Fluorescent light energy: the future for treating inflammatory skin conditions?

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Abstract:	Background We have previously reported clinical efficacy with a novel form of photobiomodulation – a biophotonic platform inducing fluorescent light energy (FLE) in both disease-affected and healthy skin, however the cellular mechanisms are largely unknown. Objective The aim of this study was to investigate the cellular mechanism of action of FLE on key skin and immune cells, which may underlie our clinical efficacy observed. Methods We examined the effect of FLE on the clinical presentation of inflammation in a representative acne vulgaris patient. The effect of FLE and a FLE-mimicking control lamp on collagen production from primary human dermal fibroblast (HDF) cells was assessed in the presence and absence of the pro-inflammatory cytokine, interferon gamma (IFN-γ). Cytokine production was assessed from HDF and human epidermal keratinocytes (HEK) exposed to M1 macrophage-conditioned media following illumination with either a blue LED or FLE. Finally, the effect of FLE on angiogenesis was assessed in human aortic endothelial (HAE) cells. Results FLE reduced inflammatory lesions and associated redness in the representative acne patient. Once the inflammation was resolved, there was a visible overall enhancement of the skin's texture. FLE enhanced collagen production from non-stressed HDF cells, decreased the inflammatory profile of HDF and HEK cells and enhanced angiogenesis in HAE cells. Conclusion FLE is a unique platform serving both aesthetic and therapeutic purposes by enhancing collagen production, modulating cutaneous inflammation and encouraging angiogenesis. Whilst further research is required, our findings have important implications for approaches to treating inflammatory skin conditions and achieving better aesthetic outcomes.
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Cover letter

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Ms. Elizabeth Klumpp,
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Dear Ms., Klumpp,

Thank you for the opportunity to submit our revised original research article JCAD-D-18-00144 entitled 'Fluorescent light energy: the future for treating inflammatory skin conditions?' by Deirdre Edge, PhD; Maiken Mellergaard, PhD; Carsten Dam-Hansen, PhD; Denis Dan Corel, MSc; Joanna Jaworska, PhD; Giovanni Scapagnini, MD, PhD; and Michael Canova Engelbrecht Nielsen, PhD, for consideration to the Journal of Clinical and Aesthetic Dermatology.

The paper has been revised to ensure it is clear to the reader that we include a single clinical case to represent an inflammatory skin disorder, *acne vulgarsis*, which has previously been investigated in our multicenter, randomized, split-face clinical trial evaluating the efficacy and safely of FLE. Additionally, we have included throughout the manuscript that further research work is required, please see line: 68, 315-316, 338, 357, and the opening line of the conclusion 357.

We would like our article published in the digital only publication of the May issue.

Thank you for your consideration.

Sincerely,

Michael Canova Engelbrecht Nielsen

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Abstract

Background We have previously reported clinical efficacy with a novel form of photobiomodulation – a biophotonic platform inducing fluorescent light energy (FLE) in both disease-affected and healthy skin, however the cellular mechanisms are largely unknown.

Objective The aim of this study was to investigate the cellular mechanism of action of FLE on key skin and immune cells, which may underlie our clinical efficacy observed.

Methods We examined the effect of FLE on the clinical presentation of inflammation in a representative acne vulgaris patient. The effect of FLE and a FLE-mimicking control lamp on collagen production from primary human dermal fibroblast (HDF) cells was assessed in the presence and absence of the pro-inflammatory cytokine, interferon gamma (IFN-γ). Cytokine production was assessed from HDF and human epidermal keratinocytes (HEK) exposed to M1 macrophage-conditioned media following illumination with either a blue LED or FLE. Finally, the effect of FLE on angiogenesis was assessed in human aortic endothelial (HAE) cells.

Results FLE reduced inflammatory lesions and associated redness in the representative acne patient. Once the inflammation was resolved, there was a visible overall enhancement of the skin's texture. FLE enhanced collagen production from non-stressed HDF cells, decreased the inflammatory profile of HDF and HEK cells and enhanced angiogenesis in HAE cells.

Conclusion FLE is a unique platform serving both aesthetic and therapeutic purposes by enhancing collagen production, modulating cutaneous inflammation and encouraging angiogenesis. Whilst further research is required, our findings have important implications for approaches to treating inflammatory skin conditions and achieving better aesthetic outcomes.

Introduction

The physiological and therapeutic effects of photobiomodulation (PBM) have recently been explored in several tissues and cell types using various low-level energy light sources, including low-level laser, light-emitting diodes (LED) and broadband visible light lamps.[1] The application of LED therapy inducing PBM in dermatology has greatly expanded in recent years, with promising results reported for slowing aging, improving inflammatory skin conditions and healing.[2]

While the complete cellular and molecular mechanisms of PBM are not fully understood, it is believed to largely affect cellular metabolism, increase adenosine triphosphate (ATP) and modulate reactive oxygen species (ROS). A change in ROS is known to affect transcription factors, (responsible for growth, inflammation, cellular proliferation and oxygenation), eventually culminating in augmented tissue repair.[3,4]

A novel technology – Kleresca® biophotonic platform, based on fluorescent light energy (FLE) produced by excited light-absorbing chromophores when illuminated with a multi-LED lamp, offers a new approach in dermatology. FLE has been shown in clinical trials to

modulate both disease-affected and healthy skin, decreasing inflammation and enhancing the skin's overall texture.[5–12]

Despite the reported clinical efficacy of FLE, the underlying cellular mechanism of action of this biphotonic platform has yet to be elucidated.

Inflammation is fundamental to many skin conditions.[13] Further, macrophages play a vital role in the inflammatory response and tissue repair process.[14] To determine if FLE could influence the inflammatory responses of cutaneous cells, cytokine concentrations were assessed in supernatant samples of cultured human fibroblasts and keratinocytes exposed to macrophage conditioned media (MCM). Additionally, as many light therapies aim to boost collagen production, this study examined the ability of FLE to enhance collagen production from human dermal fibroblasts. Finally, we explored a role for FLE in modulating angiogenesis, a final step in many healing processes.

Materials and methods

Clinical evaluation

A representative patient with acne vulgaris, Investigator's Global Assessment (IGA) grade 4 underwent the Kleresca® biphotonic treatment (consisting of a multi-light emitting diode (LED) lamp and proprietary chromophore containing gel) once per week for 6 weeks. Briefly, a 2 mm-thick layer of the chromophore containing gel was applied to a clean face and immediately illuminated with the multi-LED lamp applying approximately 30-40 J/cm^2 of blue light during the treatment.[7] This procedure was carried out with prior, informed

consent and in accordance with the Declaration of Helsinki and the International Conference of Harmonization (ICH) Guidelines for Clinical Practice. Standardized photographs of the temple region of the face were taken before the commencement of the treatment (baseline), at the end of the treatment (week 6), and at week 12 and 18 from the start date. A 'booster' treatment (1 session) was given at week 33 and photographs were taken at week 45 and 57. The percentage of pixels occupied by inflammation (% inflammation) in each photograph of facial skin was measured and expressed as a percentage of the total pixels per photograph (ImageJ, NIH, USA).[11]

Kleresca® gel and mimicking lamp

Upon illumination, the Kleresca® lamp delivers non-coherent blue light in continuous waves with a peak wavelength range of 440–460 nm. The absorbance of the chromophore gel was measured using a VideometerLab multispectral imaging device (Videometer, Hørsholm, Denmark) (Fig 1).

The fluorescence emitted from the chromophore containing gel was measured using a cosine corrected optical probe (EOP-146, Instrument Systems, München, Germany), mounted 42 mm above the output port of the setup. The optical probe was fiber coupled to an array spectroradiometer (CAS140 CT, Instrument Systems) and the system was calibrated to measure the spectral irradiance from 356 to 830 nm (Fig. 2). Upon exposure to the blue LED light, the chromophore gel acts as a photoconverter, inducing the emission of FLE in a spectrum in the range of 500–610 nm (Fig. 3).

To recreate a control light source which mimicked the output from the Kleresca® platform, we designed a mimicking lamp (i.e. a similar spectral irradiance that results in excited chromophore emissions). The mimicking lamp had an identical spectral output but was generated by continuous LED light instead of excited chromophore emissions combined with blue LED light. The blue light came from a single blue LED and the broader spectrum light, mimicking the chromophore emission spectrum, was spectrally filtered light from a cold white LED. Emission spectra were measured as above (Fig. 4).

Cell culture

Primary normal human epidermal keratinocytes (NHEK) (ATCC PCS-200—011) and human dermal fibroblasts (HDF) (ATCC-PCS-201-012) (ATCC, Manassas, VA, USA) were grown in dermal cell basal medium supplemented with a keratinocyte growth kit serum free (ATCC-PCS-200-400) and in fibroblast basal medium supplemented with a low serum fibroblast growth kit (ATCC-PCS-201-41), respectively. All cells were passed at 80% confluency and keratinocytes in the third or fourth passage were used.

Measuring collagen production

HDF cells (7.5 x 10^4 cells/ml) were seeded in a 2-well chamber slide Nunc IVF multi-dish (Nunc 167063, Thermo Fisher Scientific, Waltham, MA, USA). To test the effect of FLE on collagen production, three sets of cells were prepared: (1) a non-illuminated HDF control group; (2) HDF exposed to the mimicking lamp and; (3) HDF exposed to FLE. Illumination was performed according to manufacturer's instructions. Briefly, the cells were positioned 5 cm from the light sources and illuminated with 30–40 J/cm^2 (Fig. 5). A physical glass barrier was placed between the cell monolayer and the illuminating systems to ensure that any

biological response observed was induced by the mimicking lamp or the FLE and not through any physical or chemical interaction. Following 48 hours of incubation, cell-free supernatants were collected and assessed for total soluble collagen production using the Sircol™ Soluble Collagen Assay (Biocolor Ltd., Carrickfergus, UK), according to the manufacturer's instructions. This was carried out on two separate occasions and the results were pooled.

One subset of HDF slides (seeded at $7.5x10^4$ cells/ml) was treated with 300U/ml of IFN-γ, (human recombinant, R&D System, USA). These cells were exposed to either (1) the mimicking lamp (control) or (2) FLE. The Sircol™ Assay was subsequently carried out.

Macrophage conditioned media preparation

$CD14^+$ monocytes were isolated from fresh normal human peripheral blood mononuclear cells (PBMC) purchased from ZenBio (SER-PBMC-200; 200 x 10^6 cells/vial; Durham, NC, USA) using CD14 microbeads human MACS (30-050-201; Miltenyi Biotec, Bergisch Gladbach, Germany) and differentiated into macrophages, according to the manufacturer's protocol. Macrophage cells were then stimulated with lipopolysaccharide (LPS) (10 ng/mL; L2630, Sigma-Aldrich) + 50 ng/ml recombinant IFN-γ (285-IF-100; R&D Systems Inc.) for 3 days, polarizing them towards an M1 phenotype.[15] MCM was subsequently collected and stored at –80°C until required to test the effect of inflammatory mediators on the activity of HDF and HEK cells.

Stimulation of fibroblast and keratinocyte cells with macrophage conditioned media

Prior to exposing the HEK and HDF cells to the collected MCM, HEK at 35,000 cells/mL per

well were seeded in 4-well Corning® Biocoat™ 4-well culture glass slides (Corning®, New York, NY, USA) and HDF at 75,000 cells/mL per well were seeded in 2-well chamber slides for adherence overnight. Next, cells were stimulated with 600 µL of MCM overnight at 37^{o}C, before the media was removed and replaced by phosphate-buffered saline (PBS) prior to illumination. The cells were divided into three groups: 1) a non-illuminated control group; 2) a blue-LED lamp alone and; 3) FLE. Subsequently, cells were cultured with fresh media, supernatants were collected after 6 and 24 hours and cytokine release was assessed using ELISA, including human tumour necrosis factor alpha (TNF-α) DuoSet TNF alpha Human ELISA Kit (Invitrogen™, Waltham, MA, USA) and human interleukin-6 (IL-6) (R&D Systems Inc.). Fold changes of cytokine concentrations were calculated compared to non-illuminated controls and data were averaged from three independent experiments.

Angiogenesis and tube formation in human endothelial cells

HDF cells were 1) illuminated with blue-LED light only, or with 2) FLE. 72 hours later conditioned media (CM) was taken to assess new tube formation and branching of human aortic endothelial cells (HAEC). HAEC were seeded (0.3x10^{5}/well) onto a Matrigel® layer in a 96-well plate. Following cell adhesion, cell culture medium was replaced by 250 µl of CM. HAEC were incubated in the CM for 18 hours at 37°C with 5% CO_2; a negative control (no treatment) and a positive control, vascular endothelial growth factor (VEGF 30 ng/mL) were run in parallel. Three images per well were taken and assessed for tube formation and branching points using an inverted Olympus microscope (Olympus IX50). Group data were averaged from 3 images per well from 3 wells per condition.

Statistics

Data are expressed as the mean ± standard deviation and analyzed using a one-way analysis of variance (ANOVA) or Student's *t*-test where appropriate using GraphPad Prism® software, Version 7. All samples were compared to controls and experiments were performed up to three times in duplicate or triplicate. $P<0.05$ was deemed significant in all cases.

Results

Clinical evaluation

Figure 6 shows a woman with acne vulgaris, IGA grade 4; representative of an inflammatory skin condition. A photo taken before the treatment started (Fig. 6A) shows inflammatory lesions on the temple. There was a marked reduction in redness following 6 weeks of treatment (Fig. 6B). The redness and percentage of inflammation was resolved at the 18-week time point (Fig. 1D) and this was maintained for the remainder of time points (Fig. 6 D–H). As the inflammatory area was resolving over time (week 12 onwards) there was a concurrent decrease in visible scaring (Fig.6 E–G). The treating practitioner chose to provide a single booster treatment at week 33, after which the results obtained were maintained up to week 52, demonstrating that the overall stress and inflammatory level was reduced significantly; so small manipulations over time may support a continuous improvement of a cyclic inflammatory skin condition.

Spectral irradiance of the Kleresca® biophotonic platform and mimicking lamp

The FLE spectral output (nm) generated by Kleresca® biophotonic platform ranged from 415-700nm covering the blue, green, yellow orange and red wavelengths of the visible spectrum. The spectral output of the mimicking lamp matched this (Fig. 4).

Kleresca® biophotonic platform increases collagen production

Exposure of HDF cells to the mimicking lamp did not affect collagen production by fibroblasts ($P > 0.05$). Collagen production in fibroblasts treated with FLE was significantly increased compared to non-illuminated control cells and to cells treated with the mimicking lamp ($P < 0.0001$; Fig. 7A).

HDF cells pretreated with IFN-γ did not significantly increase their collagen production when exposed to FLE compared to cells exposed to the mimicking lamp alone ($P > 0.05$; Fig. 7B).

In subsequent experiments, the blue LED (not the mimicking lamp) was used as an appropriate control since there was no difference in the response between them. Additionally, it was more convenient to work with the blue LED due to its relatively small size and simple set-up

Kleresca® biophotonic platform modulates the release of TNF-α and IL-6

Both HDF and HEK cells were exposed to MCM before being non-illuminated (control), treated with blue LED alone, or exposed to FLE. Both blue LED-treatment ($P < 0.05$) and FLE ($P < 0.0001$) significantly reduced TNF-α release from HDF cells relative to non-illuminated control HDF cells (Fig. 8A). FLE significantly reduced TNF-α release from HDF cells compared to blue LED-treated HDF cells ($P < 0.01$; Fig. 8A). TNF-α release from HEK cells was not affected by blue LED treatment alone compared to non-illuminated control cells ($P > 0.05$; Fig. 8C). FLE-treatment decreased the production of TNF-α from HEK cells compared to non-illuminated control cells ($P < 0.01$) and blue LED-treated cells ($P < 0.05$; Fig. 8C).

IL-6 release was significantly reduced from HDF cells following both blue LED light treatment ($P < 0.02$) and FLE treatment compared to non-illuminated control cells ($P < 0.01$; Fig. 8B). IL-

6 release was not significantly different between blue LED-treated or FLE-treated HDF cells ($P > 0.05$; Fig. 8B). IL-6 release from HEK cells was not affected by blue LED light treatment alone compared to non-illuminated control cells ($P > 0.05$). However, FLE-treatment decreased IL-6 release from HEK cells compared to non-illuminated control cells ($P < 0.0001$) and blue LED-treated cells ($P < 0.0001$; Fig. 8D).

Kleresca® biophotonic platform enhances angiogenesis

Exposing HAEC to VEGF significantly increased both microvascular tube and branching formation compared to non-treated controls ($P < 0.05$); blue LED alone did not affect either tube formation ($P > 0.05$) or branching ($P > 0.05$). Treatment of HAEC with CM significantly increased both branching and tube formation compared to either non-illuminated control cells ($P < 0.01$ and $P < 0.001$) or blue LED alone ($P < 0.01$ and $P < 0.05$; Fig. 9B).

Discussion

This study sought to elucidate how FLE modulates cellular activity underlying the efficacy reported in the treatment of inflammatory dermatological conditions[5,7,9,10] and rejuvenating the skin.[6]

Here we describe the function behind a technology used on a representative patient suffering from acne vulgaris (IGA grade 4) with visible redness and inflammatory lesions. A persistent resolution of inflammation and associated redness was observed after six weeks of exposure to FLE. There was also a visible improvement in the skin's texture and a concomitant fading of existing scars. Previous clinical work using FLE has reported a significant decrease in inflammation and associated lesions in moderate and severe acne

vulgaris,[5,7] a normalization of the skin's structure (reducing the appearance of fine lines and wrinkles as a stand-alone rejuvenation therapy[6] or post-laser treatment for solar lentigines[11]) and more recently, an improvement of the signs and symptoms of rosacea.[9,10] Together, these clinical findings highlight the broad application and utility of this technology.

Although not fully elucidated, various mechanisms underpinning the beneficial clinical outcomes observed with PBM have been reported.[4,16–18] Of interest is the ability of PBM to modulate inflammation, alter cellular activation, modulate collagen synthesis and enhance blood flow.[19] We sought to investigate a role for these key biomolecular mechanisms using FLE.

To reduce the signs of aging, many modalities typically induce a controlled form of wounding in the skin epidermis to promote the induction of new collagen biosynthesis.[17] Various studies using LEDs have reported on the promising effects on skin rejuvenation by enhancing collagen production without inducing prior damage.[17] In the current study, FLE enhanced collagen production from HDF cells compared to non-illuminated control fibroblasts. Moreover, exposing HDF cells to the LED mimicking lamp (which matched the spectral output of the biophotonic platform), did not significantly alter collagen production. These results demonstrate that it is the FLE irradiating from the gel that induces this biomolecular alteration.

Multiple wavelengths of light within the visible spectrum can penetrate various depths of the skin and activate endogenous chromophores.[20] Intuitively, one might think that combining wavelengths may lead to an enhanced effect. Indeed, this has been shown by

some groups in treating acne vulgaris.[21] However, for collagen synthesis, there is still some debate in the literature.[22]

Pulsing vs continuous wavelengths of light seem to be favourable for collagen production.[23] The photoconversion of the gel leads to the production of a dynamic hyper-pulsed multi-wavelength of fluorescent energy through the phenomenon of Stokes Shift.[8] This pulsing of light appears to be the key to enhancing collagen production by the illuminated fibroblasts, as the mimicking lamp with the same spectral output failed to alter fibroblastic collagen production. Theories for enhanced collagen production with PBM suggest cytochrome *c* activation increasing mitochondrial energy production leading to downstream activation of various genes for collagen synthesis.[16] While these results show a clear differential between FLE and comparable LED light, the mechanism of enhanced collagen production remains to be elucidated.

Inflammation is an underlying feature of many dermatological indications[13] and affects normal cellular function. Prior exposure of HDF to the pro-inflammatory cytokine IFN-γ interfered with the ability of FLE-exposed fibroblasts to increase their collagen production. In our clinical case there was an improvement in the signs of visible scaring following the resolution of the visible redness and inflammatory lesions. This is likely due to an enhancement of collagen production after the attenuation of inflammation. These findings strengthen the biophotonic platform's potential for use in both therapeutic inflammatory indications and for aesthetic purposes, as well as normalization of the skin before or after treatments that cause inadvertent inflammatory responses.

The cutaneous environment is complex. Fibroblasts and other mesenchymal cells can respond to both pattern- and damage-associated molecular pathogens i.e. PAMPs and DAMPs as well as inflammatory cytokines released by resident macrophages.[24,25] Indeed, chronic skin inflammation is often triggered and maintained by production of a variety of cytokines and chemokines such as TNF-α and IL-6.[26–28] We found that both fibroblasts and keratinocytes exposed to MCM from M1-like macrophages altered their cytokine production following exposure to FLE. HDF cells responded to both LED treatment and FLE with a reduced output of both TNF-α and IL-6. For TNF-α, FLE decreased TNF-α production further compared to treatment with blue LED. Conversely, keratinocytes only responded to treatment with FLE. Taken together, these results support the idea that the FLE has an overall anti-inflammatory response, however further research is required.

Differential effects of PBM on cytokine production have been reported.[18] Although PBM therapy is noted for its anti-inflammatory effects clinically, it has been reported to activate nuclear factor kappa-light-chain-enhancer of activated B cell (NfκB) – the master regulator of inflammation in normal quiescent cells.[29] However, decreased inflammatory responses have also been reported from various cell types pretreated with pro-inflammatory cytokines.[30–32] As the skin is in a pro-inflammatory state in many conditions, light treatment may be beneficial in turning down this response. Additionally, these findings support the idea that depending on the micro-environment of the skin, PBM can have differential effects i.e. therapeutic and aesthetic. This concept supports our findings to date.

Macrophages play an integral role in the immune response i.e. upon recruitment from monocytes they can become a critical local source of various mediators including matrix metalloproteinases (MMPs), cytokines and chemokines.[24] They can have a dual reciprocal

function and can be pro- (M1) or anti-inflammatory and reparative (M2), depending on the situation and local environment.[33]

It is tempting to speculate that FLE could potentially alter the cutaneous macrophage phenotype, polarizing these cells towards an M2 phenotype. This transition is known to be a key step in the process of normal tissue repair.[24] Indeed, some groups have reported the capacity of PBM to alter macrophage phenotype,[18] which would have many implications for cutaneous inflammation. Future work will focus on a role for FLE in modulating immune metabolism.

A key and final phase of the regenerative/healing process is neovascularization (angiogenesis) from existing blood vessels. PBM has been reported to increase angiogenesis in cutaneous wounds *in vivo*.[34] In the current study, FLE increased both branching points and tubes in HAECs, which is indicative of angiogenesis. The angiogenic response was relative to that of cells stimulated with VEGF, a potent angiogenic factor. The ability of FLE to induce angiogenesis correlates with the latent persistent clinical inflammatory resolution and collagen build-up observed.[5–7] Interestingly, this was an indirect effect induced by the media taken from the 3D skin insert exposed to FLE. While it is not clear how the biophotonic platform is inducing an angiogenic response, it is clear that immune cells can release a variety of angiogenic factors.[35] Specifically, M2 macrophages are a key source of VEGF and can enhance cellular proliferation.[24] If FLE can polarize cutaneous macrophages towards the M2 phenotype, this might explain the angiogenesis observed. The ability of FLE to induce healthy vasculature and a normalized de-stressed environment in the skin could be of benefit in treating rosacea, by improving blood distribution through enhanced lateral blood flow and attenuating erythema and blushing.

Conclusion

Whilst further research work is required to elucidate the complete mechanism of action of the Kleresca® biophotonic platform, here, we report its capacity to enhance fibroblastic collagen production, attenuate the inflammatory signature of a variety of cutaneous cells and enhance angiogenesis, all contributing to normalization and de-stressing of the skin. This platform was superior to an equivalent mimicking non-fluorescent light and to conventional blue LED. These findings are relevant to a variety of inflammatory skin indications and can offer adjunct support to other, more invasive dermatological approaches.

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Authors' contributions

All the authors listed participated in the study concept and design and have viewed and approved the final version of the manuscript. Dr. Nielsen and Dr. Edge conceived the concept and design, and both take responsibility for the integrity of data and the accuracy of the data analysis. They drafted the manuscript and in collaboration with Prof. Scapagnini and Ms. Mellergaard, they participated in its critical revision for important intellectual content. Dr. Hansen and Dr. Correll designed and carried out the testing of the mimicking lamp and provided the photonic analysis. Dr. Jaworska participated in the study design of the laboratory experiments.

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Figure legends

Figure 1. Chromophore gel absorption

(A) A monochrome picture illustrating the chromophore gel absorption measured from 375 nm to 970 nm. (B) A graphic illustration of the absorption measured.

Figure 2. Measurement of fluorescence emitted from the chromophore gel

A detector probe was placed 12.6 cm away from the light source (L30935). The light from the light source was baffled by several baffles ensuring that only light that was transmitted

through the sample and the light coming from the sample itself was collected by the detector.

Figure 3. Fluorescent spectra created by illumination of the chromophore gel

Upon illumination of 2 mm of the chromophore gel, a fluorescent spectra in the range of 500–610 nm was created. The fluorescent intensity decreased over time from the onset of illumination until 10 to 15 minutes after illumination.

Figure 4. Spectral irradiance of the Kleresca® biophotonic platform and mimicking lamp

The spectral output (wavelength (λ), measured in nm) generated by Kleresca® biophotonic platform (consisting of chromophore-containing gel and lamp) is shown in black and the output of the LED lamp which matches this, is shown in blue.

Figure 5. Schematic of the cellular set-up of fluorescent light energy

Experimental set-up of exposing cells to fluorescent light energy.

Figure 6. Fluorescent light energy decreases inflammation and improves skin texture in a representative case of acne vulgaris

Clinical response of an Investigator Global Assessment (IGA) grade 4 acne vulgaris patient to fluorescent light energy (FLE). (A) inflammatory lesions and associated redness taken before FLE-treatment (baseline); (B) the end of the 6-week treatment; (C) 12 weeks; (D) 18 weeks; (E) 33 weeks; (F) 45 weeks and; (G) 57 weeks from the start of the treatment protocol. *

denotes one additional (booster) treatment. (H) The graph shows the percentage of the face occupied by inflammation, analyzed with ImageJ.

Figure 7. The effect of fluorescent light energy and mimicking lamp on collagen synthesis in primary human dermal fibroblast cells in the presence and absence of interferon gamma (IFN-γ).

(A) Collagen production (μg/ml) in non-illuminated HDF cells, HDF cells exposed to LED mimicking lamp and HDF cells exposed to fluorescent light energy. Each bar represents the mean ± SD of two independent experiments performed in duplicate. Data were statistically compared by one-way ANOVA and Tukey's post hoc test. ****$P < 0.0001$; compared to non-illuminated control and $^{\#\#\#\#}P < 0.0001$; compared to LED mimicking lamp.

(B) The experiment was repeated in the presence of the inflammatory mediator interferon gamma (IFN-γ). Data were statistically compared by unpaired Student's *t*-tests.

Figure 8. Fluorescent light energy decreases inflammatory cytokine production from macrophage conditioned media stimulated human dermal fibroblasts and epidermal keratinocytes

Fold change compared to non-illuminated control for (A) TNF-α, (B) IL-6, from HDF cells, and (C) TNF-α, (D) IL-6, from HEK cells, stimulated with M1 macrophage-conditioned media. TNF-α was measured at 6 hr post treatment and IL-6 was measured at 24 hr post treatment. Each bar represents the mean ± SD of three independent experiments performed in triplicate. * compared to non-illuminated control and, $^{\#}$ compared to blue LED lamp.

Figure 9. Fluorescent light energy enhances angiogenesis

(A) Representative micrographs of human aortic endothelial cells under various conditions. (B) Group data showing tube formation and branching points. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, unpaired Student's *t*-tests.

583 **Figure 1**

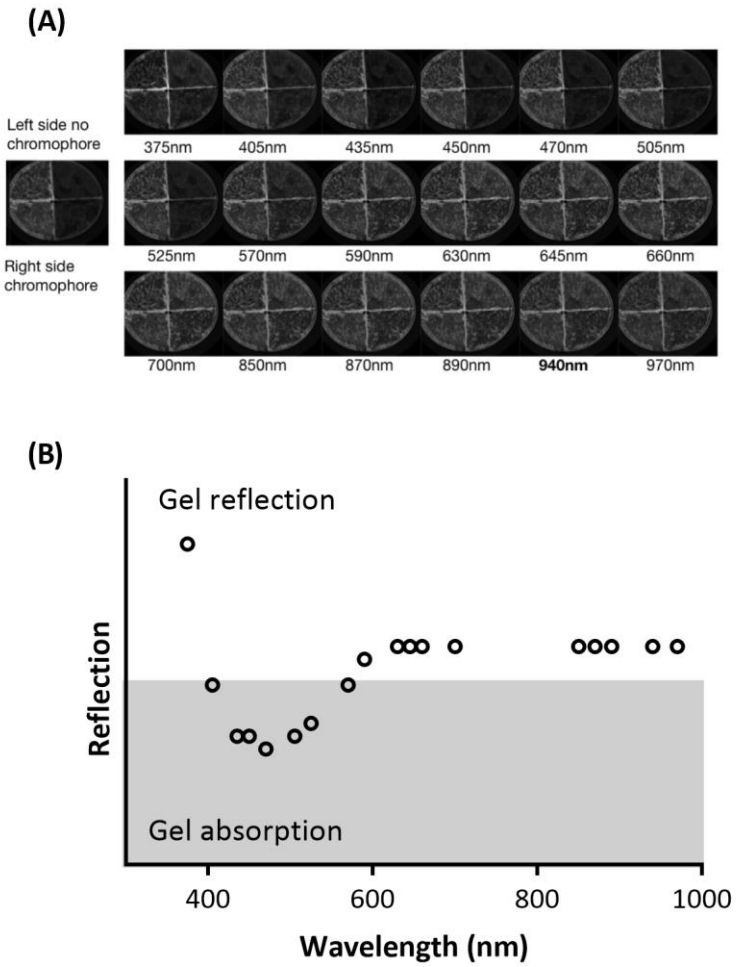

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Figure 2

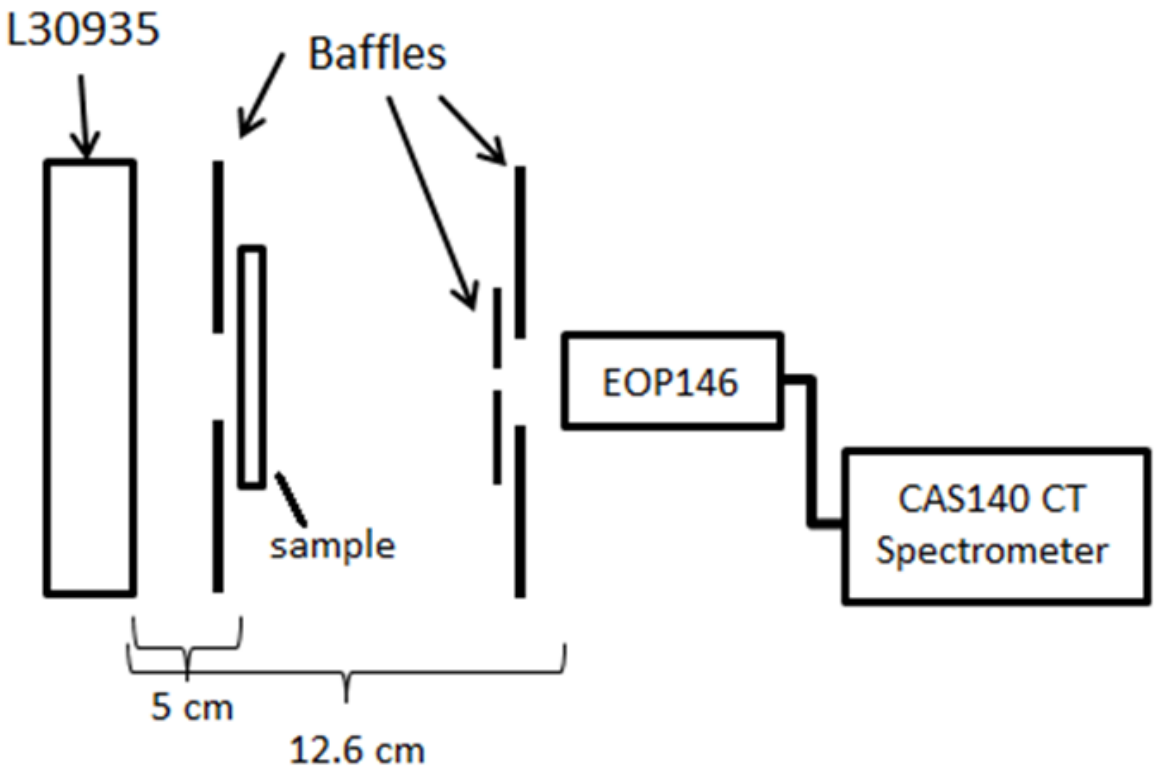

L30935
Baffles
EOP146
CAS140 CT
Spectrometer
sample
5 cm
12.6 cm

Figure 3

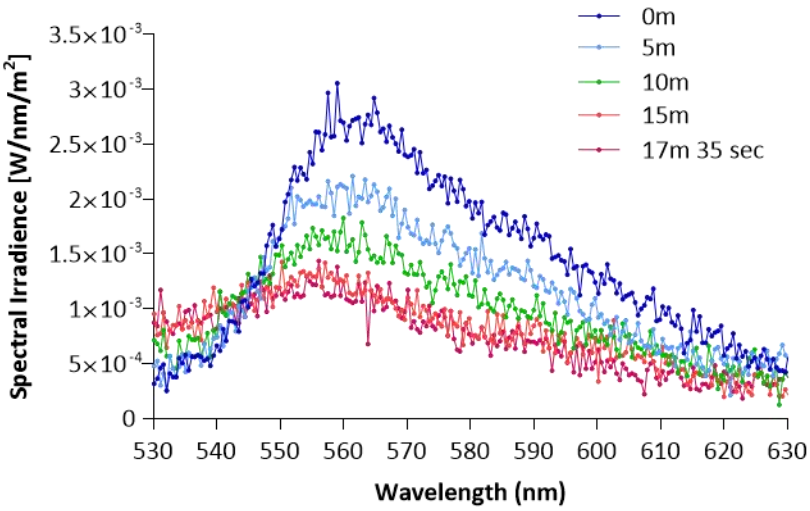

0m
5m
10m
15m
17m 35 sec
Spectral Irradience [W/nm/m²]
3.5×10⁻³
3×10⁻³
2.5×10⁻³
2×10⁻³
1.5×10⁻³
1×10⁻³
5×10⁻⁴
0
530 540 550 560 570 580 590 600 610 620 630
Wavelength (nm)

Figure 4

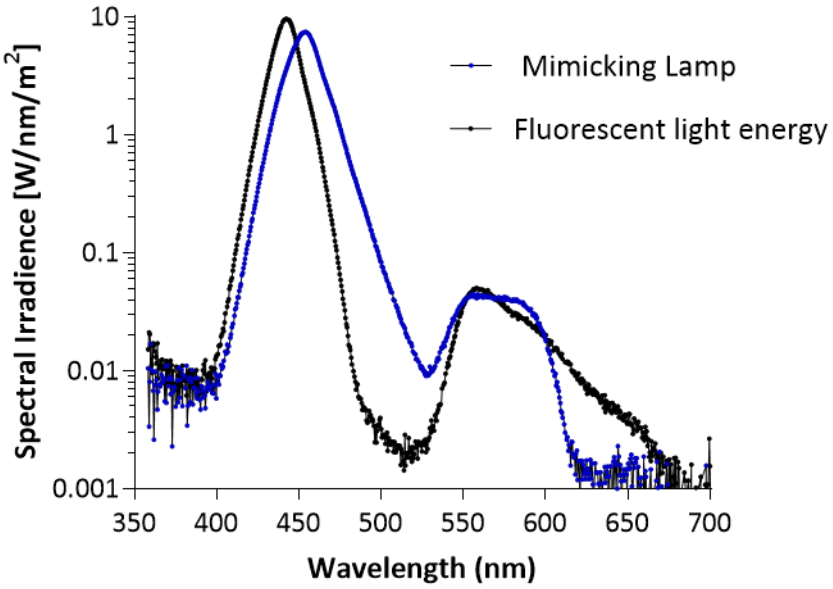

Mimicking Lamp
Fluorescent light energy
Spectral Irradience [W/nm/m^2]
10
1
0.1
0.01
0.001
350
400
450
500
550
600
650
700
Wavelength (nm)

Figure 5

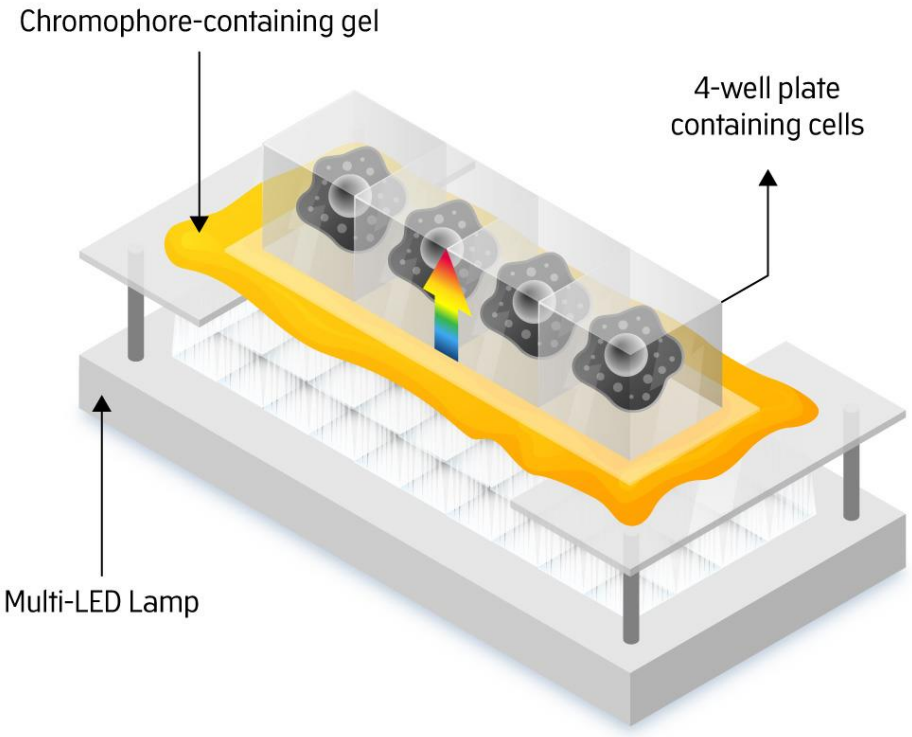

Chromophore-containing gel
4-well plate
containing cells
Multi-LED Lamp

Figure 6

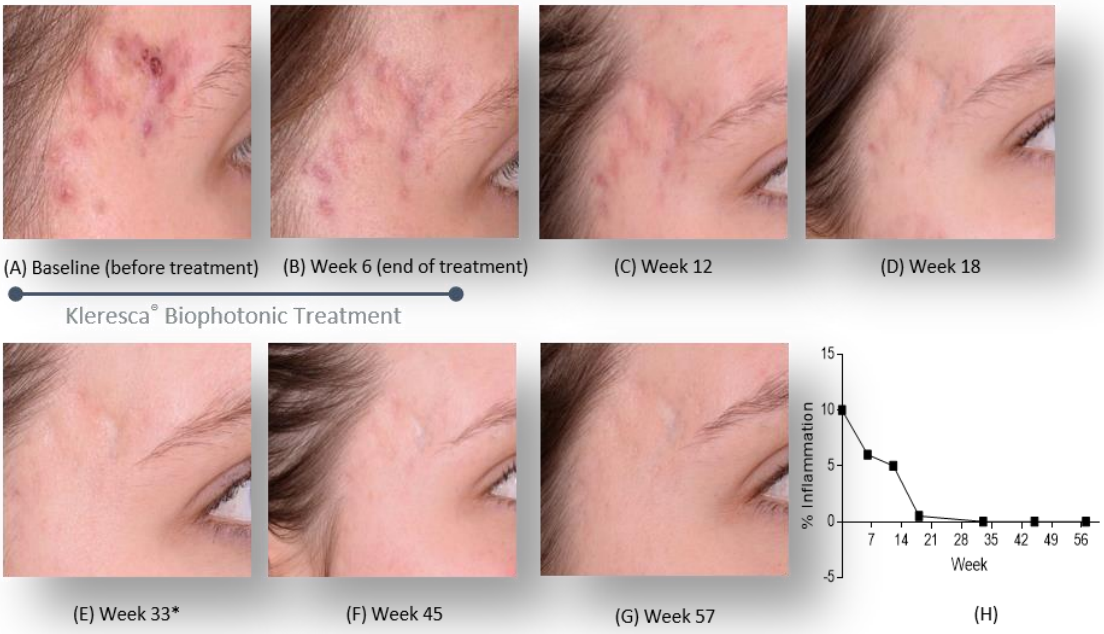
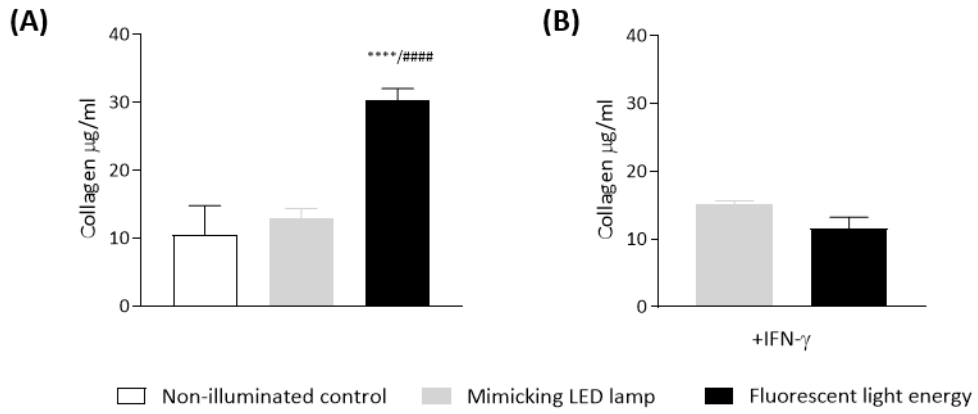
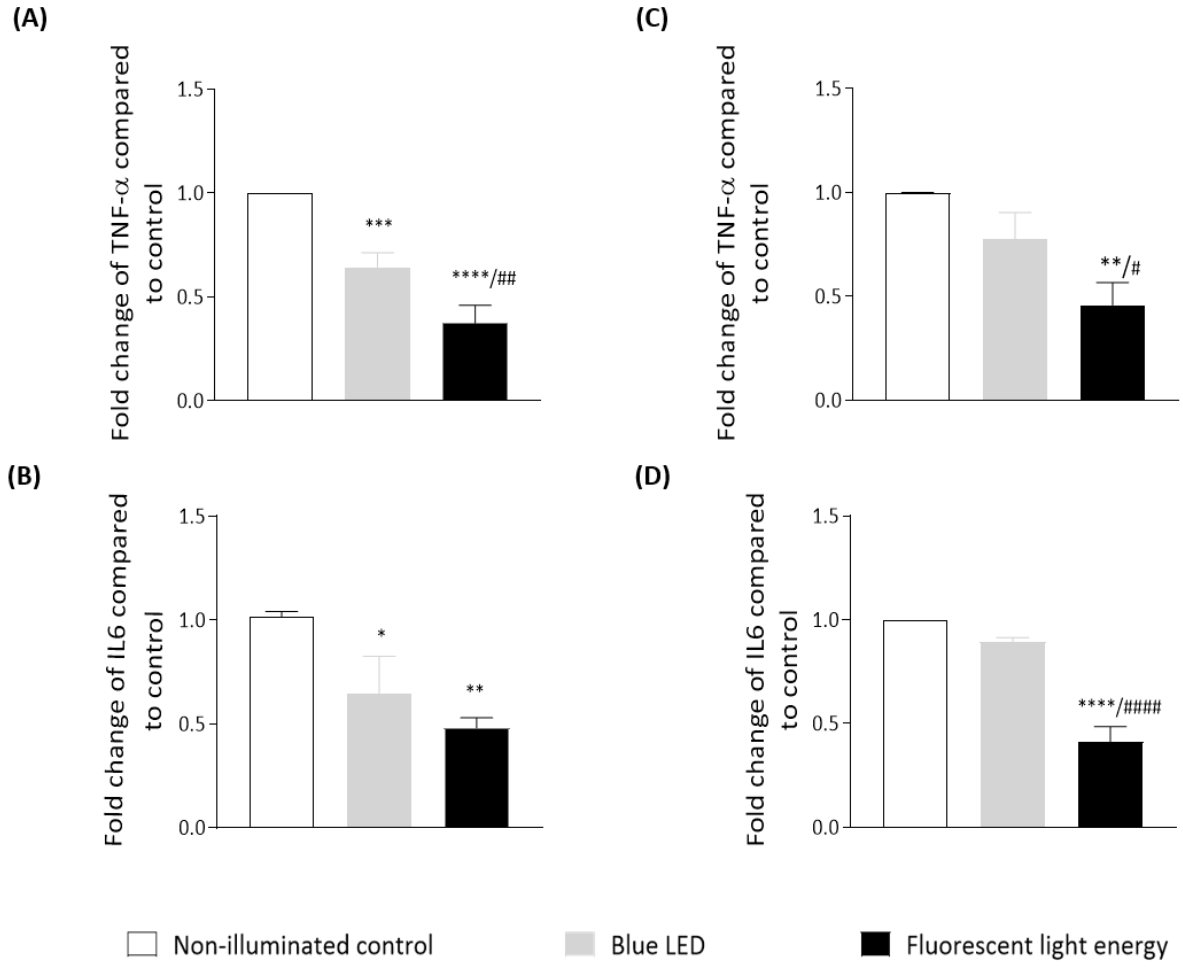

(A) Baseline (before treatment)
(B) Week 6 (end of treatment)
(C) Week 12
(D) Week 18
Kleresca® Biophotonic Treatment
(E) Week 33*
(F) Week 45
(G) Week 57
(H)
% Inflammation
15
10
5
0
-5
7
14
21
28
35
42
49
56
Week

Figure 7


(A)
(B)
Collagen µg/ml
40
30
20
10
0
****/####
+IFN-γ
Non-illuminated control
Mimicking LED lamp
Fluorescent light energy

667 **Figure 8**



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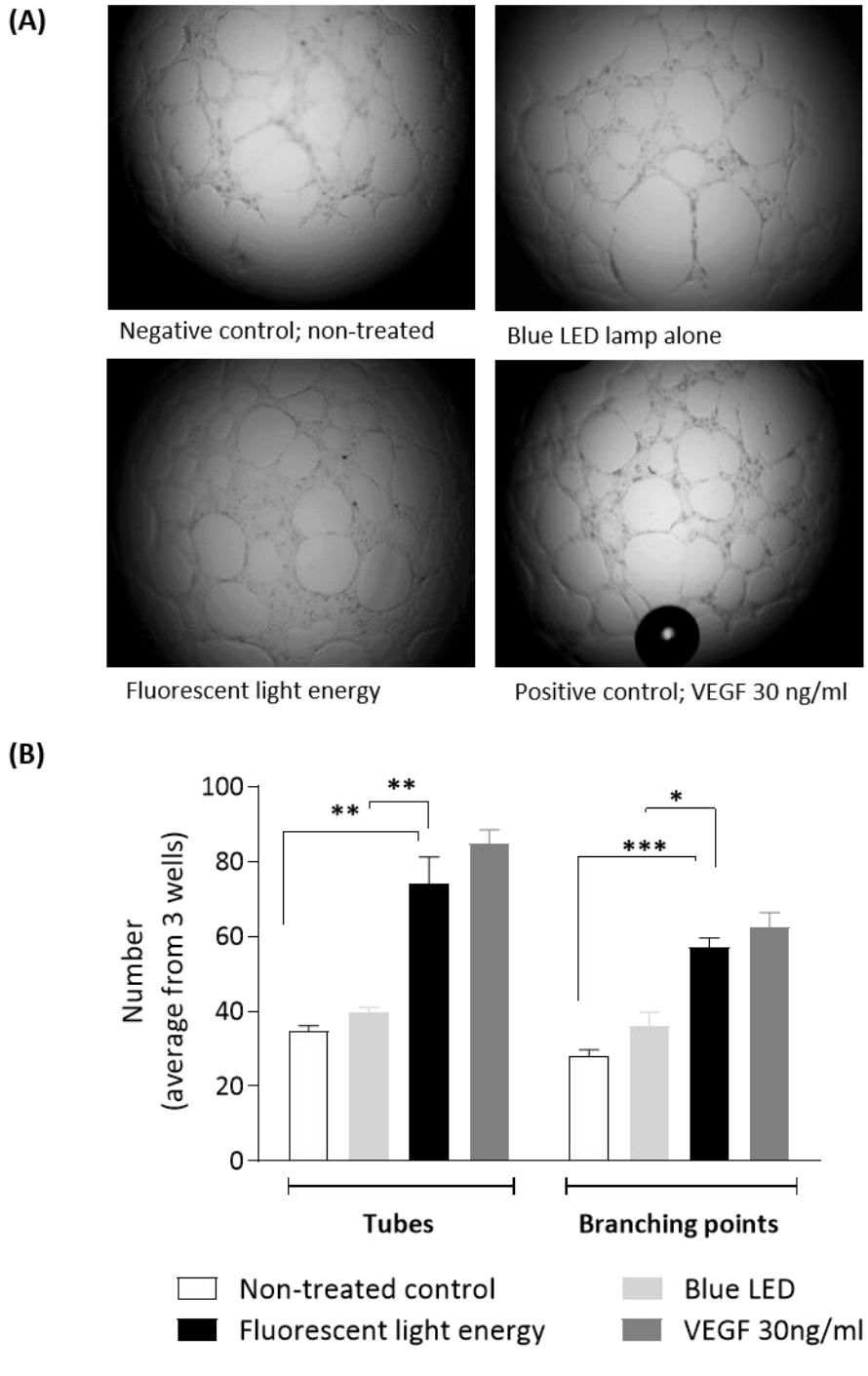
676 **Figure 9**



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