Transformation of visible light: a new perspective for skin care applications.

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Abstract

Background: A mixture of fluorochromes (*MixFl*) was designed to convert harmful wavelengths of visible light (blue light) into higher, beneficial wavelengths to offer well-being benefits to the skin.

Methods: The absorption and the emission spectrum of *MixFl* formulated in an acrylate-based gel were recorded *in vitro* and demonstrated on blue light-exposed volunteers who applied the *MixFl* gel. The skin benefits of *MixFl* were evaluated *ex vivo* by DNA oxidation quantification and measurement of photoreceptor expression on human skin explants exposed daily to blue light. Finally, a 28-day clinical study was conducted on multi-ethnic volunteers spending more than 6 hours per day in front of a digital screen, with topical application of the gel-cream containing 1% *MixFl* vs. placebo.

Results: Through *in vitro* and clinical studies, it was confirmed that this unique *MixFl* gel can capture blue light from screens and convert it into a whiter light. *Ex-vivo* skin studies demonstrated that the conversion of blue light by *MixFl* reduced DNA oxidation, and that re-emitted light modulated the expression of photoreceptors Opsin 1SW and 2, according to their specific wavelength sensitivities. Finally, these data were confirmed clinically on volunteers who applied a cream-gel containing 1% *MixFl* with heavy use of digital screens, by showing an improvement in their skin's appearance.

Conclusion: *MixFl* is an innovative ingredient able to protect the skin from blue light emitted by digital screens, and improve the skin's appearance by transforming the captured energy into higher-wavelengths, more beneficial light.

Introduction

Sunlight is an energy source that enables various chemical reactions in the living world, and its absorption at varying wavelengths [1] produces a wide range of molecules essential for biological function in plants and animals. Many living beings, from bacteria to insects, sharks, and birds, are able to light up and glow in the dark. These biofluorescent animals absorb short-wavelength electromagnetic radiation and re-emit it at longer wavelengths, resulting in a bright fluorescent glow. This phenomenon, promoting communication, is based on several types of proteins, pigments, metabolites and chemical reactions [2].

Humans do not photosynthesize, however human skin is the organ that interacts most with ambient light, some of which is able to penetrate some or all layers of the skin depending on its wavelength. Visible light is the medium used to improve the appearance of the skin through rejuvenation, anti-aging, anti-oily or acne-prone skin, as well as through effects on pigmentation or skin lightening, dandruff and other skin disorders related to *Malassezia*, and more [3]. The use of light as therapy is based on photobiomodulation which consists to modulate the chromophores exhibited in the skin and to activate their biological activities associated [4]. More recently, the retinal photoreceptors, called opsin, have emerged as new photosensor in the skin. Opsins are a large group of light-sensitive G protein–coupled receptors (GPCRs) whose activation triggers excitation wavelength-dependent signaling cascades [5]. Thus, the expression of these skin photoreceptors reflects the wavelength to which the skin is exposed [6].

Electromagnetic radiation from the sun is the main source of visible light (VL) exposure on our skin, but not the only one. Light-emitting diodes, flash lamps, computers, televisions, and cell phones are also sources of VL. Unlike the sun, which emits a balanced visible light spectrum called white light, digital screens from computers and smartphones emit an unbalanced light with an intense peak in the blue light range. While white light has beneficial effects on the skin and general well-being, artificial light is associated with biological skin perturbation [7]. In spite of this, adults spend 11 hours per day in front of a screen. In addition, many uses other devices simultaneously (second screening) especially since the COVID pandemic led to the universalization of remote work [8].

Unbalanced visible light can be modulated by means of fluorescence mechanisms, by absorbing harmful light and reemitting the energy at more beneficial wavelengths. Thus, light emitted by screens can be used as an energy source and converted into white light. Based on this concept and using a biomimetic approach, an innovative active ingredient was designed for skin care applications. A mixture of fluorochromes (*MixFl*) was created to convert harmful wavelengths of visible light (blue light) into a balanced mix of higher, beneficial wavelengths (collectively, ‘white light’), to simultaneously protect the skin from harmful wavelengths and provide skin wellness benefits.

The aim of the present work is to demonstrate how the fluorescent properties and light transformation of formulated *MixFl* can protect the skin and improve skin appearance when applied directly on the skin, under blue light exposure.

Materials and Methods.

Mix Fl ingredient: The active ingredient is composed of three fluorescent xanthenic dyes, solubilized in glycerin, and blended in a specific ratio designed to modulate and transform visible light. This blend is referred to here as *MixFl*.

The ingredient was solubilized at 1% into a transparent gel for studies related to fluorescence and characterization of its light-transforming properties, and at the same level into a gel-cream for clinical studies.

	Optical & in-vitro studies: GEL		Clinical studies: GEL-CREAM	
	MixFl	Placebo	*MixFl*	Placebo
Deionized water	97.6	98.6	83.4	84.4
Acrylates/C10-30 Alkyl Acrylate Crosspolymer	0.4	0.4	0.3	0.3
Xanthan gum	-	-	0.3	0.3
***MixFl* in glycerin**	**1**	**-**	**1**	**-**
Medium chain triglycerides	-	-	14	14
Preservative system	1	1	1	1
pH adjustment (NaOH)	5.5	5.5	5.5	5.5

Table 1: Tested formulas

Absorption and emission spectrum of MixFl: The absorption spectrum of the ingredient formulated into the transparent gel base was recorded by means of a UV-Vis spectrophotometer Cary 5 E. The emission spectrum of the gel (with excitation at 455 nm) was recorded with a Spectrofluorometer (Fluorolog FL-3).

Optical features of the MixFl illuminated with digital screens: To assess both the screen emission profile and the *MixFl* efficacy into the gel, illuminance (lux) measurements were completed according to the set-up shown in **Figure 1**. Formulated gel (with or without *MixFl*) was spread at 2 mg/cm^2 on a transparent PVC foil placed at 30 cm from a computer screen. An illuminance meter CL70 F Konica Minolta was located immediately behind the PVC foil and measured the illuminance of the digital screen light as transmitted by the gel. This design mimics the average distance between the screen and the skin of the face when a person works in front of a computer.

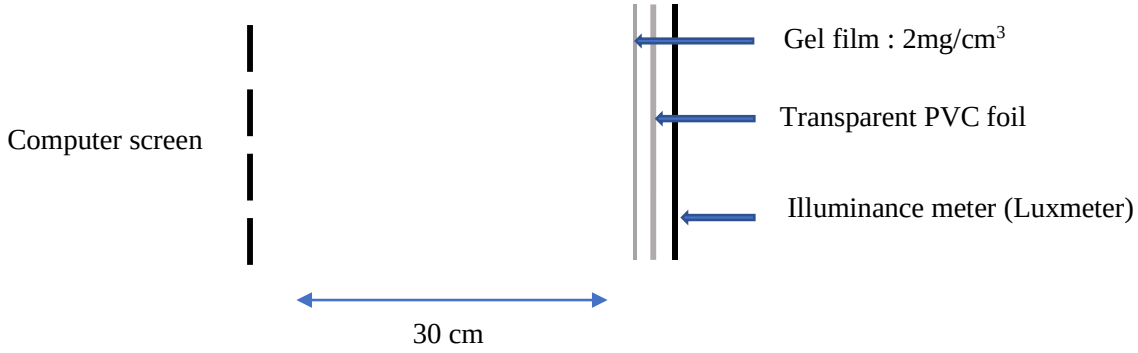

Figure 1: Experimental design for *MixFl* light transforming effect assessment

Screen emissions through gels formulated with and without *MixFl* were recorded from 380 nm to 780 nm, first in the dark, next in the vicinity of a window (natural light conditions), and finally under artificial light (LED panels). The illuminance meter records the illuminance (expressed in lux), representing the luminous flux (in lumen) received per surface unit (in m²). This measurement represents the amount of incident light illuminating a surface.

Depending on the wavelength, a conversion between lux and W/m² can be obtained based on the spectral distribution: *Illuminace = Radiant flux x S*,where S is a factor of correction obtained with the spectral distribution (ISO 18526-2). The Irradiance (expressed in W. m². nm^{-1}), represents the radiant flux of light received by a surface. It is the power per unit area received on a defined surface at a given moment.

For example, the S factor at 455nm is equal to 0.2667. Therefore, a radiant flux of 1 W/m² at this wavelength corresponds to 0.2667 lux.

Evaluation of MixFl on human skin explants: A total of 30 human skin biopsies were obtained from a plastic surgery patient (Caucasian woman, 32 years old). Each explant was kept in survival medium for 8 days and irradiated every day for 3 hours with blue light from a solarbox® device (59 W/m² at 455 nm) corresponding to a radiation dose of 63.75 J/cm². The gel containing 1% of *MixFl* and the corresponding vehicle control without *MixFl* (**Table 1**) were applied topically before irradiation, at day 0 (D0), D1, D4 and D6. An untreated, unexposed group and an untreated, exposed group served as controls. At D8, explants were fixed and embedded in paraffin for Masson's Trichrome staining and morphological studies, or directly frozen for 8-0HdG and opsins immunostaining.

8-hydroxydesoxyguanosine (8-OHdG) is one of the predominant forms of free radical-induced oxidative lesions in humans. This stable oxidative modified DNA product reflects the degree of oxidative damage to DNA [9]. 8-OHdG was labelled using a monoclonal anti-8-OHdG antibody (Gentaur, clone N45-1) coupled with biotin and revealed by substrate of peroxidase (Vector laboratories).

The Opsin 1SW and Opsin 2 receptors are expressed on the surface of the skin, and their expressions are sensitive to light excitation [6]. Opsin 1SW and Opsin 2 expressions were respectively labeled with polyclonal anti-Opsin 1SW (Merck, AB5407) and monoclonal anti-opsin 2 (Biorbyt, clone Rho1D4), both revealed by A488 secondary antibody, then the quantification was performed by image analysis using ImageJ® software.

Clinical visualization of light conversion: Healthy volunteers applied a gel-cream containing 1% *MixFI* versus placebo (**Table 1**) following a randomized hemiface design. By means of a full-face hyperspectral analysis by using spectracam®, the emissions at 560 nm were recorded after blue light excitation at 405 nm, immediately and 10 min after gel application. A negative control was performed without blue light exposure.

Clinical study on volunteers using digital screens: a multi-ethnic panel of 37 healthy female and male volunteers, (30 to 50 years old), spending at least 6 hours a day working on computers without blue light filters and who do not wear glasses was recruited. These volunteers, divided in 2 groups, applied the gel-cream containing 1% of *MixFl* or the placebo **(Table 1)** twice a day, morning and evening. At D28, the color of the volunteers' undereye dark circles was evaluated by measuring the ITA° parameter by using Visia-CR, and the skin's anti-oxidative potential was analyzed using a Corneofix®.

Statistical test: For the study of the light conversion on volunteers, a two-way Anova test was used. For *ex-vivo* and clinical studies, the Mann-Whitney T-test was used. The statistical significance thresholds were set at #p<0.1, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Results.

Absorption and emission spectrum of MixFl

Formulated into a gel, *MixFl* exhibited fluorescence properties, absorbing light between 400 and 550 nm and reemitting at higher wavelengths. Indeed, exposed to a wavelength of 455 nm, *MixFl* converted the monochromatic light into a range of 500-600 nm, yielding an overall orange light emission. **Figure 2** shows the emission spectra collected with the experimental design described above.

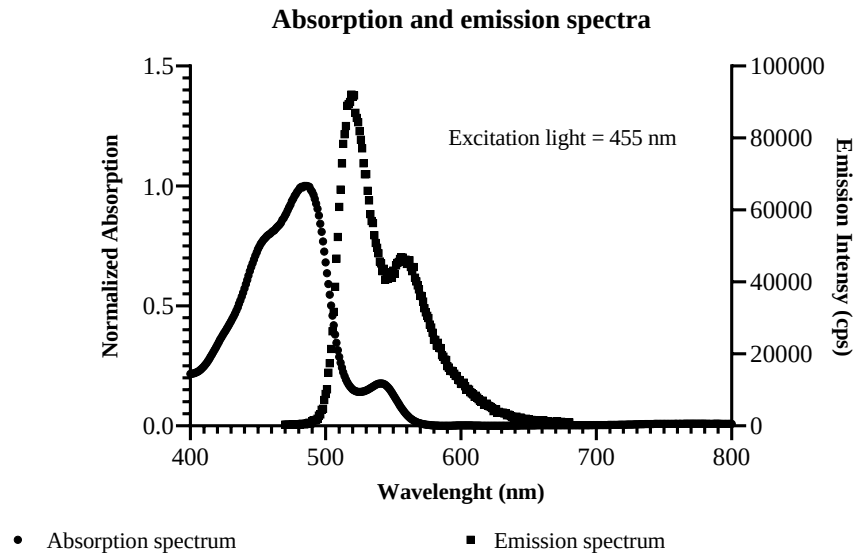

Figure 2: Coupled absorption and emission spectra of the gel containing *MixFl*. The emission spectrum was obtained by illuminating the gel with a monochromatic light at 455 nm.

Light transforming ability of MixFl

The computer screen emitted an irregular spectrum displaying 3 distinct peaks. As shown in **Figure 3**, the main peak is situated at 450-455 nm, in mid-blue light range and corresponds to the absorption range of *MixFl*. Two other peaks, with lower intensity, are positioned at 540 and 590nm.

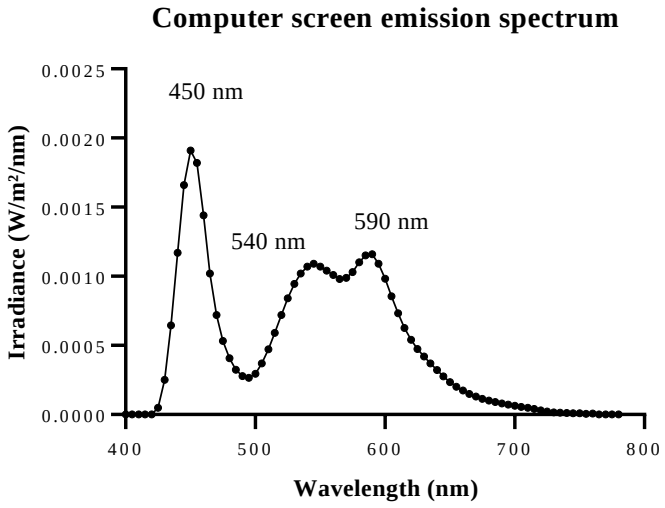

Figure 3: computer screen emission spectrum **(**30 cm).

Illuminated by the computer screen at 30 cm, *MixFl* formulated at 1% into the gel was able to decrease the irradiance at 450 nm up to -16% regardless of the light environment. At the

same time, *MixFl* provided an increase of irradiance at 600 nm particularly under natural light and dark conditions (up to +7 %) (**Figure 4**). The impact of *MixFl* on the intermediate wavelength 540 nm is quite negligible.

These data demonstrate that *MixFl* is able to balance the light emitted by a screen, protecting skin from blue light radiation while increasing lower energy radiation. In this way, the visible light received by the skin is modulated toward a white light spectrum.

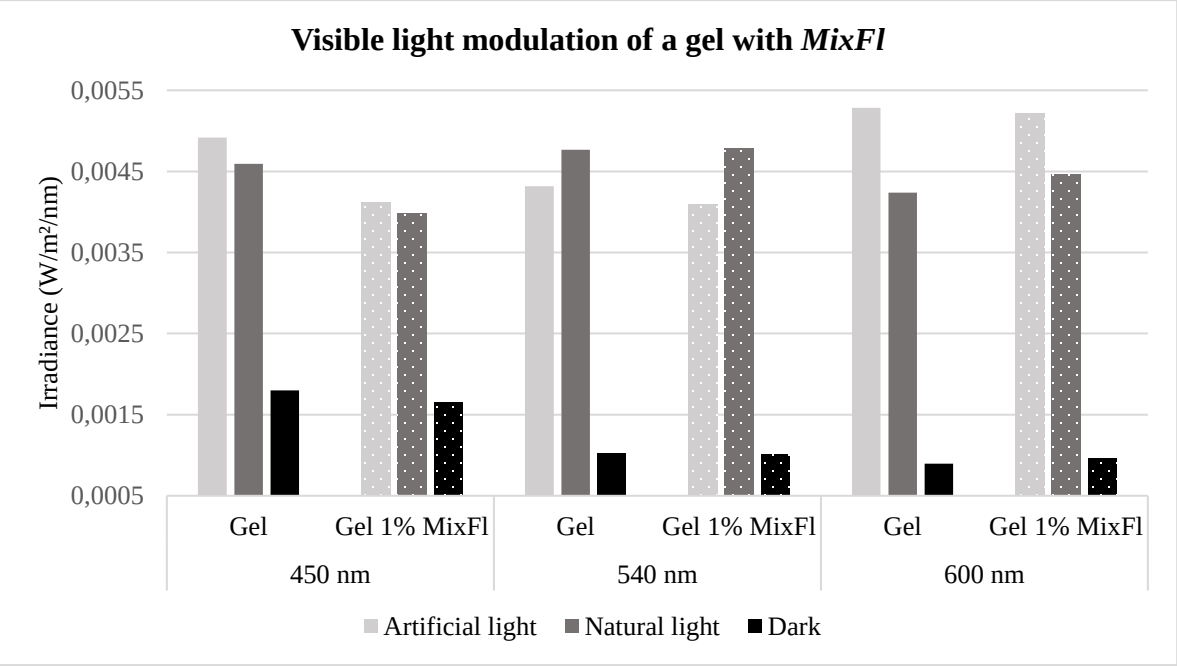

Figure 4: Visible light modulation of *MixFl* gel when illuminated by a computer screen at 30cm

Visualization of light conversion on volunteers

The ability of *MixFl* to transform blue light into higher wavelengths was evaluated in a clinical study in which volunteers applied a gel containing 1% *MixFl* vs. placebo (**Table 1**), on a split-face design. Without blue light exposure, we observed no emission at 560 nm whereas under excitation with blue light (405 nm), the hemiface that received the gel containing 1% of *MixFl* emitted light at 560 nm (**Figure 5A**). Indeed, by grayscale evaluation, we observed that compared to placebo, the emission at 560 nm under exposure to blue light increased by 73% and 96.5% on the forehead and cheek immediately after application of *MixFl* gel. This transformation was maintained over time, as we observed an increased by 77% and 94% on the forehead and cheek respectively compared to placebo side, 10 min after application (**figure 5B**).

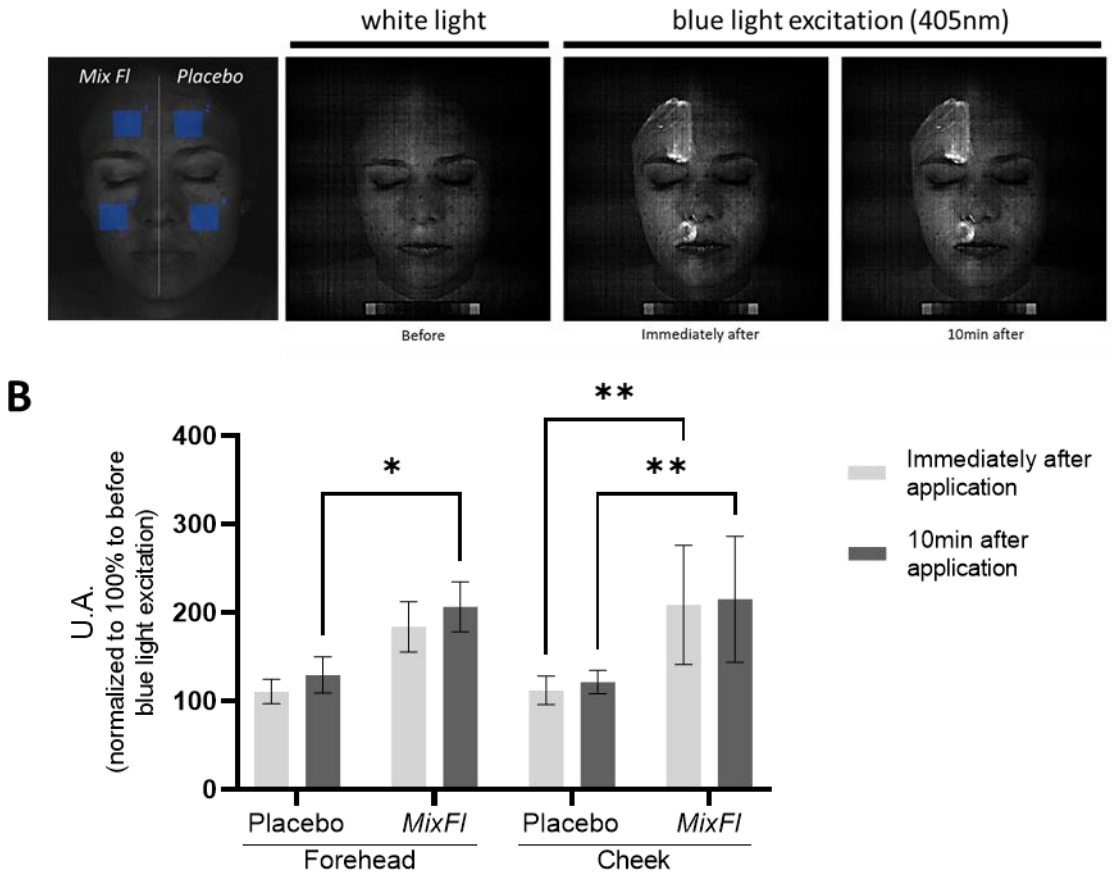

Figure 5 – Blue light transformation on volunteers' face: Volunteers applied on hemiface a gel containing 1% *MixFl* vs. placebo (**Table 1**). (A) Greyscale luminosity was evaluated on several areas of the face before and after exposure to blue light (405 nm) (blue square). Pictures illustrate the emission of light at 560 nm. (B) Average percentages ± standard deviation of greyscale luminosity at 560 nm on defined aera immediately or 10 min after application of *MixFl* gel (dark bar) or placebo (light bar) under exposure to blue light. The result was normalized to 100% of aera exposed to white light. Statistical significance was assessed using a two-way Annova test (*p<0.05; **p<0.01).

MixFl's effects on human explants exposed to blue light

Exposure of human skin to blue light is known to induce serious skin damage leading to premature aging, wrinkles, etc [7]. Thus, the beneficial ability of *MixFl* to transform blue light offers an opportunity to protect the skin from blue light exposition. To confirm this, we evaluated DNA oxidation on human explants exposed daily to blue light and treated with a gel containing 1% of *MixFl* vs. placebo (**Table 1**). As expected, compare to untreated unexposed condition, the percentage of 8-OHdG staining, a marker of DNA oxidation, increased by + 113% and +118% for untreated and placebo conditions exposed under blue, respectively (**Figure 6)**. Inversely, *MixFl* application reduced staining for 8-OHdG, by 89% compared to placebo.

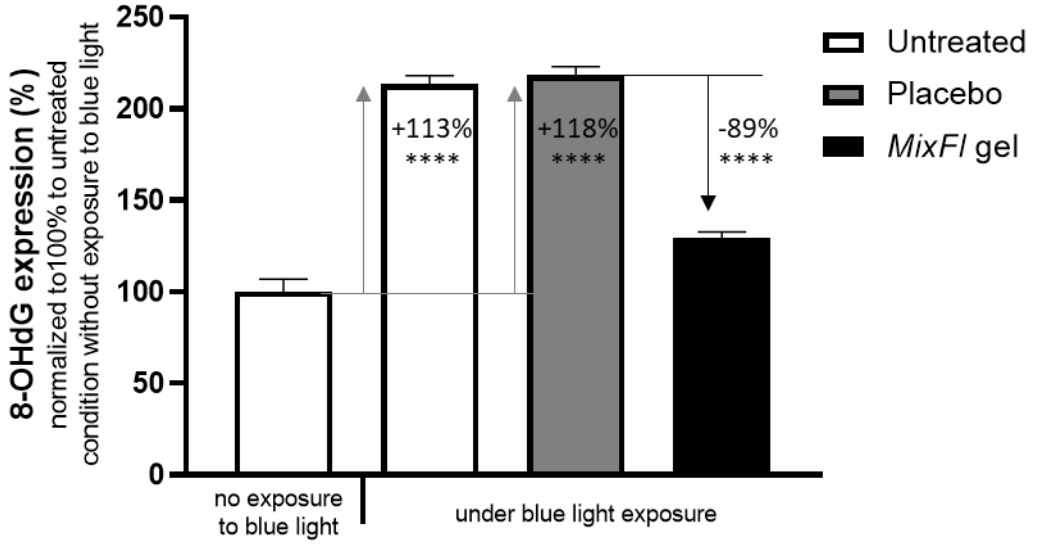

Figure 6 – DNA oxidation on human explants exposed to blue light: Average percentages ± standard deviation of positive surface for 8-0HdG staining in untreated, exposed condition (white bar), treated with placebo and exposed (grey bar), and treated with *MixFl* gel and exposed (black bar) conditions under blue light exposure. The result was normalized to to the untreated, unexposed reference condition. Statistical significance was assessed using the Mann-Whitney test (****$p<0.0001$).

The protective effect observed rests on the conversion of blue light into higher wavelengths, as demonstrated above. To observe the effects of this re-emitted light, we studied the expression of photoreceptors on the human skin surface. These cutaneous photoreceptors, are sensitive to the light absorbed by the skin and each opsin has a distinct absorption spectrum and signal transduction, and their expressions depend on the skin's exposure to light. Thus, the epidermal expression of opsin 1SW and opsin 2 was found to increase after exposure to wavelengths between 355 and 470 nm, and at 500nm [6]. To examine photoreceptor levels in our model, human explants were exposed daily to blue light, and treated with gel containing 1% of *MixFl* or placebo gel (**Table 1**). As expected, compared to the untreated, unexposed control, Opsin 1SW levels increased and Opsin 2 levels decreased in both the untreated and the placebo exposed groups. However, with *MixFl* application, Opsin 1SW expression decreased by 22% and Opsin 2 increased by 12% vs. placebo (**Figure 7**).

These results highlight the ability of *MixFl* to modify the spectrum of incoming light, protecting the skin from the effects of blue light and allowing the activation of photosensitive molecules through to the re-emission of light at higher wavelengths.

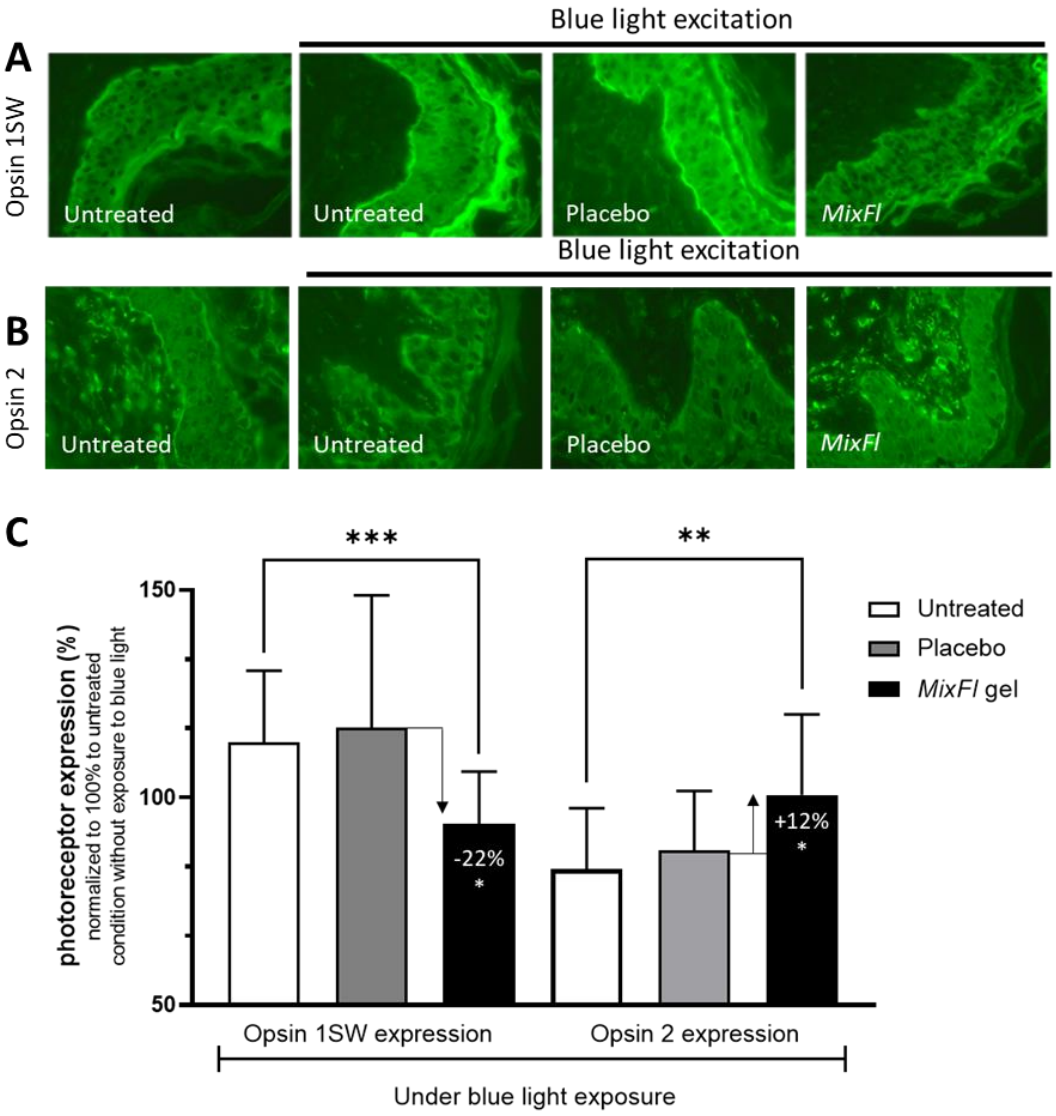

Figure 7 – Opsin expression on human skin explants: Heathy human explants were exposed daily to blue light and treated with 1% of *MixFl* gel (black bar) versus placebo (grey bar), or left untreated (white bar). After 8 days, Opsin 1SW (A) and Opsin 2 (B) were evaluated by immunostaining. (C) Average of % surface stained for Opsin 1SW and Opsin 2. The values are normalized to 100% of the untreated, unexposed condition. Significance levels are set to *p≤ 0.05, **p≤0.01, ***p≤0.001 using the Mann-Whitney test.

MixFl gel cream improves skin appearance

The beneficial effects of re-emitting the energy of absorbed blue light at higher wavelengths were investigated in a clinical study on 37 multi-ethnic volunteers who spent at least 6 hours/day in front of screens emitting blue light. Half of the volunteers applied a gel-cream containing 1% of *MixFl*, while the other half applied a placebo gel-cream (**Table 1**). After 28 days of product application, the evolution of undereye dark circle color and the skin's anti-oxidative potential were determined. As shown in **Figure 8**, compared to placebo, with

MixFl the color of dark circles lightened by 12.5% and the anti-oxidative potential increased by 14% at 28 days.
These clinical results highlight the beneficial effects of applying *MixFl* for skin protection. The transformation of blue light to higher wavelengths activates photon-sensitive signaling pathways involved protecting skin integrity.

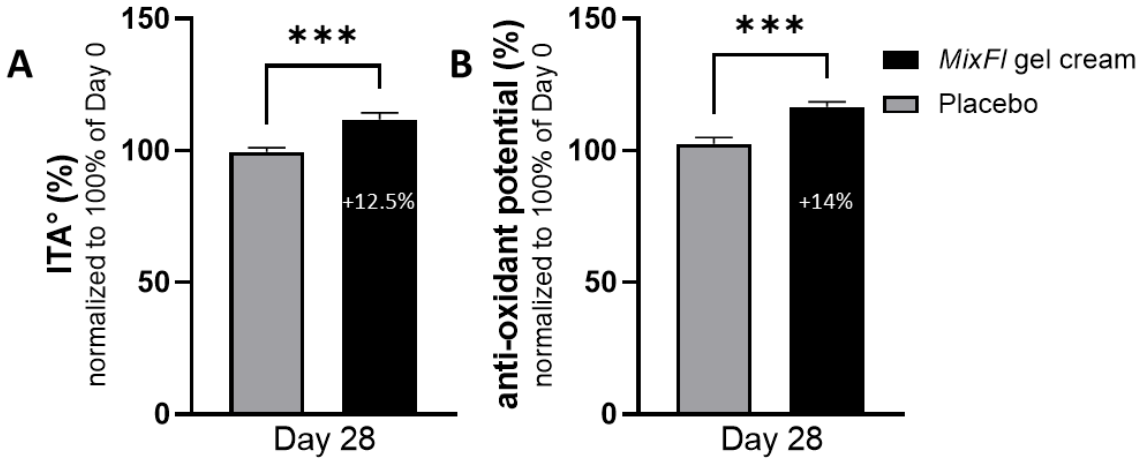

Figure 8 – Evaluation of *MixFl* gel-cream application on volunteers: healthy volunteers spending at least 6 hours daily in front of screens emitting blue light applied, morning and evening, a cream containing 1% of *MixFl* or a vs. placebo. (A) Evaluation of the color of undereye dark circles, as ITA°, at day 28 of application of a placebo gel-cream (gray bar) or a gel-cream containing 1% of *MixFl* (black bar). (B) Anti-oxidant potential after 28 days of application of a gel cream containing *MixFl* (black bar) versus placebo (gray bar). (A-B) The values were normalized to 100% of the value at Day 0. Significance thresholds ***$p \leq 0.001$using the Mann-Whitney test.

Discussion. The sun is the main source of natural light used by living organisms to generate energy, activate photon-sensitive molecules, or re-emit light. Sunlight is synonymous with wakefulness, well-being, and security. As a matter of fact, the visible white light from sun is reproduced toward luminotherapy to influence human activities and wellbeing [10]. However, due to the digital screen revolution, the adult human spends more than 11 hours in front of a digital screen. Thus, humans are mainly exposed to light sources whose emission spectrum is unbalanced in favor blue-range wavelengths. Unfortunately, this blue light overexposure is considered harmful to human health and is associated with an increase in ROS production that can lead to erythema and/or melanogenesis [7]. Moreover, blue light exposure is involved in premature skin aging, the appearance of wrinkles, etc. Current strategies counteract the blue light effect by filtration properties, for instance with organic filters or pigments forms, or skin defense enhancement with ingredients acting on skin cells. Here, the double objective was to develop an active ingredient capable of absorbing the energy emitted by digital screens and use it for producing whiter light closer to natural sunlight.

Through *in-vitro, ex-vivo*, and *in-vivo* (clinical) experimental models, we have demonstrated that *MixFl* is able to rebalance the light emitted by a screen towards a higher wavelength (**Figure 4 & 5**). This light transformation protects the skin against the harmful effects of blue light (**Figure 6**) and modulates the expression of photosensitive molecules in accordance with the emitted wavelengths **(Figure 7)**. In addition, we observed that the emission of higher-wavelength light improves skin condition, as signs of skin fatigue are reduced and the anti-oxidative potential of the skin is increased **(Figure 8)**. Finally, *MixFl* application leverages the digital screen's light into an energy source to produce a more balanced light and to mimic the beneficial effects of sunlight.

Conclusion. *MixFl* is an innovative active ingredient that protects the skin against the harmful effects of blue light exposure and improves skin condition. Indeed, *MixFl* uses the energy of blue light from digital screens to emit light at higher wavelengths, and shift the overall balance toward a more natural white light spectrum.

Conflict of Interest Statement. NONE.

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