Blue light irradiation and its beneficial effect on Dupuytren’s fibroblasts

Julia Krassovka, Annika Borgschulze, Benita Sahlender, Tim Lögters, Joachim Windolf, Vera Grotheer

Department of Trauma and Hand Surgery, Medical Faculty, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany

*vera.grotheer@med.uni-duesseldorf.de

Abstract

Dupuytren’s contracture is a fibroproliferative disorder affecting the palmar fascia of the hand. Most affected are the ring fingers, and little fingers of middle-aged men. Symptomatic for this disease is the increased proliferation and differentiation of fibroblasts to myofibroblasts, which is accompanied by an elevated α-SMA expression. The present study evaluated the therapeutic benefit of blue light (λ = 453 nm, 38 mW/cm², continuous radiance, spot size 10–12 cm²) as well as the molecular mechanism mediating this effect. It could be determined that blue light significantly diminished the induced α-SMA protein expression in both normal palmar fibroblasts and Dupuytren’s fibroblasts. The beneficial effect mediated by this irradiance, radiant exposure and wavelength was associated with an elevated reactive oxygen species generation. Furthermore, the data underlines the potential usefulness of blue light irradiation as a promising therapy option for Dupuytren’s disease, especially for relapse prevention, and may represent a useful strategy to treat further fibrotic diseases, such as keloids, hypertrophic scarring, and scleroderma.

Introduction

The cause of Dupuytren’s disease (DD) is believed to be multifactorial with a genetic disposition and concerns predominantly middle-aged Caucasian men [1]. It’s a benign fibromatosis affecting the palmar fascia of the hand, characterized by an increased subcutaneous nodule, followed by cord formation dependent on the progression of this disease. Responsible for cord or nodule formation is an excessive proliferation and differentiation of fibroblasts to myofibroblasts, so that the myofibroblasts becomes the predominant cell type in Dupuytren’s tissue [2]. And the benign but undesired growth of Dupuytren’s myofibroblasts is supported by their decreased susceptibility to undergo apoptosis [3–5]. In general, differentiated myofibroblasts show characteristics of fibroblasts and smooth muscle cells, and express α-smooth muscle actin (α-SMA), which allows the cells to contract. Therefore myofibroblasts assume mandatory functions in wound healing enabling wound closure. Hence, the common method to identify (Dupuytren’s) myofibroblasts is proving the intracellular α-SMA-amount. The pathological staging of DD is based on the definition given by Luck et al. [6], who suggested to differentiate...
between proliferative, involutional, and residual stages. In the proliferative phase, α-SMA-expression increases due to the uncontrolled proliferation of myofibroblasts, leading to nodule formation influenced by local mediators such as transforming growth factor-β1 (TGF-β1). During the progression of Dupuytren’s disease myofibroblasts synthesize an elevated level of α-SMA, collagen III and fibronectin [7]. So, the involutional stage is marked by an elevated proportion of collagen III, resulting in the formation of the characteristic cords and an increased extracellular matrix deposition. In the residual phase, nodules and myofibroblasts decrease and α-SMA and collagen III were diminished [8], and replaced by scar tissue.

Furthermore, TGF-β1 seems to play a superordinate role in the formation and maintenance of DD, especially because TGF-β1 induces the expression of α-SMA, collagen III and fibronectin, and has been shown to increase the contraction of myofibroblasts [9, 10]. So, TGF-β is localized in myofibroblasts in all phases of DD [1]. This is not surprising, since TGF-β1 is an abundant pro-inflammatory cytokine, involved in pathological scarring and fibrosis [11].

One therapy option for advanced DD is the partial fasciectomy, which is unfortunately accompanied by significant recurrence rates [12]. This surgical technique removes involved tissue, and contains extensive dissection of diseased longitudinal cords or nodules, which are removed from the surrounding fascia [13]. Needle fasciotomy or enzyme injection as collagenase [14] were performed as alternative methods, and at least 88% of the collagenase-treated patients were observed to have their movement improved by up to 48˚ movement in a one-year follow-up study [15].

The use of photobiomodulation-based therapies as a treatment option for DD is a promising alternative, which has not been applied to this day. Visible light could mediate its therapeutic effect via the interaction with endogenous photoreceptors, chromophores such as porphyrins, cytochrome c oxidase, nitrosated and flavo-proteins, opsins, and ion-gate channels [16, 17], dependent on the specific characteristics of the irradiated tissue.

It has already been shown, that blue light, dependent on the wavelength, intensity (irradiance) and radiant exposure, has anti-inflammatory effects in keratinocytes [18] and suppresses dendritic cell activation [19]. In rodents blue light inhibits skin tumors [20] and improves wound healing [21]. But, in the context of DD, blue light is particularly interesting, because mitosis and proliferation of cultured cells could be inhibited [22]. Furthermore, the proliferation and induced α-SMA-expression in human dermal myofibroblasts could be diminished [23]. These preconditions could render the irradiance with blue light (λ = 453 nm, intensity 38 mW/cm²) an interesting tool for the treatment of Dupuytren’s fibroblasts. Therefore, the therapeutic benefit of blue light (with these parameters) on Dupuytren’s disease was evaluated in the present work.

**Material and methods**

**Patients**

Study approval was obtained from the Ethics Review Board of the Medical Faculty, Heinrich-Heine-University Düsseldorf (Study No. 3634). Of the 23 patients with Dupuytren’s disease (DD), 3 were female and 20 were male (average age 62 years), all of whom underwent fasciectomy at the Department of Trauma and Hand Surgery, University Hospital, Düsseldorf, Germany. Patients who suffered from carpal tunnel syndrome served as normal palmar fascia control (NPF). Their tissue was dissected from the palmar fascia and served as control group, if it had to be removed anyway (n = 24; 16 women and 8 men; average age 52.5 years). The usage of human material was in compliance with the Declaration of Helsinki Principles. Informed written consent was gathered from all patients. During surgery, tissue specimen were deposited in PBS (1% penicillin/streptomycin) and cooled to 4˚C. Samples were processed within 24 h. All data were anonymized prior to analysis.
Isolation and culture of the cells

All chemicals were obtained from Sigma-Aldrich, and cell culture materials from Cellstar (Greiner bio-one), if not mentioned otherwise.

Minced tissue was incubated at 37°C in 10 ml collagenase-solution (0.1 M CaCl, 0.005 M Glucose, 0.1 M Heps, 0.12 M NaCl, 0.05 M KCl + 1.5% BSA and 0.2% collagenase type 1) for 45 min, filtrated through a 100 nm nylon strainer, centrifuged (1200 rpm, 5 min at 4°C) and the pellet resuspended in 30 ml 0.9% NaCl-solution. After centrifugation (5 min at 1200 rpm at 4°C) the pellet was resuspended in standard medium (RPMI 1640 Medium Biochrom with 10% FBS, 1 x NEAA, 1% penicillin/streptomycin, 1 x sodium pyruvate) and incubated at 37°C, 5% CO₂.

Irradiation and TGF-β1 treatment

Fibroblasts and NPF were used in passage 3 – 8. Cells were seeded in 3 × 10⁴/6-Well and irradiation was started after 24 h, so that procedure was started under subconfluent conditions. Fibroblasts and NPF were either treated with 2 ng/ml TGF-β1 (ImmunoTools rh TGF-β1), irradiated (0–80 Joule/cm²), treated and irradiated, or neither of those. The irradiation was performed in PBS. The appropriate control cells were put beside the irradiation unit and incubated with PBS equally for the same time. After that, PBS was replaced by culture medium. Temperature was checked with a thermometer, and the achieved maximum temperature was 37°C. Cells were harvested by cell-scraping and the α-SMA protein expression was measured with Western Blot Analysis as described down below. No technical repeats could be performed due to limited biological tissue samples.

Blue light irradiation

All irradiation experiments were accomplished with a prototype of a narrow-band light-emitting diode (LED), which emits light of the wavelength λ = 453 nm (16.7 min). The power density was 38 mW/cm². The cells were continuously irradiated from above in PBS, in 6-Well-Plates. 60 (6 × 10) LEDs were equally distributed over a total of 10–12 cm². The blue light-emitting device used in our experiments was provided by Philips GmbH, Innovative Technologies, Aachen, Germany.

Cell viability test (metabolic activity)

Fibroblasts were irradiated with different doses (5, 10, 20, 40, 60, 80 Joule/cm²). Then, effects were analyzed with CellTiter-Blue Assay (Promega, Madison, USA/G3582). CellTiter-Blue uses an indicator dye to measure the metabolic capacity of cells, as an indirect evidence for cell viability. CellTiter-Blue was added at a ratio of 1:20 into the medium. After an incubation time of 1 h, the fluorescence (540_EX/590_EM) was measured in a 1420 Multilabel Counter (Victor³, PerkinElmer).

Western blot analysis

In order to verify the differentiation from fibroblasts to myofibroblasts, the α-SMA protein expression was measured with Western Blot analysis. Protein concentration was determined with the Pierce BCA Protein Assay Kit in line with the manufacturer’s instructions. 10 μg protein were mixed with the Laemmli buffer (4 × Tris-glycin-SDS sample buffer, 252 mmol TrisHCL pH 6.8; 40% Glycerin; 8% SDS; 0.01% bromphenol blue + 20% mercaptoethanol), denaturated for 5 min at 95°C, and separated on a 12% sodium dodecyl sulphate-polyacrylamide gel (SDS-PAGE)). Separated proteins were transferred to a nitrocellulose membrane.
(25 V – 1.0 A – 30 min) in a trans-blot system (BioRad Trans-Blot Turbo). Successful transfer was verified by Ponceau red S staining. The membrane was saturated with 5% BSA in TBST for 1 h (RT) and immunolabeled with mouse anti-human-α-SMA (Abcam/ab7817) (1:1000 in 3% BSA in TBST) over night at 4 °C and mouse anti-human Glyeraldehyde-3-phosphate-Dehydrogenase (GAPDH) antibody (BioRad/#12004167) (1:6000 in 3% BSA in TBST) for 1 h (RT). After washing with TBST, the membrane was incubated with horseradish peroxidase (HRP) conjugated goat anti-mouse secondary antibody (Dako/P0447) for 1 h (RT). Bound antibodies were detected using Clarity™ Western ECL Substrate (BioRad/#170–5060) and analyzed with the Quantity One 1-D Analysis Software Version 4.6.5 (BioRad).

**Reactive oxygen species (ROS) detection**

24 h after seeding $3 \times 10^4$ cells/6-Well, ROS detection was performed using Dihydrorhodamine 123 (DHR123; Sigma-Aldrich/D1054). DHR123 is oxidized to its fluorescent derivative Rhodamine 123 by unspecific ROS. In order to trigger a strong reaction, the irradiation set up was first conducted with 60 Joule/cm$^2$, and then fibroblasts were incubated for a minimum of 30 min in DHR123 (10 μM) in standard medium. Cells were washed with PBS, and fluorescence was measured (485$_{EX}$/535$_{EM}$) in the 1420 Multilabel Counter (Victor3, PerkinElmer).

**Measuring apoptosis**

Fibroblasts and NPF were either treated with 2 ng/ml TGF-β1, irradiated (40 Joule/cm$^2$), treated and irradiated, or neither of those over the course of 5 days. After that, fibroblasts were treated with staurosporine (STS) (Sigma-Aldrich/S5921) (0.075 μM) for 18 h. Cells were then harvested and centrifuged (1200 rpm for 5 min). The supernatant was removed, and the pellet resuspended in 500 μl FACS buffer (cell wash + 3% FBS). Cells were set on ice, fixed with 70% EtOH for 20 min and centrifuged (1200 rpm for 5 min). Pellets were washed two times with FACS buffer and stained with 200 μl propidium iodide solution (20 μg/ml TBS-T; Sigma-Aldrich/P4170). Cells were incubated protected from light for 30 min (RT) and measured with FACS Calibur (BD Biosciences).

**Statistical analysis**

Statistical analysis was performed with two-tailed $t$-test or two-way ANOVA and Mann–Whitney–U test, respectively. The data were expressed as mean value and standard deviation (SD). The level of significance was considered to be $p \leq 0.05$.

**Results**

**Cell viability (metabolic activity)**

As an indicator for cell viability, metabolic activity was analyzed (CellTiter-Blue Assay). From the beginning metabolic activity of DD fibroblasts was higher compared to all NPF (normal palmar fascia control) fibroblasts (Fig 1). From 60 Joule/cm$^2$ on, metabolic activity was significantly reduced in NPF fibroblasts and in DD fibroblasts metabolic activity was notably reduced. From 80 Joule/cm$^2$ on DD fibroblasts activity was significantly down regulated and in NPF fibroblasts highly significantly degraded, compared to their appropriated untreated controls (0 Joule/cm$^2$) (Fig 1). Because of this a radiant exposure of 40 Joule/cm$^2$ was used in our subsequent experiments.
Irradiation-based modulation of \( \alpha \)-SMA protein expression

In unstimulated DD and NPF fibroblasts blue light caused no significant effect on \( \alpha \)-SMA protein expression (Fig 2A and 2C and Fig 3). TGF-\( \beta \)1 treatments over 72 h and blue light (40 Joule/cm\(^2\)) decrease \( \alpha \)-SMA protein expression in DD fibroblasts significantly (Fig 2B and Fig 3). The effect was even stronger after a 120 h incubation time (Fig 2D and Fig 3).

A blue light irradiation with the intensity of 38 mW/cm\(^2\) and with a radiant exposure of 40 Joule/cm\(^2\) with a narrow-band LED, which emits light of the wavelength \( \lambda = 453 \) nm down regulated \( \alpha \)-SMA protein expression significantly in DD fibroblasts after TGF-\( \beta \)1-treatment. The \( \alpha \)-SMA protein expression decreases the longer the treatment continues.

ROS expression after irradiation

From the beginning on DD fibroblasts have generated more ROS in tendency. Absolute ROS increased after irradiation in both fibroblasts’ groups NPF and DD. Control DD fibroblasts as well as irradiated ones generated more ROS compared to their appropriate NPF controls (Fig 4A). So, ROS expression measured with DHR123 rose continuously over 24 h. The ROS expression of irradiated NPF fibroblasts was significantly enhanced after 24 h, compared to irradiated NPF fibroblasts after 0.5, 1, and 2 h. After 24 h irradiated NPF fibroblasts showed a significantly higher amount of ROS in comparison to not irradiated NPF fibroblasts.

Furthermore, the use of blue light appears to elevate ROS -especially in Dupuytren’s fibroblasts-.

The apoptotic potential of DD fibroblasts

In untreated DD and NPF fibroblasts apoptosis rate was similar. Same effect could be shown if fibroblasts were treated with STS, even if the rate of apoptosis was higher overall. If cells were
treated daily with TGF-β1, the rate of apoptosis was significantly higher in DD fibroblasts, compared to NPF. Overall the results demonstrated that the treatment with blue light did not induce apoptosis. And the treatment with blue light and an additional application with TGF-β1 slightly reduced apoptosis in NPF and DD fibroblasts. So, in conclusion the therapeutic benefit of blue light is not regulated via apoptosis.

Discussion

Partial fasciectomy, enzyme injection, and needle fasciotomy are today’s treatment options for Dupuytren’s disease (DD) [24]. Due to the limitations of these treatment methods, researchers and clinicians are forced to search for better therapies. The usage of photobiomodulation strategies may provide a solution to this problem, because the combination of intensity, radiant...
exposure, wavelength, and their specific interaction with the absorption properties of the target tissue can have a therapeutic effect [25]. So, blue light (50 mW/cm²; 15, 30 Joule/cm²; λ = 420 nm) has already been proven to inhibit TGF-β1-induced α-SMA expression in differentiated human dermal fibroblasts [23]. Especially in Dupuytren’s fibroblasts, the increased and pathological differentiation of fibroblasts to myofibroblasts, is associated with an elevated α-SMA-expression. Hence, in this study the therapeutic benefit of blue light irradiation on Dupuytren’s fibroblasts was analyzed for the first time.

Dupuytren’s fibroblasts and normal palmar fascia (NPF) fibroblasts were irradiated (with a wavelength of λ = 453 nm and an intensity of 38 W/cm²), and as the most promising radiant exposure 40 Joule/cm² was used for the subsequent experimental procedures (Fig 1). Since the

---

**Fig 3.** A representative western blot analysis showing α-SMA expression of DD fibroblasts with TGF-β1 stimulation (+), without stimulation (-), with (+) or without (-) irradiation at 453 nm (either about 72 or 120 h). The untreated DD control harvested after 24 h C α-SMA expression was measured and normalized to GAPDH.

https://doi.org/10.1371/journal.pone.0209833.g003

---

**Fig 4.** Analysis of ROS in DD compared to NFF. A ROS generation of irradiated (60 Joule/cm²) or resting NPF (n = 5) compared to DD fibroblasts (n = 5). Measurement after 0.5, 1, 2, 4, and 24 h with DHR123. Absolute ROS expression of fibroblasts increased after irradiation. Both irradiated and non-irradiated fibroblasts of DD always revealed the highest ROS expression compared to irradiated and non-irradiated NPF fibroblasts (Fig 4A). ROS expression rose over 24 h continuously. Nevertheless, the ROS expression of irradiated NPF fibroblasts was significantly enhanced after 24 h compared to irradiated NPF fibroblasts after 0.5, 1, and 2 h. The irradiated NPF fibroblasts showed a significant boost after 24 h compared to non-irradiated NPF fibroblasts.

https://doi.org/10.1371/journal.pone.0209833.g004
irradiation with 40 Joule/cm² hardly inhibited the cell viability/metabolic activity of Dupuytren’s fibroblasts and especially of NPF, which is required for a therapeutic application of blue light in DD.

Up to 60 Joule/cm² irradiation, metabolic activity of Dupuytren’s fibroblasts was increased compared to NPF (Fig 1). This result is supported by several research groups, demonstrating that the proliferation of Dupuytren’s fibroblasts is elevated compared to control cells by the activation of the TGF-β/SMAD- and extracellular signal-regulated kinase (ERK) signaling [26], and an autocrine regulation through epidermal growth factor (ERBB)-2 and insulin growth factor (IGF)-1 receptors, as well as Akt-signaling [27].

Furthermore, it could be demonstrated that after 24 h cell viability of Dupuytren’s fibroblasts appeared to be considerably higher than NPF fibroblasts viability (Fig 1, 0 Joule), although in both groups the same cell number was seeded. This is a remarkable result, because “cell viability” was indirectly determined by measuring metabolic activity (CellTiter-Blue Assay). These results and the elevated ROS amount in Dupuytren’s fibroblasts compared with NPF fibroblasts shown in Fig 4 indicate, that Dupuytren’s fibroblasts have, by nature, a higher metabolism than the respective controls from patients suffering from carpal tunnel syndrome. And this may be an explanation why DD fibroblasts were more affected by the blue light irradiation, than the NPF fibroblasts. This assumption is solidified by the findings, that the expression of the housekeeping gene Glyeraldehyd-3-phosphat-Dehydrogenase (GAPDH) is increased in Dupuytren’s fibroblasts (data not shown) treated with TGF-β1 compared to NPF.

It could be ascertained that the exposure with λ = 453 nm does not change α-SMA expression in neither resting NPF nor in Dupuytren’s fibroblasts after 72 h and 120 h (Fig 2A and 2C and Fig 3). (It has to be considered, that only one donor causes those high standard deviations of irradiated Dupuytren’s fibroblasts.) Furthermore, to tighten standard deviation is difficult in Dupuytren’s research, because it is a multifactorial disease, and tissue specimen were not classified in regard to secondary diseases like diabetes, alcohol abuse, smoking, or just relapse. And to exacerbate these issues, the amount of tissue specimen (quantity of Dupuytren’s tissue specimen is low, but quantity of NPF tissue specimen is even lower) is not sufficient for technical replicates. But interestingly, if NPF were pretreated with TGF-β1 and irradiated with blue light, α-SMA protein expression was significantly diminished after 72 h, and this effect could be further enhanced after 120 h (Fig 2B and 2D and Fig 3). And special attention should be paid to the observation, that in TGF-β1 pretreated Dupuytren’s fibroblasts, α-SMA protein expression is significantly decreased by the blue light irradiation (Fig 2B and 2D and Fig 3).

It was assumed that the effect of the blue light irradiation is mediated by the generation of reactive oxygen species (ROS), such as singlet oxygen [28] or hydrogen peroxide (H₂O₂) [23]. And it was presumed, that photons interact with endogenous photoreceptor molecules, such as lipofuscin [29], cytochrome c oxidase [30], or flavin-based photo sensors [31]. In fact, Taflinski et al. determined that an irradiation with blue light (420 nm) led to an increased amount of ROS as well as α-SMA reduction, and the induction of intracellular oxidative stress was thought to be the cause for that [23]. These findings intrigued the present work to examine ROS in Dupuytren’s fibroblasts after the exposure to blue light. It could be assured, that Dupuytren’s fibroblasts tended to produce more ROS, which was detected (after 24 h seeding the same cell number) with DHR123, an unspecific ROS indicator (Fig 4). As mentioned before, this might be due to a higher metabolic activity of Dupuytren’s fibroblasts. And the irradiation in general induced increased ROS formation in DD and NPF fibroblasts in tendency (Fig 4). As postulated by Taflinski et al., we assume that repeated smaller doses of blue light irradiation induce subtoxic levels of intracellular oxidative stress (as shown here in Fig 4), which may induce energy-consuming cellular responses against oxidative stress, which may in turn result in a proliferation stop and interfere with myofibroblasts differentiation [23].
The excessive proliferation of myofibroblasts [32], combined with the diminished capability to undergo apoptosis [3, 4] in the proliferative and involutional stages are further characteristics determining the phenotype of DD. In order to proof, if the beneficial effect of blue light is mediated via apoptosis, and to investigate, if photobiomodulation makes Dupuytren’s disease fibroblasts (n = 4–6) more sensitive to apoptotic signals, the apoptotic capacity of (Dupuytren’s) fibroblasts was analysed after five days of irradiation and TGF-β1 treatment. To induce cell death staurosporine was used. Interestingly, it was measured that in Dupuytren’s fibroblasts incubated for five days with TGF-β1, apoptosis was significantly higher compared to NPF (Fig 5). But even more important is, that blue light irradiation with a wavelength of $\lambda = 453$ nm, intensity of 38 mW/cm$^2$, and a radiant exposure of 40 Joule/cm$^2$, does not reinforce apoptosis in neither Dupuytren’s fibroblasts nor in NPF fibroblasts.

The present study highlights, that the application of blue light could inhibit the differentiation of Dupuytren’s fibroblasts into myofibroblasts and the accompanied $\alpha$-SMA expression. Our data suggests that, especially in the proliferative and involutional stages, or after surgery when TGF-β concentration is increased, an irradiation with blue light could be beneficial. Consequently, blue light therapy could be a promising therapy option for DD, and especially for relapse prevention. Our results suggest that the usage of blue light is a promising tool for therapeutic treatment for further fibrotic diseases such as keloids, hypertrophic scarring, and scleroderma.
Acknowledgments

Data were provided from the doctoral thesis submitted by Annika Borgschulze, manufactured in the Department of Trauma and Handsurgery in the Faculty of Medicine of the Heinrich-Heine-University Düsseldorf, Germany. We thank Jutta Schneider, Christa-Maria Wilkens, and Samira Seghrouchni for technical assistance. We also thank Prof. Dr. Matthias Born and Dr. rer. nat. Jörg Liebmann (Philips GmbH, Innovative Technologies, Aachen, Germany) for providing the prototype of the blue light-emitting device used in our experiments.

Author Contributions

Conceptualization: Tim Lötgers, Vera Grotheer.
Data curation: Benita Sahlender, Vera Grotheer.
Investigation: Benita Sahlender.
Methodology: Julia Krassovka, Annika Borgschulze.
Supervision: Joachim Windolf, Vera Grotheer.
Writing – original draft: Vera Grotheer.

References

1. Picardo NE, Khan WS. Advances in the understanding of the aetiology of Dupuytren's disease. The surgeon: journal of the Royal Colleges of Surgeons of Edinburgh and Ireland. 2012; 10(3):151–8. Epub 2012/02/03. https://doi.org/10.1016/j.surge.2012.01.004 PMID: 22297148.

2. Shih B, Bayat A. Scientific understanding and clinical management of Dupuytren disease. Nature reviews Rheumatology. 2010; 6(12):715–26. Epub 2010/11/10. https://doi.org/10.1038/nrrheum.2010.180 PMID: 21060335.

3. Jemec B, Grobbelaar AO, Wilson GD, Smith PJ, Sanders R, McGrathner DA. Is Dupuytren's disease caused by an imbalance between proliferation and cell death? Journal of hand surgery (Edinburgh, Scotland). 1999; 24(5):511–4. Epub 1999/12/22. https://doi.org/10.1054/jhsb.1999.0251 PMID: 10597921.

4. Meek RM, McLellan S, Reilly J, Crossan JF. The effect of steroids on Dupuytren's disease: role of programmed cell death. Journal of hand surgery (Edinburgh, Scotland). 2002; 27(3):270–3. Epub 2002/06/21. https://doi.org/10.1054/jhsb.2001.0742 PMID: 12074617.

5. Wilutzky B, Berndt A, Katenkamp D, Koshmehl H. Programmed cell death in nodular palmar fibromatosis (Morbus Dupuytren). Histology and histopathology. 1998; 13(1):67–72. Epub 1998/02/26. https://doi.org/10.14670/HH-13.67 PMID: 9476635.

6. Luck JV. Dupuytren’s contracture; a new concept of the pathogenesis correlated with surgical management. The Journal of bone and joint surgery American volume. 1959; 41-a(4):635–64. Epub 1959/06/01. PMID: 13664703.

7. Tomasek JJ, Schultz RJ, Episalla CW, Newman SA. The cytoskeleton and extracellular matrix of the Dupuytren’s disease "myofibroblast": an immunofluorescence study of a nonmuscle cell type. The Journal of hand surgery. 1986; 11(3):365–71. Epub 1986/05/01. PMID: 3519746.

8. Bisson MA, McGrathner DA, Mudera V, Grobbelaar AO. The different characteristics of Dupuytren’s disease fibroblasts derived from either nodule or cord: expression of alpha-smooth muscle actin and the response to stimulation by TGF-beta1. Journal of hand surgery (Edinburgh, Scotland). 2003; 28(4):351–6. Epub 2003/07/10. PMID: 12849947.

9. Vaughan MB, Howard EW, Tomasek JJ. Transforming growth factor-beta1 promotes the morphological and functional differentiation of the myofibroblast. Experimental cell research. 2000; 257(1):180–9. Epub 2000/06/15. https://doi.org/10.1006/excr.2000.4869 PMID: 10854066.

10. Roberts AB, Sporn MB, Assoian RK, Smith JM, Roche NS, Wakefield LM, et al. Transforming growth factor type beta: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. Proceedings of the National Academy of Sciences of the United States of America. 1986; 83(12):4167–71. Epub 1986/06/01. PMID: 2424019; PubMed Central PMCID: PMCPC0323692.

11. Border WA, Noble NA. Transforming growth factor beta in tissue fibrosis. The New England journal of medicine. 1994; 331(19):1286–92. Epub 1994/11/10. https://doi.org/10.1056/NEJM199411103311907 PMID: 7935686.
12. Gudmundsson KG, Arngrimsson R, Jonsson T. Eighteen years follow-up study of the clinical manifestations and progression of Dupuytren’s disease. Scandinavian journal of rheumatology. 2001; 30(1):31–4. Epub 2001/03/17. PMID: 11252689.

13. Shaw RB Jr., Chong AK, Zhang A, Hentz VR, Chang J. Dupuytren’s disease: history, diagnosis, and treatment. Plastic and reconstructive surgery. 2007; 120(3):44e–54e. Epub 2007/08/19. https://doi.org/10.1097/01.prs.0000278455.63546.03 PMID: 17700106.

14. Badalamente MA, Hurst LC. Enzyme injection as nonsurgical treatment of Dupuytren’s disease. The Journal of hand surgery. 2000; 25(4):629–36. Epub 2000/07/27. https://doi.org/10.1053/jhsu.2000.6918 PMID: 10913202.

15. Stromberg J, Ibsen-Sorensen A, Friden J. Comparison of Treatment Outcome After Collagenase and Needle Fasciotomy for Dupuytren Contracture: A Randomized, Single-Blinded, Clinical Trial With a 1-Year Follow-Up. The Journal of hand surgery. 2016. Epub 2016/07/31. https://doi.org/10.1016/j.jhsa.2016.06.014 PMID: 27473921.

16. Shnitkind E, Yaping E, Geen S, Shalita AR, Lee WL. Anti-inflammatory properties of narrow-band blue light. Journal of drugs in dermatology: JDD. 2006; 5(7):605–10. Epub 2006/07/27. PMID: 16865864.

17. Wang Y, Huang YY, Wang Y, Lyu P, Hamblin MR. Photobiomodulation (blue and green light) encourages osteoblastic-differentiation of human adipose-derived stem cells: role of intracellular calcium and light-gated ion channels. Scientific reports. 2016; 6:33719. Epub 2016/09/22. https://doi.org/10.1038/srep33719 PMID: 27650508; PubMed Central PMCID: PMCPMC5030629.

18. Shnitkind E, Yaping E, Geen S, Shalita AR, Lee WL. Anti-inflammatory properties of narrow-band blue light. Journal of drugs in dermatology: JDD. 2006; 5(7):605–10. Epub 2006/07/27. PMID: 16865864.

19. Fischer MR, Abel M, Lopez Kostka S, Rudolph B, Becker D, von Stebut E. Blue light irradiation suppresses dendritic cells activation in vitro. Experimental dermatology. 2013; 22(8):558–60. Epub 2013/07/25. https://doi.org/10.1111/exd.12193 PMID: 23879817.

20. Ohara M, Kawashima Y, Kitajima S, Mitsuoka C, Watanabe H. Blue light inhibits the growth of skin tumors in the v-Ha-ras transgenic mouse. Cancer science. 2003; 94(2):205–9. Epub 2003/04/24.

21. Adamskaya N, Dungel P, Mittermayer R, Hartinger J, Feichtinger G, Wassermann K, et al. Light therapy by blue LED improves wound healing in an excision model in rats. Injury. 2011; 42(9):917–21. https://doi.org/10.1016/j.injury.2010.03.023 PMID: 20081819.

22. Gorgidze LA, Oshemkova SA, Vorobjev IA. Blue Light Inhibits Mitosis in Tissue Culture Cells. Bioscience Reports. 1998; 18(4):215–24. https://doi.org/10.1023/a:1020104914726 PMID: 9877234.

23. Tafelinski L, Demir E, Kauczok J, Fuchs PC, Born M, Suschek CV, et al. Blue light inhibits transforming growth factor-beta1-induced myofibroblast differentiation of human dermal fibroblasts. Experimental dermatology. 2014; 23(4):240–6. Epub 2014/02/19. https://doi.org/10.1111/exd.12353 PMID: 24533842.

24. Feldman G, Rozen N, Rubin G. Dupuytren’s Contracture: Current Treatment Methods. The Israel Medical Association journal: IMAJ. 2017; 19(10):648–50. Epub 2017/11/06. PMID: 29103246.

25. Niemz MH. Laser-Tissue Interactions: Fundamentals and Applications: Springer; 2007.

26. Krause C, Kloen P, Ten Dijke P. Elevated transforming growth factor beta and mitogen-activated protein kinase pathways mediate fibrotic traits of Dupuytren’s disease fibroblasts. Fibrogenesis & tissue repair. 2011; 4(1):14. Epub 2011/06/30. https://doi.org/10.1186/1755-1536-4-14 PMID: 21711521; PubMed Central PMCID: PMCPMC3148569.

27. Kraljevic Pavelic S, Sedic M, Hock K, Vucinic S, Jurisic D, Gehrig P, et al. An integrated proteomics approach for studying the molecular pathogenesis of Dupuytren’s disease fibroblasts. Fibrogenesis & tissue repair. 2011; 4(1):14. Epub 2011/06/30. https://doi.org/10.1186/1755-1536-4-14 PMID: 21711521; PubMed Central PMCID: PMCPMC3148569.

28. Oplander C, Hidding S, Werners FB, Born M, Pallua N, Suschek CV. Effects of blue light irradiation on human dermal fibroblasts. Journal of photochemistry and photobiology B, Biology. 2011; 103(2):118–25. Epub 2011/03/23. https://doi.org/10.1016/j.jphotobiol.2011.02.016 PMID: 21421326.

29. Boulton M, Rozanowska M, Rozanowski B, Wess T. The photoactivity of ocular lipofuscin. Photobiological sciences: Official journal of the European Photobiology Association and the European Society for Photobiology. 2004; 3(8):759–64. Epub 2004/08/06. https://doi.org/10.1039/b400108g PMID: 15295632.

30. Karu T, Pyatibrat L, Kalendo G. Irradiation with He—Ne laser can influence the cytotoxic response of HeLa cells to ionizing radiation. International journal of radiation biology. 1994; 65(6):691–7. Epub 1994/06/01. PMID: 7912719.
31. Losi A. Flavin-based Blue-Light photosensors: a photobiophysics update. Photochemistry and photobiology. 2007; 83(6):1283–300. Epub 2007/11/22. https://doi.org/10.1111/j.1751-1097.2007.00196.x PMID: 18028200.

32. Rayan GM. Dupuytren disease: anatomy, pathology, presentation, and treatment. The Journal of bone and joint surgery American volume. 2007; 89.