PROCEEDINGS OF SPIE

SPIEDigitalLibrary.org/conference-proceedings-of-spie

Blue light effects in human keloid fibroblasts

Giada Magni, Federica Cherchi, Elisabetta Coppi, Marco Fraccalvieri, Francesca Tatini, et al.

Giada Magni, Federica Cherchi, Elisabetta Coppi, Marco Fraccalvieri, Francesca Tatini, Irene Fusco, Roberto Pini, Anna Maria Pugliese, Felicita Pedata, Antongiulio Mangia, Stefano Gasperini, Francesco Pavone, Duccio Rossi Degl'Innocenti, Cristina Tripodi, Domenico Alfieri, Lorenzo Targetti, Francesca Rossi, "Blue light effects in human keloid fibroblasts," Proc. SPIE 10861, Mechanisms of Photobiomodulation Therapy XIV, 1086107 (7 March 2019); doi: 10.1117/12.2509504

SPIE.

Event: SPIE BiOS, 2019, San Francisco, California, United States

Blue light effects in human keloid fibroblasts

Giada Magni*[a], Federica Cherchi[b], Elisabetta Coppi[b], Marco Fraccalvieri[c], Francesca Tatini[a], Irene Fusco[b], Roberto Pini[a], Anna Maria Pugliese[b], Felicita Pedata[b], Antongiulio Mangia[c], Stefano Gasperini[d], Francesco S. Pavone[e,f,g,h], Duccio Rossi Degl'Innocenti[h], Cristina Tripodi[h], Domenico Alfieri[h], Lorenzo Targetti[h], Francesca Rossi[a].

[a]Istituto di Fisica Applicata, CNR, Via Madonna del Piano 10, Sesto Fiorentino, Florence, Italy 50019; [b]Dept. NEUROFARBA, Division of Pharmacology and Toxicology, University of Florence, Viale Pieraccini 6, Florence, Italy 50139; [c]A.O.U. Città della Salute e della Scienza di Torino, Ospedale San Lazzaro, Corso Bramante 88, Torino, Italy 10126; [d]Medical Advisor SaS, Via Cavour 4, Pisa, Italy 56127; [e]National Institute of Optics, National Research Council, Via Nello Carrara 1, Sesto Fiorentino, Italy 50019; [f]European Laboratory for Non-linear Spectroscopy (LENS), Via Nello Carrara 1, Sesto Fiorentino, Italy 50019; [g]Department of Physics, University of Florence, Via G. Sansone 1, Sesto Fiorentino, Italy 50019; [h]EmoLed S.r.l., Via di Rimaggio 141, Sesto Fiorentino, Florence, Italy 50019

ABSTRACT

Keloids scars are an abnormal overgrowth of fibrotic tissue in response to an injury. The current treatments show several limits and do not represent a definitive solution or a prevention protocol. In a preliminary study, we irradiated two samples of human keloid fibroblasts with a Blue LED light, evidencing a possible modulation of their activity *in vitro*. In the current study, we use primary fibroblasts cultures from eight keloid tissues (from seven selected patients undergoing aesthetic surgery). The fibroblasts were irradiated with a Blue LED light and the treatment time was varied in the range 5÷60s. After irradiation, cell metabolism and cell proliferation were studied by the use of two colorimetric tests, CCK-8 and SRB (Sigma-Aldrich, Saint Louis, Missouri, USA). The analysis was performed 24 and 48h after the treatment. We thus evidenced that the Blue LED light induces a modulation of the fibroblasts metabolism; this effect is particularly evident at 30s irradiation time. We also evaluated the impact of Blue LED light on membrane currents by performing whole-cell patch-clamp recordings. We observed a significant increase of voltage dependent outward currents activated by a depolarizing ramp-protocol upon Blue LED light irradiation (@30s exposure). This effect was maintained in K^+ free-solutions, thus ruling out the involvement of K^+ channels. In conclusion, we demonstrated that the Blue LED light has a photobiomodulation effect in fibroblasts from human keloids. This effect can be proposed as a possible treatment of the wound site in human patients to prevent keloid scars occurrence.

Keywords: Wound Healing, Blue LED light, keloid, fibroblasts.

1. INTRODUCTION

The wound healing process is made up of four correlated phases: haemostasis, inflammation, proliferation and remodeling phase. At the end of the last phase, the scar tissue is generated and remains visible on the surface of the skin for the next few years, when the remodeling of the underlying tissue will go further. In relation to the physiological or abnormal wound healing mechanism, the scar tissue can lead to different outcomes, healthy or pathological, from unaesthetic results to severe reduction of movements. The physiological processes underlying wound healing are well known in the literature [1-3], while the events leading to hypertrophic or keloid scar formation are still unclear: no specific growth factors or proteins are identified as responsible for their development.

*g.magni@ifac.cnr.it; phone 055 5225327; http://bnlab.ifac.cnr.it

Mechanisms of Photobiomodulation Therapy XIV, edited by Michael R. Hamblin, James D. Carroll, Praveen Arany,
Proc. of SPIE Vol. 10861, 1086107 · © 2019 SPIE · CCC code: 1605-7422/19/$18 · doi: 10.1117/12.2509504

Keloids scars are described as a benign fibro-proliferative dermal tumor with no malignant potential. The anatomic sites typically involved in keloids formation are usually chest, shoulders, upper back, back of the neck and earlobes [4]. They are unique in humans, and they seem to present a preferential incidence in dark-skinned people [5], with a high show up in specific ethnic populations, such as Afro-Americans, and in individuals with familiarity [6-7]. The keloid tissue is characterized by a high level of collagen deposition in the site of the lesion: this produces a scar extending beyond the boundaries of the original wound. In keloids, the collagen fibres are larger, thicker and wavier than those in normal skin and they show a random orientation. The exaggerated synthesis of collagen is probably due to the increase in fibroblasts density and to their higher proliferation index, which arise the production of collagen and components of extracellular matrix (ECM) in respect to the normal skin [8-9].

Currently, keloid treatments can be divided into three main categories: i) non-invasive medical therapies, ii) surgical and other invasive therapies, and iii) new therapies under investigations [10]. To the best of our knowledge, there is no pharmacological treatment to control (or prevent) keloid formation. Among the principal non-invasive medical therapies are Pressure Garments Therapy (PGT), Silicone Gel Sheeting, Onion Extract and Heparin Gel, Intralesional Corticosteroid Injections, 5-Fluorouracil (5-FU), Bleomycin and Mitomycin C. Intralesional corticosteroid injections represent the main treatment for keloids scars, but in almost the totality of cases it is not a definitive solution. The second category of keloid treatment approaches concerns surgical and other invasive therapies: surgery, cryotherapy and radiotherapy. Surgical excision of keloid scarring is a very popular option; however, if surgical excision is provided alone, the recurrence rate of the scar will be high [11]. Intralesional cryosurgery is used to remove keloid scar tissue with minimal dermal injury. Cryotherapy is the most popular classic treatment for both keloid and hypertrophic scars, but a common side effect is the permanent hypopigmentation. As concern the new therapies under investigations, cell and tissue-based therapies and physical treatments are recently proposed [12]. Physical treatments regained an important role in the management of the wounds, and new technologies and devices have been developed to address this issue. The approach that we present in this paper is one of these new physical treatment.

In preliminary *in vivo* studies on rodent models, we demonstrated that a 30s treatment 22 J/cm^2 Blue LED light (@ 420 nm) induces an early increase of inflammatory infiltrate with improved healing in superficial abrasions, and also an early activation of fibroblasts and myofibroblasts. [13-23]. In the present study, we used an *in vitro* model of primary human dermal fibroblast cultures obtained from human keloid samples in order to investigate whether human derma keloid fibroblasts are influenced by a Blue LED light treatment at different irradiation times [24]. In particular, we studied the effects of Blue LED light on cell metabolism, cell proliferation and membrane ionic currents.

2. MATERIALS AND METHODS

2.1 Primary cultures cell

Human Keloid Fibroblasts Cell (HKFCs) cultures were set up from eight keloids tissues by seven patients after aesthetic surgeries performed at the AOU Città della Salute e della Scienza di Torino. The study was approved by the Hospital Ethical Board. The keloid tissue was used within 5h from the excision and during this time it was maintained in Dulbecco Modified Eagle Medium (DMEM) at a temperature of 4°C. Each keloid was treated according to the literature [25]. HKFCs were prepared with a surgical punch in order to obtain sections of approximately 4-6 mm in diameter. Sections were collected in scratch-Petri dishes and maintained in DMEM low glucose medium (Pan-React Applichem, Milan, Italy) supplemented with 10% Foetal Bovine Serum (FBS), 1% of Glutamine and 1% Penicillin-Streptomycin (EuroClone, Milan, Italy), keeping it at 37°C and 5% CO_2 (Figure 1A). Within two weeks following the preparation, fibroblasts migrated from the tissue at the bottom of the dish (Figure 1B-C-D). All the experiments were performed between the second and the fifth passages in culture; during this time, the cultures were maintained under standard culture conditions and the medium was refreshed every 48h. When possible, fibroblasts from the wound periphery were also harvested. After this procedure, HKFCs were treated with the Blue LED light emitting for of 5, 10, 20, 30, 45, 60s irradiation times. The LED (Figure 1E) is coupled with a 1.2m long flexible polymeric fiber and mounted on a benchtop device equipped with a touchscreen to control all the irradiation parameters. The illuminated area corresponds to a 5 mm radius circle with a resulting power density of about 1.2W/cm^2, homogeneously distributed on the spot area as from a top-hat source.

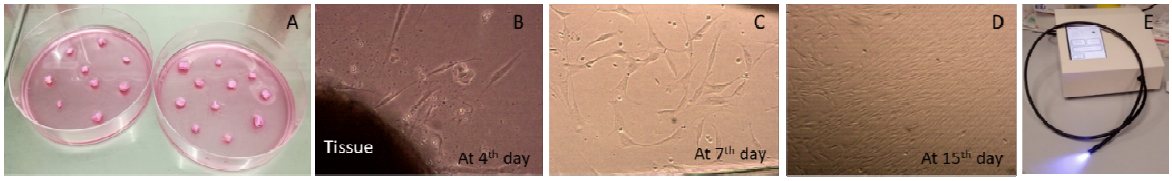

Figure 1. A: The sections of keloid tissue after surgical punch use. B-C-D: Images obtained from the optical microscope (Eurotek Orma, INV100T) of Human Keloid Fibroblasts Cells (HKFCs) at different time-points after culture set up, B and C 20x, D 10x. E: The Blue LED light device used for the irradiation of fibroblasts.

2.2 Confocal microscopy

Confocal microscopy (Leica SP8, Mannheim, Germany) was used to characterize the cell cultures. HKFCs were seeded in treated-glass bottom dishes (Ibidi, GMBH, Martinsried, Germany) and an immunostaining protocol was performed. Cells were permeabilized using 0.25% Triton X100 (Sigma-Aldrich, St. Louis, MO, USA) in Phosphate Buffer Saline (PBS) (Pan-React Applichem, Milan, Italy). The blocking of unspecific sites was obtained by 1% of Albumine Bovine Serum (BSA) (Sigma-Aldrich, St. Louis, MO, USA) in PBST (PBS in which added 0,1% of Tween20) (Sigma-Aldrich, St. Louis, MO, USA). Anti-Alpha-smooth muscle actin (α-SMA), anti-HSP-47 and anti-cytokeratin were diluted 1:250, while anti-type I collagen was diluted 1:500. All secondary antibodies, AlexaFluor555, AlexaFluor488 and AlexaFluor647, were diluted 1:400. All the primary and the secondary antibodies were purchased from AbCam (UK, Cambridge), while DAPI (used to stain cell nuclei) was purchased from Sigma-Aldrich, (St. Louis, MO, USA). All the markers and fluorescent antibodies were used according to the manufacturer instructions.

2.3 Colorimetric tests

Cell metabolism was evaluated using Cell Counting Kit Assay (CCK-8) purchased from Sigma-Aldrich (St. Louis, MO, USA). CCK-8 use WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt), which produces a water-soluble formazan dye upon bioreduction in the presence of an electron carrier, 1-Methoxy PMS. WST-8 is bioreduced by cellular dehydrogenases to an orange formazan product that is soluble in tissue culture medium. The cell proliferation was measured by the use of SRB (Sulforhodamine B based Assay) purchased from Sigma-Aldrich (St. Louis, MO, USA) which binds stoichiometrically to proteins under mildly acidic conditions and then can be extracted using basic conditions; thus, the amount of bound dye can be used as a proxy for cell mass, which can then be extrapolated to measure cell proliferation [26-27-28]. $5x10^3$ HKFCs were seeded in a multiwell plate (Corning, Sigma Aldrich, Milan, Italy) in triple of wells were irradiated with the Blue LED light for 5, 10, 20, 30, 45 and 60s. For each experiment, three wells were left untreated, to be used as a control. The power of radiation was regulated at 235mW, while the irradiation was performed by keeping the fiber tip 1cm far from the bottom of the well. All the treatments were performed in Serum Free Medium (SFM) without red phenol in order to avoid light absorption from cell medium. Tests analysis were performed 24h and 48h after irradiation: the absorbance at 450nm and 570nm for CCK-8 and SRB respectively was read with an automatic microplate absorbance reader (LT-4000 Labtech, Heathfield, East Sussex, England) and the values were processed by specific commercial software (GraphPad Prism 8th edition).

2.4 Electrophysiology experiments

Whole-cell patch-clamp recordings were performed in -60 mV clamped-cells as previously described [29-30]. The following solutions were used: standard K^+-containing extracellular solution (mM): NaCl 147; KCl 4; $MgCl_2$ 1; $CaCl_2$ 2; HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) 10; D-glucose 10 (pH 7.4 with NaOH). Standard K^+-based pipette solution (mM): K-gluconate 130; NaCl 4.8; KCl 10; $MgCl_2$ 2; $CaCl_2$ 1; Na_2-ATP 2; Na_2-GTP 0.3; EGTA 3; HEPES 10 (pH 7.4 with KOH). For K^+-replacement experiments, extracellular and intracellular K^+ were substituted with equimolar Cs^+. Keloid's cells were grown in culture as described above. Confluent cells were suspended, plated into 13 mm diameter coverslips and allowed to adhere for at least 2 h before starting electrophysiological recordings. Each cover-slip was then transferred to a 1 ml recording chamber mounted on the platform of an inverted microscope (Olympus CKX41, Milan, Italy) and superfused at a flow rate of 1.5 ml/min by a three-way perfusion valve controller (Harvard Apparatus). Borosilicate glass electrodes (Harvard Apparatus, Holliston, MA) were pulled with a Sutter Instruments puller (model P-87) to a final tip resistance of 1–3 MΩ. Data were acquired with an Axopatch 200B amplifier (Axon Instruments, CA), low-pass filtered at 10 kHz, stored and analysed with pClamp 9.2 software (Axon Instruments, CA). All the experiments were carried out at room temperature (RT: 20–22°C). Series resistance (Rs), membrane resistance (Rm), and membrane capacitance (Cm) were routinely measured by fast hyperpolarizing voltage

pulses (from -60 to -70 mV, 40 ms duration). Only cells showing a stable Cm and Rs before, during, and after light application were included in the analysis. Immediately after seal breaking-through, cell resting membrane potential was determined by switching the amplifier to the current-clamp mode. A voltage ramp protocol (800 ms depolarization from -80 to +80 mV) was used to evoke overall voltage-dependent currents before, during, and after light treatments. The equilibrium potential for K^+ (E_K) and Cl^- (E_{Cl}) ions was calculated by applying Nernst equation as follow: E=E0-(RT/nF) ln([K^+]o/[K^+]); were R = ideal gas constant = 8.314 J/mol(K); F = Faraday's constant = 95,484.56 C/mol; ln([K^+]o/[K^+]in) = natural log of the ion concentration quotient across cell membrane. Data were expressed as mean ± SEM.

3. RESULTS AND DISCUSSION

3.1 Confocal microscopy

Microscopy analysis evidenced that fibroblasts and myofibroblasts are present in the entire keloid tissue (figures 2A-C-D), while only in the superficial derma we identified also keratinocytes (figure 2B). The confocal images were acquired using LAS-X Software (Leica, Mannheim, Germany), while optical images (Figure 2A) were acquired with an inverted microscope (Eurotek Orma, INV100T). Both types of images were processed with an open source software (ImageJ, imagej.nih.gov/ij/).

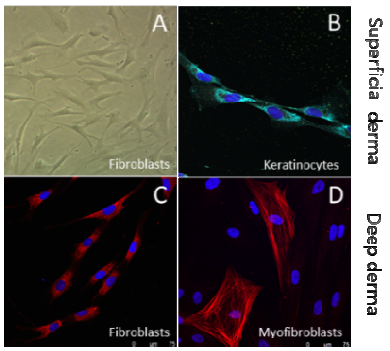

Figure 2. A: Cultured keloid fibroblasts at optical microscope (10x); B: Keratinocytes in superficial derma (cyano = cytokeratin), obtained with confocal microscopy (63x). C: Type I collagen and D: Alpha Smooth Muscle Actin (α-SMA) (63x), corresponding to fibroblasts and myofibroblasts, respectively. Cellular nuclei in blue (DAPI).

3.2 Colorimetric assays results

We conducted 132 tests (66 CCK-8 and 66 SRB) at 24h and 88 tests (44 CCK-8 and 44 SRB) at 48h after Blue LED light irradiation on keloid fibroblast cultures obtained from superficial and deep derma of keloid tissues and from boundary tissues. Data on CCK-8 test in superficial and deep derma fibroblasts demonstrated that cell metabolism decreases at increasing irradiation times, 24h after the Blue LED light treatment. The same effect was even more evident 48h after the treatment (Figure 3). The decrease in cell metabolism was significant in a time window of 10÷60s at 24h after irradiation and 20÷60s at 48h. Only at 48h, 5s of irradiation induces a weak increase of cell metabolic activity, when compared to untreated cells. Fibroblasts proliferation, evaluated by SRB assay, remains constant at the irradiation times until 30s, while at 45s and 60s there is a significant decrease, when evaluated 24h after the treatment. On the other hand, 48h after irradiation, cell proliferation decreases at increasing irradiation times, with significant values observed from 20s to 60s (Figure 4).

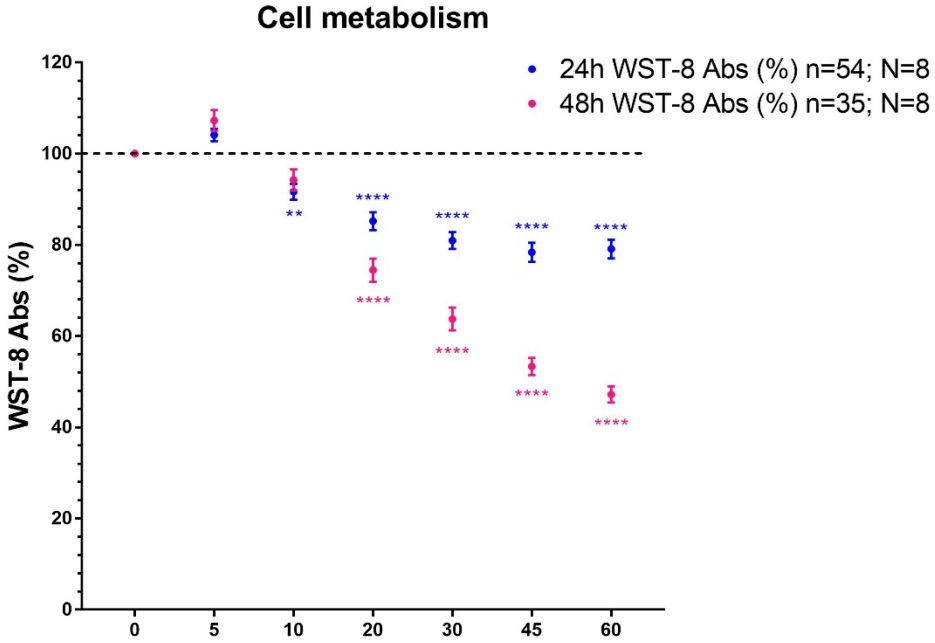

Figure 3. CCK-8 test results in keloid fibroblasts (N = 8 keloids from seven patients). Cell metabolism was evaluated 24h (blue) and 48h (violet) after treatment. Data are expressed as mean ± SEM, n = 54 at 24h and n = 35 at 48h after treatment. Each measure is repeated in triplicate. Significant values: **P ≤ 0.01; ****P ≤ 0.0001 *vs* control (0s of irradiation with Blue LED light), one-way ANOVA followed by Dunnett's multiple comparison test.

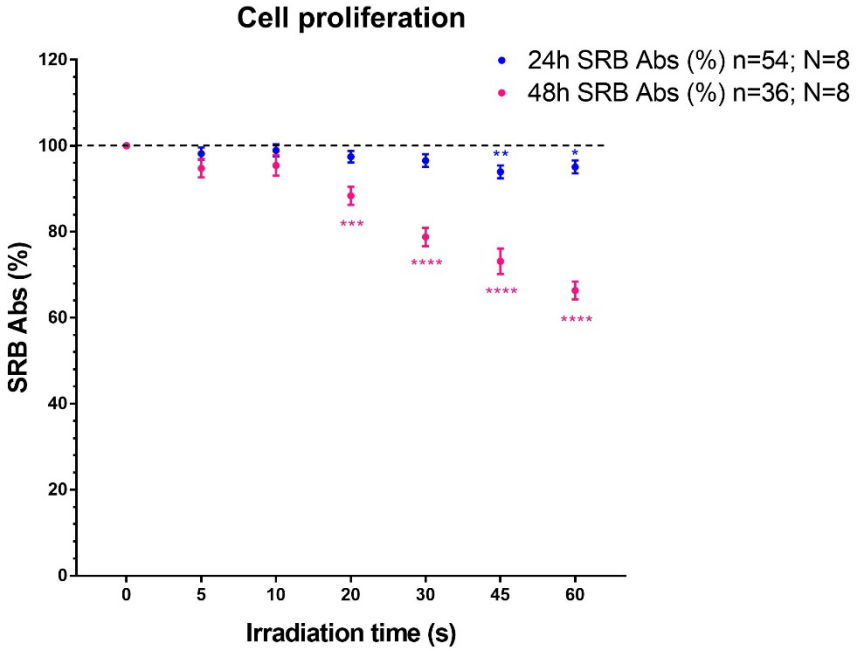

Figure 4. SRB assay results in keloid fibroblasts (N = 8 keloids from seven patients). Cell proliferation was evaluated 24h (blue) and 48h (violet) after treatment. Data are expressed as mean ± SEM, n = 54 at 24h and n = 35 at 48h after treatment. Each measure is repeated in triplicate. Significant values: *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; ****P ≤ 0.0001 *vs* control (0s of irradiation with Blue LED light), one-way ANOVA followed by Dunnett's multiple comparison test.

In boundary derma fibroblasts (N = 4 from four patients), significant metabolic inhibition was revealed 24h after treatment only for 30s and 60s irradiation times whereas, 48h after the treatment, a significant effect was observed only in the range of 20÷60s irradiation (data not shown). Cell proliferation did not show significant variations after 24h, while it was significantly reduced 48h after Blue LED light exposure for irradiation times in the range 20÷60s (data not shown).

3.3 Electrophysiological results

Whole-cell patch clamp recordings were performed on 12 HKFCs showing a Cm= 29.8 ± 8.2 pF; Rm= 752.0 ± 281.8 MΩ; Vm_{K+} = -53.4 ± 3.7 mV; Vm_{Cs+} = -17.1 ± 6.5 mV. According to our previous results [24], Blue LED light application (30s) increased the amplitude of outward currents evoked by a voltage ramp protocol (Figure 5A-B). This effect was observed in 8 HKFCs from three different keloid samples and, on average, a 23 ± 8% current increase at +80 mV was observed (Figure 5B). In order to disclose the ion carrying Blue light-activated current, we blocked all K^+ currents by replacing intra- and extra-cellular K^+ ions with equimolar Cs^+. In these experimental conditions, 30s Blue LED light application induced again an increase, on average 94 ± 38 % (n=4), in outward ramp current amplitude (Figure 5C-D). This effect was not statistically different when compared to K^+ experiments (Figure 5E). These results indicate that K^+ channels are not involved in Blue LED light-mediated effect.

It is known that ramp-evoked outward currents can be carried only by K^+ or Cl^- ions and that fibroblasts express different kinds of Cl^- channels [33], such as Cystic fibrosis transmembrane conductance regulator (CTRF) [31] and Calcium Activate Chloride Channels (CaCCs) [32]. Ruling out the involvement of K^+ currents implies that Cl^- channels are probably major players in the Blue LED light effect. Recent evidence suggests that Cl^- channels are critical in cell proliferation, migration, and differentiation. For this reason, in our future work, we will investigate the nature of this Blue light-activated current. In particular, we will test the involvement of Cl^- channels by applying selective blockers, such as glycine hydrazides or 3-acyl-2-aminothiophene and 5-aryl-2-aminothiazole classes [34].

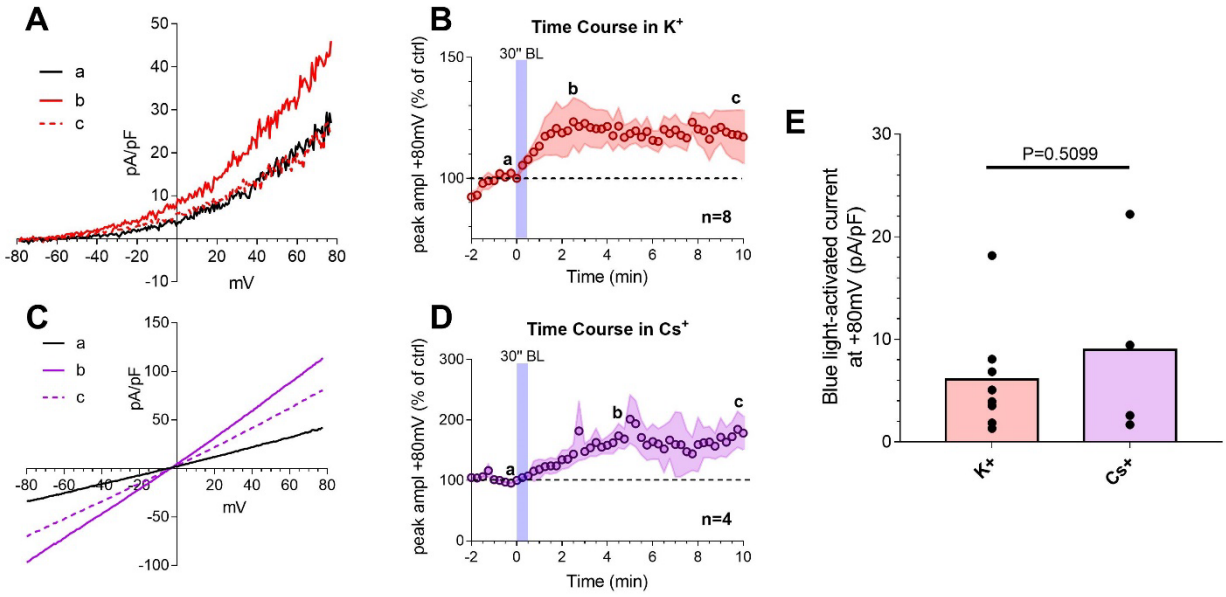

Figure 5. A: Original ramp current traces recorded before (a), 2 minutes (b) or 10 minutes (c) after the application of 30s Blue LED light in the presence of K^+ ions. B: Averaged time course of ramp evoked currents at +80 mV in the presence of K^+ (n = 8). C: Original ramp current traces recorded before (a), 2 minutes (b) or 10 minutes (c) after the application of 30s Blue LED light when intra- and extra-cellular K^+ ions were replaced with equimolar Cs^+. D: Averaged time course of ramp evoked currents at +80 mV, obtained by subtraction of the control value from that recorded at the peak of effect (between 2 and 3 min after light exposure), in Cs^+-replacement experiments (n = 4). E: Pooled data of Blue LED light-activated currents in the same cells, in K^+ (n = 8) or in Cs^+ (n = 4). P = 0.5099, unpaired Student's t-test.

4. CONCLUSION

In this work we studied the effects of Blue LED light on human keloid fibroblasts cells, showing that these cells are sensitive to Blue light at 420nm. In particular, we pointed out that the Blue LED light induces an early biochemical effect in fibroblasts, that reduce their metabolism and, only after 48h from irradiation, this effect is followed by a reduction of their proliferation rate. The electrophysiological experiments evidenced that a 30s treatment with Blue LED light on HKFCs induced an increase in outward ramp currents, that it is not prevented in absence of K^+ ions. For this reason, we exclude the involvement of K^+ channel in this effect. More experiments are necessary to investigate the nature of this current. Since outward ramp-evoked currents can be carried either by K^+ or Cl^- ions, we will test the involvement of Cl^- channels with selective blockers, such as blocker of Calcium Activate Chloride Channels (CaCCs) that are involved in several fibroblasts processes. Further studies will be performed, in order to investigate about the photoacceptor molecules and their possibly role underling the Blue LED light effects.

5. ACKNOWLEDGMENTS

The authors wish to thank the Fondazione Cassa di Risparmio 2018.

REFERENCES

[1] Zhao, R., Liang, H., Clarke, E., Jackson, C., Xue, M., “Inflammation in chronic wounds”, Int. J. Mol. Sci. 17 (2016).
[2] Seo, B.F., Lee, J.Y., Jung, S.N., “Models of abnormal scarring”, Biomed. Res. Int. 423147 (2013).
[3] Zeng, Q., Macri, L.K., Prasad, A., Clark, R.A.F., Zeugolis, Hanley, D.I., Garcia, C., Pandit, Y. A., “Skin tissue engineering”, in: P. Ducheyne (Ed.), Comprehensive Biomaterials, Elsevier, Oxford, pp. 467–499 (2011).
[4] Seifert, O., Mrowietz, U., “Keloid scarring: bench and bedside,” Arch Dermatol Res 301, 259–272 (2009).
[5] Bayat, A., Arscott, G., Ollier, W.E., et al., “Description of site-specific morphology of keloid phenotypes in an Afrocaribbean population,” Br J Plast Surg 57, 122–133 (2004).
[6] Shih, B., Bayat, A., “Genetics of keloid scarring,” Arch Dermatol Res 302, 319–339 (2010).
[7] Fujiwara, M., Muragaki, Y., Ooshima, A., “Keloid-derived fibroblasts show increased secretion of factors involved in collagen turnover and depend on matrix metalloproteinase for migration,” Br J Dermatol 153, 295–300 (2005).
[8] Andrews, J.P., Marttala, J., Macarak, E., Rosenbloom, J., Uitto, J., “Keloids: The paradigm of skin fibrosis – pathomechanisms and treatment,” Matrix biology: journal of the International Society for Matrix Biology. 51, 37-46 (2016).
[9] Gauglitz, G.G., Korting, H.C., Pavicic, T., Ruzicka, T., Jeschke, M.G., “Hypertrophic Scarring and Keloids: Pathomechanisms and Current and Emerging Treatment Strategies,” Molecular Medicine 17(1-2), 113-125 (2011).
[10] Ma, X., Chen, J., Xu, B., Long, X., Qin, H., Zhao, R.C., Wan, X., “Keloid-derived keratinocytes acquire a fibroblast-like appearance and an enhanced invasive capacity in a hypoxic microenvironment in vitro,” International Journal of Molecular Medicine 35(5), 1246-1256 (2015).
[11] Huang, C., Ogawa, R., “Roles of lipid metabolism in keloid development”, Lipids Health Dis., 12(1):60 (2013).
[12] Kuffler, D.P., “Photobiomodulation in promoting wound healing: a review”, Reg Med. 11:1 (2015).
[13] Rossi, F., Pini, R., De Siena, G., Massi, D., Pavone, F.S., Alfieri, D., Cannarozzo, G., "A Blue-LED-based device for selective photocoagulation of superficial abrasions: theoretical modeling and in vivo validation", Proc. SPIE 7548, (2010).
[14] Rossi, F., Tatini, F., Pini, R., Bacci, S., De Siena, G., Cicchi, R., Pavone, F.S., Alfieri, D., "Improved wound healing in Blue LED treated superficial abrasions", Proc. SPIE 8803, (2013).
[15] Alfieri, D., Bacci, S., Cicchi, R., De Siena, G., Lotti, V., Pavone, F.S., Pini, R., Rossi, F., Tatini, F., "Blue LED treatment of superficial abrasions", Proc. SPIE 8565, (2013).
[16] Cicchi, R., Rossi, F., Tatini, F., Bacci, S., De Siena, G., Alfieri, D., Pini, R., Pavone, F.S., "Improvement of the healing process in superficial skin wounds after treatment with EMOLED", Proc. SPIE 9129, (2014).
[17] Cicchi, R., Rossi, F., Tatini, F., Bacci, S., De Siena, G., Alfieri, D., Pini, R., Pavone, F.S., "Irradiation with EMOLED improves the healing process in superficial skin wounds", Proc. SPIE 8926, (2014).
[18] Cicchi, R., Rossi, F., Tatini, F., Bacci, S., De Siena, G., Alfieri, D., Pini, R., Pavone, F.S., "Improvement of the healing process in superficial skin wounds after treatment with EMOLED", Proc. SPIE 9303, (2015).
[19] Rossi, F., Cicchi, R., Tatini, F., Bacci, S., Alfieri, D., De Siena, G., Pavone, F.S., Pini, R., "Healing process study in murine skin superficial wounds treated with the Blue LED photocoagulator EMOLED", Proc. SPIE 9542, (2015).
[20] Cicchi R, Rossi, F., Alfieri D., Bacci, S., Tatini, F., De Siena G., Paroli, G., Pini, R.., Pavone, F.S. Observation of an improved healing process in superficial skin wounds after irradiation with a Blue-LED haemostatic device. J. Biophoton, 9, 645–655 (2016).
[21] Rossi, F., Cicchi, R., Magni, G., Tatini, F., Bacci, S., Paroli, G., Alfieri, D., Tripodi, C., De Siena, G., Pavone, F.S., Pini, R., "Blue LED induced thermal effects in wound healing: experimental evidence in an in vivo model of superficial abrasions", Proc. SPIE 10066, (2017).

[22] Rossi, F., Cicchi, R., Magni, G., Tatini, F., Bacci, S., Paroli, G., Alfieri, D., Tripodi, C., De Siena, G., Pavone, F.S., Pini, R., "In-vivo wound healing modulation after irradiation with a Blue LED photocoagulator", Proc. SPIE 10417, (2017).
[23] Magni, G.,Tatini, F., Cavigli, L., Pini, R., Cicchi, R., Bacci, S., Paroli, G., De Siena, G., Alfieri, D., Tripodi, C., Targetti, L. Pavone, F.S., Rossi, F., "Blue LED treatment of superficial abrasions: in vivo experimental evidence of wound healing improvement", Proc. SPIE. 10685, (2018).
[24] Magni, G., Rossi, F., Tatini, F., Pini, R., Coppi, E., Cherchi, F., Fusco, I., Pugliese, A.M., Pedata, F., Fraccalvieri, M., Gasperini, S., Pavone, F.S., Tripodi, C., Alfieri, D., Targetti, L. "Blue light-irradiated human keloid fibroblasts: an in vitro study", Proc. SPIE 10477, (2018).
[25] Keira, S.M., Ferreira, L.M., Gragnani, A., Duarte, I.S., Santos, I.A.N., Experimental model for fibroblast culture. Acta Cir Bras Vol 19 Special Edition (2004).
[26] Orellana, E.A., Kasinski, A.L. Sulforhodamine B (SRB) Assay in Cell Culture to Investigate Cell Proliferation. Bio Protocol 6(21), doi:10.21769/BioProtoc.1984 (2016).
[27] Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J.T., Bokesch, H., Kenney, S., Boyd, M.R. New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst. 82(13):1107–1112 (1990).
[28] Vichai, V., Kirtikara, K. Sulforhodamine B colorimetric assay for cytotoxicity screening. Nat Protoc.; 1(3):1112–1116. (2006).
[29] Coppi E., Maraula G., Fumagalli M., Failli P., Cellai L., Bonfanti E., Mazzoni L., Coppini R., Abbracchio M.P., Pedata F., Pugliese A.M. UDP-glucose enhances outward K plus currents necessary for cell differentiation and stimulates cell migration by activating the GPR17 receptor in oligodendrocyte precursors. Glia; 61(7):1155-1171 (2013).
[30] Coppi E., Pedata F., Gibb A.J. P2Y(1) receptor modulation of Ca2+-activated K+ currents in medium-sized neurons from neonatal rat striatal slices. Journal of Neurophysiology; 107(3):1009-1021 (2012)
[31] Hwang, T. C., Yeh, J. T., Zhang, J., Yu, Y. C., Yeh, H. I., & Destefano, S. (2018). Structural mechanisms of CFTR function and dysfunction. The Journal of general physiology, 150(4), 539-570.
[32] Tian X.Q., Ma K.T., Wang X.W., Wang Y., Guo Z.K., Si J.Q. Cell Physiol Biochem. 2018;49(2):706-716. doi: 10.1159/000493036. Epub 2018 Aug 30.
[33] Estacion M. Characterization of ion channels seen in subconfluent human dermal fibroblasts. J Physiol. 1991;436:579-601.
[34] Verkman A.S, Galietta L.J. Chloride channels as drug targets. Nat Rev Drug Discov. 2008;8(2):153-71.