagen, induction of apoptosis as a result of altering activity of a varieties of proteins, including MMPs, and so forth has raised concerns [79, 102–105].

While the value of in vitro and ex vivo cell and tissue culture data is considerable, for example, to identify fundamental molecular mechanisms of light interaction with cutaneous cells, we must avoid the temptation to extrapolate these data to physiologically relevant clinical contexts like skin aging and other phenotypic manifestations, especially, given the proven clinical benefits of blue light therapy for a range of cutaneous disorders, helping patients. This has never been more relevant, given our fuller understanding of the limitations of in vitro data. Indeed, in a recent in-depth analysis of this issue by Klein et al. [106], it is now clear that standard cell cultures consistently exhibit environmental instability, and these authors propose that this is a "pervasive issue" affecting experimental findings.

Therefore, the key objective of this study was to identify and to review clinical studies using blue light, specifically where safety aspects were investigated in intact human skin (as excised human skin biopsies), using histological and immunohistochemical analysis.

Surprisingly, in contrast to the reported in vitro data, the results obtained using skin biopsies excised after blue light exposure over the entire bandwidth (400–495 nm) at fluences up to 124 J/cm^2 failed to support a blue light association with hallmarks of photodamage, such as induction of apoptosis, arrest of cell proliferation, DNA damage, cell damage including presence of sunburnt, dyskeratotic cells (see Table 4).

No cell death by apoptosis was reported at a wide range of light parameters spanning 10 J/cm^2 at 417 nm to 100 J/cm^2 at 400–495 nm. Moreover, there was no evident impact on cell proliferation at 415–417 nm wavelength at a dose up to 90 J/cm^2. These data are especially convincing as they are derived from experimental designs that included a comparison with UVA and UVB, where the impact of both on DNA damage and cell damage was clearly present [86, 87].

Using the same parameter range as described above, these blue light studies in intact human skin also failed to show histological evidence of frank photoaging (see Table 5), such as damage of collagen, elastin, fibrillin and activation of MMPs [88, 89].

Importantly, no evidence of recruitment or activation of inflammatory immunocytes was found after a single exposure of skin to 420 nm blue light at 20 J/cm^2 and also at a cumulative dose of 100 J/cm^2 over several days [88]. There was no reduction in the number or viability of T cells using 400–500 nm light at 28.9 J/cm^2 daily dose and cumulative dose up to 433.5 J/cm^2 and also no alteration in the number of mast cells and their degranulation was reported [93].

LCs number and their dendricity were not affected by 417 nm light at 10 J/cm^2. [86] A higher dose of 28.9 J/cm^2 and cumulative dose up to 433.5 J/cm^2 of 400–500 nm light, increased APC, CD1+ epidermal LCs and HLA-DR dermal DCs [93].

Interestingly, both blue and red light at much lower doses (420 nm 0.9 J/cm^2 and 660 nm 1.22 J/cm^2) were reported to exhibit an anti-inflammatory effect, characterized by reductions in mast cell count, IL-8, TLR-2, NF-kB, IL-1a, IGF-1 receptor, and SREBP-1 [65].

As for clinical observations, not supported by skin biopsies analysis, the data by Jo et al. [100, 107] reported that 456 nm light repeated irradiation at total doses 958, 1597, and 447 J/cm^2 delivered in 3, 10, and 14 days resulted in decreased skin elasticity and hydration. To our knowledge, however, none of the existing commercial FDA and CE-approved commercial devices for blue light therapy delivers such a high dose of 269 J/cm^2 and 112 mW/cm^2 irradiance in a single treatment. Here, next to direct photochemical reactions induced by light, one must be careful with the "collateral impact" due to temperature increase as a result of light absorption by melanin in darker skin types. Therefore, the reported "adverse" effects on skin hydration and elasticity could be attributed to a suboptimal experimental study design. Clinical treatment protocols with blue light and also recently developed protocols for ex vivo and in vitro studies all need to pay careful attention to the selection of irradiance, light dose and thermal management [71, 81, 82] to avoid undesired effects due to heating.

This review of the clinical studies, where assessment of pigmentary change was conducted in intact skin, not in isolated cultured melanocytes in 2D assays, shows that application of blue light (400–495 nm) in vivo causes a clinical hyperpigmentation, detected by both noninvasive optical methods, and confirmed histologically [87–89].

What is very clear is that this effect has a dose-dependent character, with a gradual increase in pigmentation upon an increase in a dose, manifested both at a clinical level and also in immune-histochemical analysis [67, 87–89, 98,100].

Also, dependence of blue light induced hyperpigmentation on skin phototype is clearly documented [87, 89]. Hyperpigmentation was mainly reported in melano-competent skin types III to VI and to be absent in fairer skin (phototypes I and II) at an equivalent blue light dose (Mahmoud et al. [89]). However, the recent study by Moreiras et al. [43] using a modified and sensitive Warthin-Starry stain for specific melanin detection [108] demonstrated that 140 J/cm^2 of blue light (peak wavelength 450 nm) and 140 J/cm^2 of green light (peak wavelength 530 nm) both induce melanin production in healthy human skin in ex vivo histoculture of skin types II and III. While UVR (at 6 J/cm^2) failed to induce a histologically detectable pigmentation in the palest skin type I skin (despite this radiation causing DNA damage in the epidermis), remarkably, a histologically detectable increase in melanin was evident in the epidermis of blue and green light irradiated phototype I skin. Of note, this was not detectable as a color change at the skin surface. This study results may explain the lack of detectable surface pigmentation in skin type II in response to visible light (up to 120 J/cm^2 of blue-green light) reported by Mahmoud et al. [89] Very importantly, the Moreiras et al. study shows that in marked contrast to UVR, no detectable increase in DNA damage or cell apoptosis (as assessed by cyclobutane pyrimidine dimers and caspase-3 expression) was detected even at these rather high doses of blue and green light (140 J/cm^2).

Duteil et al. [87] reported MPD of 60 J/cm^2 for 415 nm blue light in skin types III–VI, when assessed 1 day after the first treatment and Jo et al. [100] defined MPPD as 135–180 J/cm^2 at 456 nm light, in the same skin types.

As for the minimum threshold dose of blue light that induces the minute pigmentation detectable in spectroscopic measurements this value in skin types IV and V was as low as a sum of 10 J/cm^2 of blue light and 18 J/cm^2 of green light (where both wavelength are potent pigmentation inductors [43]), when detected already 30 min after treatment [89]. This result is in line with the data by Falcone et al. [99], reporting on pigmentation response already detectable at 18 J/cm^2 of 453 nm light. Also, Duteil et al. [87] reported changes in ITA and skin lightness at as low as 10 J/cm^2 of 415 nm light.

It is not however, possible to establish a well-defined MPD dose or minimal change in skin lightness dose at a selected wavelength due to multiple factors, such as differences in the skin types of the recruited subjects, different measurement methods (i.e., spectrophotometry vs. diffuse reflectance), different irradiances, where the latter is suggested to play a role in defining pigmentary response [109] as well as different definitions of a "threshold" and MPD.

Speaking of time dynamics of pigmentary response, immediate pigment darkening occurs 30 min after irradiation where redistribution of melanin can be detected histologically 2 h after treatment [89]. At the same time, de novo melanin production is also reported as early as 3 h postexposure [88] or 24 h after 3 daily exposures [43]. Such pigmentation was sustained longer than 14 days after the last exposure [67, 68, 88, 89, 98] and even up to 3 months postirradiation [87].

Perhaps, the most intriguing question, as far as the skin pigmentary response is concerned, is whether the published data reveal any information allowing us to define physiologically relevant "bands" of blue light and also whether we may relate them to skin safety and to photochemical reactions underlying skin response to light. Unfortunately, here, we still remain in dark, with only scattered bits of the puzzle becoming available. For example, Weinstable et al. [68] reported the results of treatment of psoriasis vulgaris in patients with skin phototypes I–IV, where hyperpigmentation occurred in 59% of patients' treatment with 420 nm light at 90 J/cm^2, while this occurred in 50% of patients treated with 453 nm light at the same dose. The data on MPD or MPPD, were 60 J/cm^2 for 415 nm blue light [87] and 135–180 J/cm^2 at 456 nm light [100]. These data highlight a potential wavelength-dependency of the propigmentary effect.

5 | CONCLUSIONS AND PERSPECTIVES

In contrast to reported in vitro data results obtained using skin biopsies excised after blue light exposure (400–495 nm) at fluences from 10 to 124 J/cm^2 failed to support a blue light association with hallmarks of photodamage, such as induction of apoptosis, arrest of cell proliferation, DNA damage, cell damage including presence of sunburnt, dyskeratotic cells. Similarly, no histological evidence of frank photoaging, such as damage of collagen, elastin, fibrillin, activation of MMPs and no evidence of recruitment or activation of inflammatory immunocytes was found.

Induction of human skin pigmentation was the only unintended and yet consistent endpoint of blue light exposure, which is driven by different mechanisms compared with UV light [40] and can occur already immediately after blue light irradiation. With this in mind, we hypothesize that blue-green visible light may even protect healthy individuals with pale skin (skin phototypes I and II) from damaging effects of UVR, by increasing the mixed melanin content of the epidermis with low, nondeleterious light doses, as suggested by Moreiras et al. [43] and as is the case when using α-MSH analog for induction of sustained increased melanocyte dendricity, skin pigmentation and enhanced photoprotection in patients with urticaria [110].

The majority of currently available blue light-based therapies operate within a dose window below 100 J/cm^2, which is equivalent to ∼2 h of exposure with the sun in zenith during summer [87], and which was previously considered as not requiring light absorbing sunscreens, except perhaps for patients taking photosensitizing medication or suffering from photosensitive diseases such as patients with melasma, where protection against blue-green part of the visible spectrum under natural sunlight (but not computer screens) may become important [109, 111].

Therefore, blue light may indeed be beneficial in treatment of skin and hair growth disorders. Future research should be focused on clarification of delineation between the specific bands of blue light (e.g., 380–420 nm, 420–450 nm, 450–495 nm), on understanding how they affect human skin and its appendages (including microbiome), what are the governing photochemical reactions and what are the most effective and safe therapeutic parameters sets.

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DATA AVAILABILITY STATEMENT

We don't present original experimental data.

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REFERENCES

[1] A. T. Slominski, M. A. Zmijewski, P. M. Plonka, J. P. Szaflarski, R. Paus, *Endocrinology* **2018**, *159*, 1992.
[2] N. E. Uzunbajakava, R. Verhagen, A. Vogel, N. Botchkareva, B. Varghese, Highlighting the nuances behind interaction of picosecond pulses with human skin: relating distinct laser-tissue interactions to their potential in cutaneous interventions. in *Optical Interactions with Tissue and Cells XXIX* (Eds: E. D. Jansen, H. T. Beier), SPIE, San Francisco, CA **2018**, p. 44. https://doi.org/10.1117/12.2307804
[3] S. L. Jacques, *Surg Clin North Am* **1992**, *72*, 531.
[4] M. H. Niemz, *Laser-tissue interactions*, Springer, Berlin Heidelberg **2007**. https://doi.org/10.1007/978-3-540-72192-5
[5] C. A. Gueymard, D. Myers, K. Emery, *Solar Energy* **2002**, *73*, 443.
[6] J. Cadet, C. Anselmino, T. Douki, L. Voituriez, *J Photochem Photobiol B* **1992**, *15*, 277.
[7] M. A. Sheraz, S. H. Kazi, S. Ahmed, Z. Anwar, I. Ahmad, *Beilstein J Org Chem* **2014**, *10*, 1999.
[8] Z. C. F. Garza, M. Born, P. A. J. Hilbers, N. A. W. van Riel, J. Liebmann, *Curr Med Chem* **2019**, *25*, 5564.
[9] C. Opländer, A. Deck, C. M. Volkmar, et al., *Free Radical Biol Med* **2013**, *65*, 1363.
[10] A. W. Girotti, *J Lipid Res* **1998**, *39*, 1529.
[11] J. P. Bouly, E. Schleicher, M. Dionisio-Sese, et al., *J Biol Chem* **2007**, *282*, 9383.
[12] T. Karu, *J Photochem Photobiol B* **1999**, *49*, 1.
[13] M. R. Hamblin, *Photochem Photobiol* **2018**, *94*, 199.
[14] A. St-Jacques, J. Anichina, B. B. Schneider, T. R. Covey, D. K. Bohme, *Anal Chem* **2010**, *82*, 6163.
[15] L. O. Björn, in *Photobiology* (Ed: L. O. Björn), Springer, New York **2015**. DOI: 10.1007/978-1-4939-1468-5
[16] B. Lehmann, M. Meurer, *Dermatol Ther* **2010**, *23*, 2.
[17] K. Rajakumar, S. L. Greenspan, S. B. Thomas, M. F. Holick, *Am J Public Health* **2007**, *97*, 1746.
[18] N. Charoenngam, A. Shirvani, M. F. Holick, *J Clin Orthop Trauma* **2019**, *10*, 1082.
[19] G. Ahluwalia, in *In Personal Care & Cosmetic Technology, Cosmetics Applications of Laser and Light-Based Systems* (Ed: G. S. Ahluwalia), William Andrew Publishing, **2009**.
[20] F. Greiter, F. Gschnait, *Photochem Photobiol* **1984**, *39*, 869.
[21] J. D'Orazio, S. Jarrett, A. Amaro-Ortiz, T. Scott, *Int J Mol Sci* **2013**, *14*, 12222.
[22] R. M. Slominski, T. Sarna, P. M. Płonka, C. Raman, A. A. Brożyna, A. T. Slominski, *Front Oncol* **2022**, *12*, 842496. https://doi.org/10.3389/fonc.2022.842496
[23] A. Slominski, D. J. Tobin, S. Shibahara, J. Wortsman, *Physiol Rev* **2004**, *84*, 1155.
[24] J. B. Kerr, *Opt Eng* **2005**, *44*, 041002.
[25] ISO 20473:2007(en) Optics and photonics — Spectral bands. Published online **2007**.
[26] D. H. Sliney, *Eye* **2016**, *30*, 222.
[27] C. R. Taylor, R. S. Stern, J. J. Leyden, B. A. Gilchrest, *J Am Acad Dermatol* **1990**, *22*, 1.
[28] M. Yaar, B. A. Gilchrest, *Br J Dermatol.* **2007**, *157*, 874.
[29] T. B. El-Abaseri, S. Putta, L. A. Hansen, *Carcinogenesis* **2006**, *27*, 225.
[30] B. A. Gilchrest, *J Invest Dermatol.* **2013**, *133*, E2.
[31] B. A. Gilchrest, N. A. Soter, J. S. Stoff, M. C. Mihm, *J Am Acad Dermatol* **1981**, *5*, 411.
[32] B. A. Gilchrest, H. Y. Park, M. S. Eller, M. Yaar, *Photochem Photobiol* **1996**, *63*, 1.

[33] A. Slominski, J. Wortsman, D. J. Tobin, *FASEB J* **2005**, *19*, 176.
[34] G. S. Rogers, B. A. Gilchrest, *Br J Dermatol* **1990**, *122*, 55.
[35] M. B. Totonchy, M. W. Chiu, *Dermatol Clin* **2014**, *32*, 399-, ix-x.
[36] T. F. Anderson, T. P. Waldinger, J. J. Voorhees, *Arch Dermatol* **1984**, *120*, 1502.
[37] J. Jacob, A. Pona, A. Cline, S. Feldman, *Dermatol Clin* **2020**, *38*, 109.
[38] R. E. Bird, C. Riordan, *J Climate Appl Meteorol* **1986**, *25*, 87.
[39] C. Mignon, N. V. Botchkareva, N. E. Uzunbajakava, D. J. Tobin, *Exp Dermatol* **2016**, *25*, 745.
[40] C. Regazzetti, L. Sormani, D. Debayle, et al., *J. Invest. Dermatol.* **2018**, *138*, 171.
[41] L. E. Olinski, E. M. Lin, E. Oancea, *Adv Biol Regul* **2020**, *75*, 100668.
[42] S. Suh, E. H. Choi, M. N. Atanaskova, *Photodermatol Photoimmunol Photomed* **2020**, *36*, 329.
[43] H. Moreiras, C. O'Connor, M. Bell, D. J. Tobin, *Exp Dermatol* **2021**, *30*, 1324.
[44] S. Buscone, A. N. Mardaryev, G. E. Westgate, N. E. Uzunbajakava, N. V. Botchkareva, *Exp Dermatol* **2021**, *30*, 271.
[45] S. Buscone, A. N. Mardaryev, B. Raafs, et al., *Lasers Surg. Med.* **2017**, *49*, 705.
[46] I. Castellano-Pellicena, N. E. Uzunbajakava, C. Mignon, B. Raafs, V. A. Botchkarev, M. J. Thornton, *Lasers Surg Med* **2019**, *51*, 370.
[47] P. P. C. Toh, M. Bigliardi-Qi, A. M. Y. Yap, G. Sriram, P. Bigliardi, *Exp Dermatol* **2016**, *25*, 1002.
[48] M. Sadowska, J. Narbutt, A. Lesiak, *Life* **2021**, *11*, 670.
[49] A. M. Scott, P. Stehlik, J. Clark, et al., *Ann Fam Med* **2019**, *17*, 545.
[50] P. Woodgate, L. A. Jardine, *BMJ Clin Evid* **2015**, *2015*, 0319.
[51] G. Lodi, M. Sannino, G. Cannarozzo, et al., *Lasers Med Sci* **2021**, *36*, 1719.
[52] I. Albers, E. Zernickel, M. Stern, et al., *Free Radical Biol Med* **2019**, *145*, 78.
[53] M. Stern, M. Broja, R. Sansone, et al., *Eur J Prev Cardiol* **2018**, *25*, 1875.
[54] A. M. Reuss, D. Groos, R. Scholl, M. Schröter, C. Maihöfner, *Pain Rep* **2021**, *6*, e968.
[55] A. Spinella, M. de Pinto, C. Galluzzo, et al., *Rheumatol Ther* **2022**, *9*, 891.
[56] V. B. Khoo, S. Soon, C. J. Yap, S. P. Chng, T. Y. Tang, *Cureus* **2021**, *13*, e17703.
[57] M. Fraccalvieri, G. Amadeo, P. Bortolotti, et al., *Ital J Dermatol Venereol* **2022**, *157*, 187. https://doi.org/10.23736/S2784-8671.21.07067-5
[58] T. Omi, P. Bjerring, S. Sato, S. Kawana, R. W. Hankins, M. Honda, *J Cosmet Laser Ther* **2004**, *6*, 156.
[59] J. F. Tremblay, D. J. Sire, N. J. Lowe, R. L. Moy, *J Cosmet Laser Ther* **2006**, *8*, 31.
[60] M. Elman, M. Slatkine, Y. Harth, *J Cosmet Laser Ther* **2003**, *5*, 111.
[61] M. H. Gold, W. Sensing, J. A. Biron, *J Cosmet Laser Ther* **2011**, *13*, 308.
[62] H. Gold, A. Andriessen, J. Biron, H. Andriessen, M. H. Gold, *J Clin Aesth Dermatol* **2009**, *2*, 44.
[63] S. Y. Lee, C. E. You, M. Y. Park, *Lasers Surg Med* **2007**, *39*, 180.
[64] C. Ash, A. Harrison, S. Drew, R. Whittall, *J Cosmet Laser Ther* **2015**, *17*, 170.
[65] H. H. Kwon, J. B. Lee, J. Y. Yoon, et al., *Br J Dermatol* **2013**, *168*, 1088.
[66] A. C. Barolet, I. V. Litvinov, D. Barolet, *Nitric Oxide* **2021**, *117*, 16.
[67] M. M. Kleinpenning, M. E. Otero, P. E. J. van Erp, M. J. P. Gerritsen, P. C. M. van de Kerkhof, *J Eur Acad Dermatol Venereol* **2012**, *26*, 219.
[68] A. Weinstabl, S. Hoff-Lesch, H. F. Merk, V. von Felbert, *Dermatology* **2011**, *223*, 251.
[69] A. Lesiak, I. A. Bednarski, J. Narbutt, *Postepy Dermatol Alergol* **2021**, *38*, 446.
[70] S. Pfaff, J. Liebmann, M. Born, H. F. Merk, V. von Felbert, *Dermatology* **2015**, *231*, 24.
[71] K. Keemss, S. C. Pfaff, M. Born, J. Liebmann, H. F. Merk, V. von Felbert, *Dermatology* **2016**, *232*, 496.
[72] N. W. Bellono, L. G. Kammel, A. L. Zimmerman, E. Oancea, *Proc Natl Acad Sci U S A* **2013**, *110*, 2383.
[73] N. W. Bellono, J. A. Najera, E. Oancea, *J Gen Physiol* **2014**, *143*, 203.
[74] N. L. Wicks, J. W. Chan, J. A. Najera, J. M. Ciriello, E. Oancea, *Curr Biol* **2011**, *21*, 1906.
[75] Y. Lan, Y. Wang, H. Lu, *Br J Dermatol* **2020**, *182*, 1228.
[76] Y. Lan, W. Zeng, X. Dong, H. Lu, *Br J Dermatol.* **2021**, *185*, 391.
[77] N. M. Dalesio, S. F. Barreto Ortiz, J. L. Pluznick, D. E. Berkowitz, *Front Physiol* **2018**, *9*, 1673. https://doi.org/10.3389/fphys.2018.01673
[78] D. Malacara, *Color Vision and Colorimetry: Theory and Applications*, 2nd ed., SPIE, **2011**.
[79] M. Bonnans, L. Fouque, M. Pelletier, et al., *J Photochem Photobiol B* **2020**, *212*, 112026. https://doi.org/10.1016/j.jphotobiol.2020.112026
[80] C. Mignon, N. E. Uzunbajakava, I. Castellano-Pellicena, N. v. Botchkareva, D. J. Tobin, *Lasers Surg Med* **2018**, *50*, 859.
[81] C. Mignon, N. E. Uzunbajakava, B. Raafs, N. v. Botchkareva, D. J. Tobin, *Sci Rep* **2017**, *7*, 2797.
[82] C. Mignon, N. E. Uzunbajakava, *Mol Dermatol* **2020**, *2154*, 255. https://doi.org/10.1007/978-1-0716-0648-3_22
[83] C. Hirsch, S. Schildknecht, *Front Pharmacol* **2019**, *10*, 1484. https://doi.org/10.3389/fphar.2019.01484
[84] C. Mignon, D. J. Tobin, M. Zeitouny, N. E. Uzunbajakava, *Biomed Opt Express.* **2018**, *9*, 852.
[85] A. Slominski, J. Wortsman, *Endocr Rev* **2000**, *21*, 457.
[86] P. Ramaswamy, J. G. Powers, J. Bhawan, I. Polyak, B. A. Gilchrest, *Dermatol Surg* **2014**, *40*, 979.
[87] L. Duteil, N. Cardot-Leccia, C. Queille-Roussel, et al., *Pigment Cell Melanoma Res* **2014**, *27*, 822.
[88] Kleinpenning MM, Smits T, Frunt HA, et al. *Photodermatol Photoimmunol Photomed* **2010**, *26*, 16.
[89] B. H. Mahmoud, E. Ruvolo, C. L. Hexsel, et al., *J Invest Dermatol* **2010**, *130*, 2092.
[90] D. E. Brash, A. Ziegler, A. S. Jonason, J. A. Simon, S. Kunala, D. J. Leffell, *J Investig Dermatol Symp Proc* **1996**, *1*, 136.
[91] D. Decraene, P. Agostinis, A. Pupe, P. de Haes, M. Garmyn, *J Photochem Photobiol B* **2001**, *63*, 78.

[92] M. J. Zhou, L. Zheng, L. Guo, et al., *Biomed Environ Sci* **2012**, *25*, 583.
[93] D. Becker, E. Langer, M. Seemann, et al., *PLoS One* **2011**, *6*, e20566. https://doi.org/10.1371/journal.pone.0020566
[94] G. A. Frost, G. M. Halliday, D. L. Damian, *J Invest Dermatol* **2011**, *131*, 962.
[95] G. Evangelou, M. D. Farrar, L. Cotterell, et al., *Br J Dermatol* **2012**, *166*, 1112.
[96] Y. J. Matthews, D. L. Damian, *Br J Dermatol* **2010**, *162*, 637.
[97] G. Evangelou, M. D. Farrar, R. D. White, et al., *Br J Dermatol* **2011**, *165*, 513.
[98] R. Campiche, S. J. Curpen, V. Lutchmanen-Kolanthan, et al., *Int J Cosmet Sci* **2020**, *42*, 399.
[99] D. Falcone, N. E. Uzunbajakava, F. van Abeelen, et al., *Photodermatol Photoimmunol Photomed* **2018**, *34*, 184.
[100] H. L. Jo, Y. Jung, B. F. Suh, E. Cho, K. Kim, E. Kim, *J Cosmet Dermatol* **2020**, *19*, 2438.
[101] C. Maari, G. Viau, R. Bissonnette, *J Am Acad Dermatol* **2003**, *49*, 55.
[102] R. Schütz, *Curr Probl Dermatol* **2021**, *55*, 354.
[103] J. G. Coats, B. Maktabi, M. S. Abou-Dahech, G. Baki, *J Cosmet Dermatol* **2021**, *20*, 714.
[104] T. Christensen, B. J. Johnsen, E. M. Bruzell, *Photochem Photobiol Sci* **2021**, *20*, 615.
[105] K. Dong, E. C. Goyarts, E. Pelle, J. Trivero, N. Pernodet, *Int J Cosmet Sci* **2019**, *41*, 558.
[106] S. G. Klein, S. M. Alsolami, S. Arossa, et al., *Commun Biol* **2022**, *5*, 119.
[107] H. L. Jo, Y. Jung, Y. K. Kim, et al., *J Cosmet Dermatol* **2022**, *21*, 1270.
[108] I. Castellano-Pellicena, C. G. Morrison, M. Bell, C. O'Connor, D. J. Tobin, *Int J Mol Sci* **2021**, *22*, 3143.
[109] L. Duteil, C. Queille-Roussel, J. P. Lacour, H. Montaudié, T. Passeron, *J Am Acad Dermatol* **2019**, *83*, 913.
[110] A. K. Haylett, Z. Nie, M. Brownrigg, R. Taylor, L. E. Rhodes, *Br J Dermatol* **2011**, *164*, 407.
[111] F. Boukari, E. Jourdan, E. Fontas, et al., *J Am Acad Dermatol* **2015**, *72*, 189.

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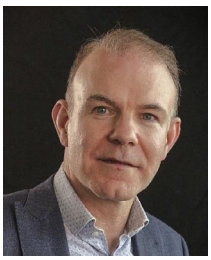

Dr Desmond J. Tobin is Full Professor of Dermatological Science and Director of The Charles Institute of Dermatology at the UCD School of Medicine, Dublin. Prior to September 2018 he was Professor of Cell Biology and Director of the Centre for Skin Sciences at Bradford University (UK). He holds a BSc from the National University of Ireland (Maynooth), a PhD from the University of London (St. John's Institute of Dermatology) and post-doctorate from New York University Medical School's Department of Dermatology. Over the past ~25years he has researched in basic and applied skin/hair sciences, with a focus on the biology of human melanocytes/pigmentation in health and disease (including melanoma) and of hair growth disorders, especially those with an immune basis. Des is Fellow of the Royal College of Pathologists, Royal Society of Biology, Institute of Trichologists, Royal Microscopical Society, Institute of Biomedical Science, and Member of the Royal Irish Academy. He is immediate past president of the British Society for Investigative Dermatology. Des serves(ed) on several editorial boards, scientific advisory panels, and is a subpanel member of UK-based Research Excellent Framework (REF2021). He has published over 200 publications, incl. 3 books. His H-Index is 65 (Scopus @Summer 2022).

Dr Natalia Botchkareva, MD, has extensive experience in both Industrial and Academic research with interests in the area of molecular mechanisms of hair growth and light-based technologies for skin and hair benefits. She obtained a PhD degree from Humboldt University of Berlin (Germany)/Peoples' Friendship University of Russia under supervision of Dr Ralf Paus, followed by post-doctoral training in the Boston University Department of Dermatology (Massachusetts, USA). She continued her scientific career as a Scientist at Gillette/P&G exploring signalling molecules applicable for the development of novel products for correction of skin and hair growth abnormalities (Needham, Massachusetts). In 2007–2018, she has led her research lab at the University of Bradford, UK, delineating the role of noncoding RNAs involved in skin and hair regeneration, as well as the effects of light on human hair growth jointly with Dr Natallia Uzunbajakava (PHILIPS NV). Later, she served as a Head of Innovation Research and Principal Consultant at the Monasterium Laboratory GmbH, Munster, Germany. To date, Dr Botchkareva has 61 original publications and reviews on different aspects of skin and hair growth in the leading interdisciplinary and specialized journals, her H-index is 35 (Scopus @Summer 2022), patent/patent applications, several book chapters, and edited a book "Molecular Dermatology. Methods in Molecular Biology" (Nature/Springer 2020). The quality and novelty of Dr Botchkareva's research were also acknowledged by numerous oral presentations at major national and international meetings, as well as by the Juergen Schweizer Award at the European Hair Research Society meeting (2015).

Dr Christine C. Dierickx, MD, finished her dermatology residency in Belgium. Subsequently, she completed a fellowship in phlebology in the Netherlands with Professor H. Neuman, a clinical laser fellowship with Dr R. Fitzpatrick in San Diego, California and a Mohs fellowship with Dr F. Mohs in Madison, Wi. During a 2-year laser fellowship with Dr R. Anderson at Massachusetts General Hospital of Harvard University, she developed research laser expertise in various fields. For her work on the thermal relaxation time of ectatic blood vessels, she received the Best Basic Science Award of the American Society for Laser Medicine and Surgery. Subsequently, she became a full-time staff member of the Wellman Laboratories at Harvard. During this time, she conducted the research projects with long pulsed ruby and diode lasers for hair removal that led to approval for permanent hair reduction. She returned to Europe in 2000 where she is in private practice in Luxembourg. In 2006, Dr Dierickx was vice-president of the "American Society of Laser Medicine and Surgery, Inc" and afterwards a board member of the International Committee. In 2013, she was the recipient of the Vasant Orwal Oration van de British Medical Laser Society. In April 2016, she was granted the Leon Goldman memoriam award. This award recognizes a practicing physician whose career demonstrates excellence in clinical research, patient care, or education, related to medical lasers. Dr Dierickx remains actively involved in research on various energy-based devices and its applications. She has published and lectured nationally and internationally; her H-index is 25 (Scopus @Summer 2022).

Professor Peter Bjerring, MD, is a Board Certified Dermatologist. Born March 19, 1953 in Aarhus, Denmark, he graduated from the University of Aarhus, Denmark. After serving 24 years at the Department of Dermatology, Aarhus University Hospital, he was appointed Medical Director and Head of the Department of Dermatology at the Molholm Hospital, Vejle, Denmark and Honorary Professor at Swansea University, Wales, UK. He has published more than 200 scientific papers, his H-index is 46 (Scopus @Summer 2022) with the main interest being on laser-treatment and IPL-treatment, and he has contributed with more than 400 lectures at international congresses and scientific meetings. Professor Bjerring is Past President of the European Society for Lasers in Dermatology (ESLD), Past Vice President of the European Society for Laser Aesthetic Surgery (ESLAS) and Fellow of EADV, AAD and ASLMS.

Dr Godfrey Town, PhD, Registered Clinical Technologist, is RPA2000 certified in Non-Ionising Radiation Protection, a UK Registered Clinical Technologist, holds a Cardiff University Law School Expert Witness Certificate, is a Fellow of the American Society for Lasers in Medicine and Surgery (ASLMS), a member of the European Society for Lasers and Energy Based

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