Journal of Photochemistry and Photobiology 8 (2021) 100087

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology

journal homepage: www.sciencedirect.com/journal/journal-of-photochemistry-and-photobiology

# The effect of a geometric-shaped tool with blue led light on the activation of human dermal fibroblasts and cancer cells

Ebru Kilicarslan Toruner [a,*], Handan Kayhan [b], Fatih Suheyl Ezgu [c]

[a] Department of Nursing, Gazi University Health Sciences Faculty, Ankara, Turkey
[b] Department of Adult Hematology, Gazi University Faculty of Medicine, Ankara, Turkey
[c] Department of Pediatrics, Gazi University Faculty of Medicine, Ankara, Turkey

ARTICLE INFO

*Keywords:*
Light
Geometrical spiral
Neuroblastoma
Fibroblast
Apoptosis
Wound closure

ABSTRACT

Light-emitting diode (LED) irradiation has been used as a therapeutic technique for several diseases. It is known that energy levels of light change according to different surfaces, colors and geometric shapes. However, more information is needed regarding the effect of this change on the human body. This study was aimed to investigate the effect of blue LED light applied through an optical path with special geometry on the human dermal fibroblast (HDF) and cancer cell proliferation, apoptosis, migration, and cell cycle. The number of cancerous cells was found to be lower and the rate of necrosis was determined to be significantly higher in the geometrical light-treated group than in the direct light and non-light groups. The migration of HDF cells in blue LED light in the geometrical tool group increased more than the other groups after 72 h of light irradiation. In cancer cells, the wound closure rate of the non-light group was higher than light-exposed groups after 72 h. Blue LED light induces a G1 phase peak followed by apoptosis in cancer cells 24 h after light irradiation, especially in the geometrical tool group. These results indicate that blue LED irradiation applied through an optical path with a special geometrical tool inhibits cancer cell proliferation, migration, and induces cell necrosis.

## 1. Introduction

Health promotion is one of the primary health issues in the world. There are different types of approaches and interventions for health promotion. Light therapy is one of these interventions. When light is used properly, it has positive effects on human health. In the early 1900s, hospitals started using ultraviolet (UV) rays to kill bacteria in patients. UV light has been used in the treatment of tuberculosis, streptococcal infections, viral pneumonia, mumps, fungal infections in the skin [1]. Nowadays, light, especially at 405–470 nm, has the potential for the inactivation of various bacteria including Staphylococcus aureus, Staphylococcus epidermidis, methicillin-resistant S. aureus, and Escherichia coli without adversely affecting wound healing, in comprasion to ultraviolet irradiation [2–5].

Light-emitting diode (LED) has been widely used for light therapy since it is a cost-effective, reliable and efficient solid-state light source, and less harmful than UV, radiation therapy, chemotherapy, or magnetic treatment [6]. Therapeutic applications of blue (400–495 nm), green (500–565 nm), yellow (570–590 nm), and red (630–700 nm) LED could be observed in medical treatments for various physical problems, such as acne, mucositis, wound healing, seasonal affective disorder, skin calming, and photoageing [7]. The efficacy of LED is dependent on various factors, such as applied power, energy density, wavelength, and exposure time. For example, blue light penetrates least efficiently through tissue while green and red lights penetrate more deeply [8].

Some studies have investigated the effects of red LED light therapy on fibroblast proliferation. Red LED light therapy is promising for the treatment of keloid fibroblast cells because it decreases fibroblast proliferation [9,10]. On the other hand, blue LED light therapy was investigated for its role in fibroblast proliferation. In one study, it was found that blue LED inhibits the proliferation of keloid fibroblasts and reduces cell viability [11].

LED light therapy has been investigated for cancer cells in recent years [12–16]. In one study, blue LED light reduced the viability of human cancer cells compared to green and red LED light colors [12]. In another study, it was found that blue LED light decreased the cell density in human cervical cancer cells compared to red and green LED lights [13]. In addition, He et al. found that blue LED light reduced cell proliferation and increased cell apoptosis in hepatoma cells by inducing DNA damage [17].

* Corresponding author.
*E-mail address:* ebrutoruner@gazi.edu.tr (E.K. Toruner).

https://doi.org/10.1016/j.jpap.2021.100087
Received 17 November 2020; Received in revised form 7 November 2021; Accepted 11 November 2021
Available online 16 November 2021
2666-4690/© 2021 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Light is an electromagnetic wave; therefore, light acts as a synchronised oscillation of electric and magnetic fields. Frequency, wavelength, and the speed of light can change as it passes through different surfaces and shapes [18]. Colors are different frequencies of light (some high, some low). Therefore, as a quantum effect, light can have major effects on the clockwise or counter-clockwise spins of electrons in certain surfaces or substances. This effect is called "double effect in spin-orbit" [19].

The effect of light therapy with LED irradiation on fibroblast and cancer human cells was searched through the literature, and it was noticed that LED light is directly applied to human cell lines without any guided optical path. We could not locate a study that used an optical path with a special geometrical tool to apply light to human fibroblasts or cancer cells. Although it is known that the energy levels of light in different colors and wavelengths change, more information is needed regarding the effect of this change with respect to geometry of irradiation, materials, and duration of light at the cellular level in the human body.

### 1.1. Purpose of the study

This study is aimed to investigate the effect of blue LED light applied through an optical path with a special geometry on the activation of dermal fibroblasts and cancer cells. The study questions the differences between the dermal fibroblasts and cancer cell cultures in the experimental group and control groups in terms of the following parametres: (a) cell proliferation, (b) cell apoptosis test results, (c) cell cycle analysis results (cell cycle), and (d) scratch wound test results (migration of cells).

## 2. Method

This study was an experimental study with an innovative content. The research was conducted in a molecular haematology laboratory of a university hospital between the Spring and Fall semester of 2020. Human dermal fibroblasts (HDF) and human neuroblastoma cancer (SR-SY5Y) cells were used for the study. Ethical committee approval of the university (No: 24074710–033) was obtained for the research. The flow chart of the study was given in Fig. 1.

*Experimental groups:*

E1A: Human dermal fibroblast (HDF) cell line irradiated with blue LED light guided through an optical path with a special geometrical tool.
E1B: Human neuroblastoma cancer (SR-SY5Y) cell line irradiated with blue LED light guided through an optical path with a special geometrical tool.

*Control groups:*

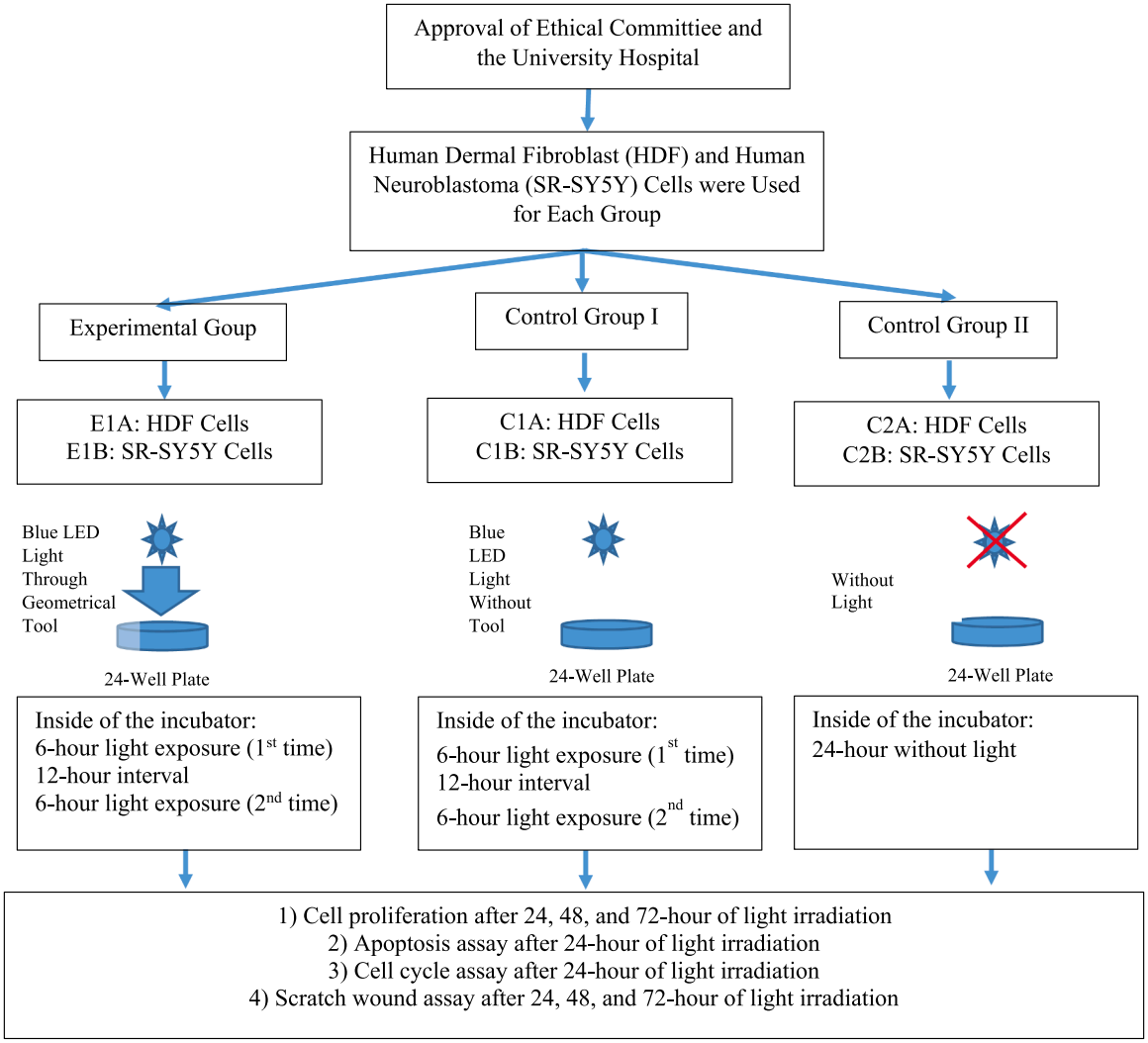



**Fig. 1.** Flow chart of the study.

C1A: HDF cell line irradiated with a direct optical path with blue LED light.
C1B: SR-SY5Y cell line irradiated with a direct optical path with blue LED light.
C2A: HDF cell line in the dark.
C2B: SR-SY5Y cell line in the dark.

### 2.1. Cell culturing

Both cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with phenol red, containing 10.0% fetal bovine serum (FBS), 1.0% penicillin/streptomycin, and 1.0% L-glutamine. Cells were cultured in an incubator (95% humidified, temperature set up 37 °C, and 5.0% $CO_2$) in two separate flasks for each cell type and subcultured nearly once in three days (1:3 ratio) upon reaching 80% confluency. After reaching confluency, the dermal and cancer cells were trypsinized with 0.25% trypsin-EDTA, washed with PBS, and transferred to 3 separate 24-well plates with a refreshed medium. The cells were seeded at $2 \times 10^5$ cells per well 24 h before the light exposure. They have cultured for two days in these plates. The 1st plate was for the experimental group, the 2nd plate was for the 1st control group, and the 3rd plate was for the 2nd control group. HDF cells were placed in half (12-well) of each plate and SR-SY5Y cells were in the other half.

### 2.2. Blue LED irradiation and placement of plates according to the groups in an incubator

Two separate 6 h light irradiation to the cells of E1 and C1 groups with 12 h interval (total 12 h light irradiation) was applied two days after planting. Blue LED light, 3 W, 460 nm to 470 nm (Manufacturer: Wayjun Technology, Shenzen, China) was used inside of the incubator with a temperature of 37 °C. An aluminum cooler was installed to prevent the blue LEDs from heating up. The plates of the E1 and C1 groups were horizontal under the blue light sources with a distance of 5 cm. A photo-radiometer was used to measure light intensity. The mean of the light intensity was similar for E1 and C1 groups (10,540 lux vs 10,560 lux, respectively). The blue LED light temperature and light intensity were measured via a real-time data collection system.

There is a specially drilled 2.0 cm diameter hole at the side of the incubator's wall to allow cable passage. After the cables of LED have passed, the insulation of this hole has been provided to preserve the $CO_2$, heat, and humidity inside of the incubator. Three plates were placed at the same time in the incubator. The plates were placed in specially designed black, plastic boxes with holes to prevent light exposure between the groups. Also, black clothes were covered on boxes to prevent light exposure but allow to pass air and humidity. The blue LED light was given to the E1 and C1 groups in the boxes. C2 group was excepted from light exposure and provided a dark environment.

### 2.3. Geometric-shaped tool

The geometric-shaped tool was designed according to a special spiral geometry that guided light through an optical path via plexiglass (Picture 1). Blue LED light source was placed inside of the spiral. Heat shrink flexible tubing was used to cover the plexiglass to prevent the leakage of light apart from the guided optical path. Blue LED light coming out from the other end of the spiral was applied to the plate of the experimental group.

### 2.4. Cells proliferation

After the light irradiation, the viability of the cells was determined by trypan blue dye and the number of cells was counted via an automated cell counter device (Invitrogen Countess). Cell proliferation was studied according to the manufacturer's instructions with an XTT-based kit (Biological Industries, cat. No: 20-300-1000) and measured spectrophotometrically at 450/670 nm with the TECAN Sunrise instrument three times at 24, 48 and 72 h after the end of light application.

### 2.5. Apoptosis assay

Apoptosis test was performed 24 h after the light application and it was done to all groups. The cells were collected and washed with cold PBS. Pharmingen FITC Annexin V Apoptosis Detection Kit was used for the test according to the manufacturer's instructions. In summary, the obtained cells were suspended in 500 μl annexin V binding buffer with 5 μl of Annexin V-FITC Conjugate and 10 μl of Propidium Iodide Solution. The cells were incubated in the tubes at room temperature for 15 min and protected from light. The cells' fluorescences were determined immediately after the incubation with a flow cytometer (Beckman Coulter - Navios). The apoptosis test was performed in triplicate.

### 2.6. Cell cycle assay

It was aimed to determine the G1-S and G2 phases of the cell cycle. Abcam Cell Cycle Assay Kit (Fluorometric - Green) was used for the cell cycle test according to the instructions. The test was performed on all groups 24 h after the light application. The adherent cells ($10^6$ cells/ml) were collected and washed with warm PBS. 200X Nuclear Green CCS1 (component A) was added to the washed cells. The cells were incubated (37 °C, 5% $CO_2$) for 30 min. After the incubation, the cells were washed three times with a serum-containing growth medium. Then, they were centrifuged at 1000 rpm for 4 min and they were re-suspended in 0.5 ml of Assay Buffer (component B). The cell cycle analysis was done with Beckman Coulter - Navios flow cytometer using the FL1 channel. Cells in the G1, S, and G2 phases were analyzed with the Kaluza software (Beckman Coulter).

### 2.7. Wound healing assay

A wound healing test was used to examine the effects of light application on the migration of dermal fibroblasts and cancer cells. After transferring of cells to three separate 24-well plates (for E1, C1, and C2 groups) at cell culture, the apparatus included in the Abcam Wound Healing Assay Kit was placed in 3-well of HDF cells and 3-well of SR-SY5Y cells at each plate. The standard protocol specified for wound formation in the kit was applied to these wells. The apparatuses were removed from the wells before applying light to the cells. The apparatuses formed horizontal and vertical lines on the surface. The locations of the wells where the scratches were created were the same on 3 plates to be able to view migration in wound closure (Zeiss 4 - Carl microscope was used). The photos (AxioCam Icc 3, Axiovert 135 M) were taken from the same distance immediately after the wound was created and 24, 48, and 72 h later of the light irradiation. The images were transferred to the AxioVison Rel 4.8 LE program. Image J 1.52v program (NIH, USA) was used to monitor the rate of the wound closure.

### 2.8. Statistical analysis

Data obtained in the study were analyzed with the SPSS 20.0 (Chicago, IL, USA), and the variables' compliance with the normal distribution was examined with visual (histogram and probability graphics) and analytical (Kolmogorov-Smirnov) methods. For the comparison of the data in the three groups which had continuous variables (ordinal), the Kruskal–Wallis test was used for not normally distributed data and one way ANOVA test was used for normally distributed data. Tukey test was used for post-hoc analysis. The significance level was set at 0.05.

## 3. Results

Cell proliferation assay results performed 24, 48, and 72 h after light

irradiation are presented in Table 1. The cell proliferation of healthy human dermal fibroblast cells was determined to be higher in the non-light-applied (C2A) group than the E1A and C1A groups after 24 h of light irradiation (3.00 ± 0.15, 2.84 ± 0.04, and 2.50 ± 0.06, respectively) and the difference between the groups was statistically significant ($p < 0.05$). The average proliferation in the C2A group decreased 48 h after light application and approached the other groups in the subsequent hours. The mean proliferation of the HDF cells was 2.60 ± 0.13 in the C2A group, 2.56 ± 0.04 in the E1A group, and 2.51 ± 0.04 in the C1A group after 72 h of light irradiation. In the SR-SY5Y cells, cell proliferation was found to be lower in the C1B group compared to the E1B and C2B groups at 24, 48, and 72 h and the difference was found to be statistically significant ($p < 0.05$).

The mean apoptosis scores of the groups of healthy human dermal fibroblast and human neuroblast cells 24 h after light irradiation are shown in Table 2. In human dermal fibroblast cells, the number of healthy cells was similar in the non-light group (C2A = 71.60 ± 0.34) and light group with the geometrical tool (E1A = 69.76 ± 0.87), and the means were determined to be higher than for the direct light group (C1A = 57.93 ± 1.61) ($p < 0.05$). In the SR-SY5Y cells, the number of cancerous cells was found to be lower in the geometrical light-treated group (E1B) compared to the C1B and C2B groups (E1B = 69.56 ± 0.25, C1B = 77.3 ± 0.75, and C2B = 72.3 ± 6.21) ($p > 0.05$). In addition, the rate of necrosis was determined to be higher in the geometrical light-treated group (E1B = 27.8 ± 0.11) than in the direct light (C1B = 19.9 ± 0.40) and non-light (C2B = 14.5 ± 5.25) groups ($p < 0.05$).

After 24 h light irradiation, the HDF and SR-SY5Y cells were subjected to flow cytometric analyses for the cell cycle. The results showed that the percentage of HDF cells in the G1 phase was lower in the light group with the geometrical tool than the other groups (Table 3 and Fig. 2A). Interestingly, a distinct G1 phase peak was found following a 24 h period of blue LED light treatment in the E1B and E2B groups, compared to the C2B cells. Beside, the percentage of SR-SY5Y cells in the S phase was lower in the light group with the geometrical tool (3.98%) compared to the direct light (6.86%) and non-light (19.38%) groups (Table 3 and Fig. 2B).

Images of the scratch wounds of the HDF (A) and SR-SY5Y (B) cells before and 24, 48 and 72 h after light irradiation are presented in Fig. 3A and B. We noticed that HDF cells in the E1A group migrated through the direction of light in the well (light was coming from left sight) after 48 h of light exposure. On the other hand, SR-SY5Y cells in the E1B and C1B groups mostly migrated through the center of the wells and the cells at the edges of the wells were very reduced after 72 h of light irradiation.

Closed wound area percentages of HDF (A) and SR-SY5Y (B) cells before and after 24, 48, and 72 h of light irradiation are shown in Fig. 4A and B. At baseline, the means of the scratch wound areas were similar in all groups. The mean of the closed wound area of C1A group was higher than the E1A and C2A groups after 24 h of light irradiation ($p < 0.05$). The difference was detected between the E1A and C2A ($p = 0.001$), and the C1A and C2A ($p = 0.001$) groups (post-hoc). The migration of the HDF cells in the E1A group increased more (77.02%) than in the C1A (73.83%) and C2A (68.15%) groups after 72 h of light irradiation ($p < 0.05$). According to the post-hoc analysis, differences were determined between the E1A and C1A ($p = 0.004$), and the E1A and C2A ($p = 0.007$) groups. In the SR-SY5Y cells, the wound closure rate of the C1B group (28.91%) was higher than the E1B (26.06%) and C1B (25.91%) groups after 72 h of light exposure ($p > 0.05$).

## 4. Discussion

In this innovative study, we investigated the effect of blue LED light applied through an optical path with special geometry on the human cells. For the first time, we discovered that blue LED irradiation applied through an optical path inhibits cancer cell proliferation and migration, and induces cell necrosis. Light irradiation also showed an anti-tumour effect on human cancer cells. Furthermore, we identified that the migration of human fibroblast cells in the blue LED light group with geometrical tool increased more than the other groups after 72 h of light irradiation.

Some studies have investigated the effects of red LED light therapy on fibroblast proliferation and viability in scar treatment *in vitro*. Red LED light has been found to decrease fibroblast proliferation [9,10]. Therefore, this color light therapy is promising for the treatment of keloid fibroblast cells. Blue LED light therapy has also been investigated for fibroblast proliferation, viability, and migration. In one study, it was found that blue LED inhibited the proliferation of and decreased the migration speed of human skin fibroblasts [20]. In our study, we only used blue LED light. The cell proliferation of healthy human dermal fibroblast cells was determined to be higher in the non-light group than the light group with the geometrical tool and direct light groups after 24 h of light irradiation ($p < 0.05$). The average proliferation in the non-light-applied group decreased 48 h after light application and the average proliferation approached the other groups in the following hours. During human dermal fibroblast cells apoptosis, the number of healthy cells was found to be similar in both the non-light group and light group with the geometrical tool, and the means were determined to be higher than for the direct light group ($p < 0.05$). It seems that blue LED light applied with a geometrical tool has an effect on cell proliferation and apoptosis of dermal fibroblasts after 48 h of light exposure.

Cancer cells exposed to LED light have been mostly investigated in recent years. Hopkins et al. examined the effects of blue, green, and red LED light irradiation on the viability of human cancer cell lines. In their study, they found that only blue light exhibited a reduction in viability of some cancer types compared to dark control groups [12]. Three colored LEDs were also investigated in another study and the cell density exposed to blue LED light (11.19 ± 2.51%) was much lower than for red and green LED lights (81.71 ± 9.92% and 61.81 ± 4.09%, respectively) for human cervical cancer cells [13]. The viability of colon cancer cells was lower in the blue LED-irradiated cultures than in control cultures in another study [14]. In our study, cancer cell proliferation was found to be lower in the direct light group compared to the other groups at 24, 48, and 72 h ($p < 0.05$). In addition, the number of cancerous cells was found to be lower in the geometrical light-treated group compared to the other groups. The rate of necrosis was also determined to be higher in

**Table 1.**
Comparison of cell proliferation averages of healthy fibroblast and neuroblast cells in groups after 24, 48, and 72 h of light irradiation.

| | 24 h | | 48 h | | 72 h | |
|---|---|---|---|---|---|---|
| | X±SS | Min-max | X±SS | Min-max | X±SS | Min-max |
| HDF | | | | | | |
| E1A | 2.84 ± 0.04 | 2.80–2.92 | 2.57 ± 0.03 | 2.54–2.63 | 2.56 ± 0.04 | 2.50–2.63 |
| C1A | 2.50 ± 0.06 | 2.41–2.58 | 2.54 ± 0.03 | 2.51–2.59 | 2.51 ± 0.04 | 2.48–2.56 |
| C2A | 3.00 ± 0.15 | 2.82–3.17 | 2.65 ± 0.14 | 2.49–2.84 | 2.60 ± 0.13 | 2.45–2.78 |
| Test | K-W = 10.063, $p = 0.007$ | | K-W = 2.463, $p = 0.292$ | | K-W = 1.423, $p = 0.491$ | |
| SR-SY5Y | | | | | | |
| E1B | 2.87 ± 0.01 | 2.87–2.89 | 2.61 ± 0.04 | 2.56–2.64 | 2.61 ± 0.55 | 2.55–2.66 |
| C1B | 2.37 ± 0.21 | 2.13–2.63 | 2.35 ± 0.10 | 2.20–2.47 | 2.32 ± 0.11 | 2.18–2.46 |
| C2B | 2.86 ± 0.03 | 2.83–2.89 | 2.57 ± 0.02 | 2.54–2.61 | 2.55 ± 0.06 | 2.45–2.60 |
| Test | K-W = 8.736, $p = 0.013$ | | K-W = 9.284, $p = 0.01$ | | K-W = 8.949, $p = 0.01$ | |

**Table 2.**
Comparison of healthy fibroblast and neuroblast cells in groups 24 h after light irradiation according to the apoptosis stages.

| | Healthy Cells | | Apoptosis* | | Necrosis | |
|---|---|---|---|---|---|---|
| | X±SS | Min-max | X±SS | Min-max | X±SS | Min-max |
| HDF | | | | | | |
| E1A | 69.76 ± 0.87 | 68.8–70.5 | 8.36 ± 1.04 | 7.20–9.20 | 21.93 ± 0.98 | 20.8–22.6 |
| C1A | 57.93 ± 1.61 | 56.9–59.8 | 26.6 ± 1.81 | 24.5–27.7 | 15.46 ± 0.20 | 15.3–15.7 |
| C2A | 71.60 ± 0.34 | 71.4–72.0 | 8.86 ± 0.20 | 8.70–9.10 | 19.5 ± 0.32 | 19.3–19.9 |
| Test | K-W= 7.261, $p = 0.027$ | | K-W= 5.355, $p = 0.65$ | | K-W= 7.200, $p = 0.027$ | |
| SR-SY5Y | | | | | | |
| E1B | 69.56 ± 0.25 | 69.3–69.8 | 2.63 ± 0.32 | 2.40–3.00 | 27.8 ± 0.11 | 27.8–28.0 |
| C1B | 77.3 ± 0.75 | 76.6–78.1 | 2.70 ± 0.36 | 2.40–3.10 | 19.9 ± 0.40 | 19.5–20.3 |
| C2B | 72.3 ± 6.21 | 65.2–72.6 | 13.06 ± 2.17 | 10.6–14.7 | 14.56 ± 5.25 | 9.50–20.0 |
| Test | K-W= 5.132, $p = 0.77$ | | K-W= 5.535, $p = 0.063$ | | K-W= 6.305, $p = 0.43$ | |

*Early and late apoptosis.

**Table 3.**
Percentage of cells in groups 24 h after light irradiation according to the cell-cycle stages.

| Groups | G1% | S% | G2% | G2/G1 |
|---|---|---|---|---|
| HDF | | | | |
| E1A | 69.67 | 17.96 | 12.37 | 1.54 |
| C1A | 72.90 | 13.91 | 13.19 | 1.54 |
| C2A | 72.09 | 19.13 | 8.78 | 1.54 |
| SR-SY5Y | | | | |
| E1B | 82.96 | 3.98 | 13.06 | 1.51 |
| C1B | 80.64 | 6.86 | 12.5 | 1.54 |
| C2B | 65.6 | 19.38 | 15.02 | 1.51 |

the geometrical light-treated group than in the direct light and non-light groups ($p < 0.05$). Blue light with a geometrical tool has a therapeutic benefit of inducing necrosis of cancer cells. Thus, blue LED light has been shown to induce the increase of the G1 phase, followed by apoptosis, in cancer cells.

In 2016, Oh et al. found that blue LED light reduced cell viability and induced apoptotic cell death in B cell lymphoma cells [15]. For colorectal cancer cells, blue LED light intervention increased apoptosis and decreased cell proliferation as well as cell migration [16]. Another study investigated the migration and invasion of tumour cells after blue LED light irradiation and showed that blue LED light inhibited cell migration and the invasion of cancer cells [21]. In our study, the migration of HDF

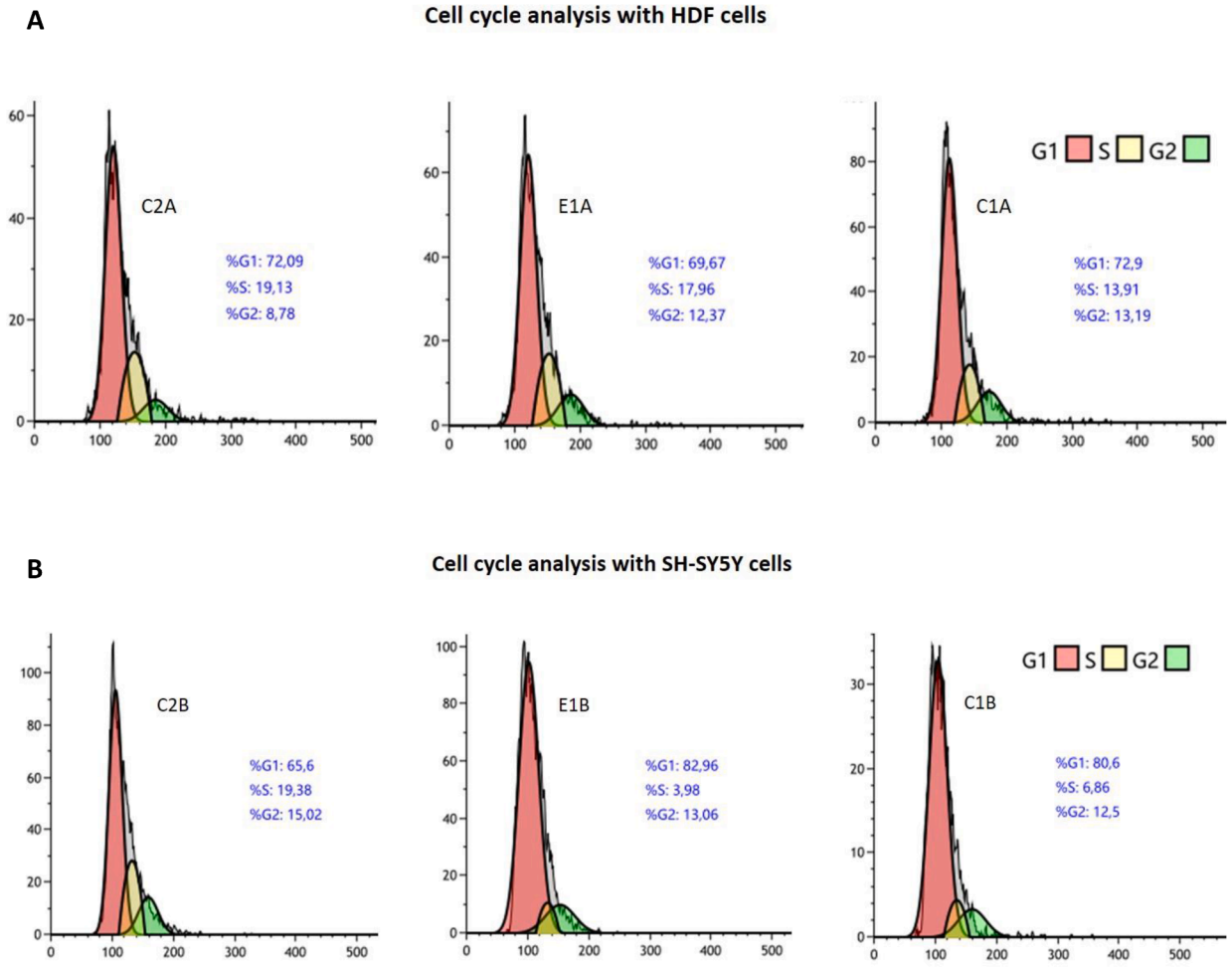



**Fig. 2.** Percentage of HDF (A) and SR-SY5Y (B) Cells After 24 h Light Irradiation According to the Cell Cycle Stages.

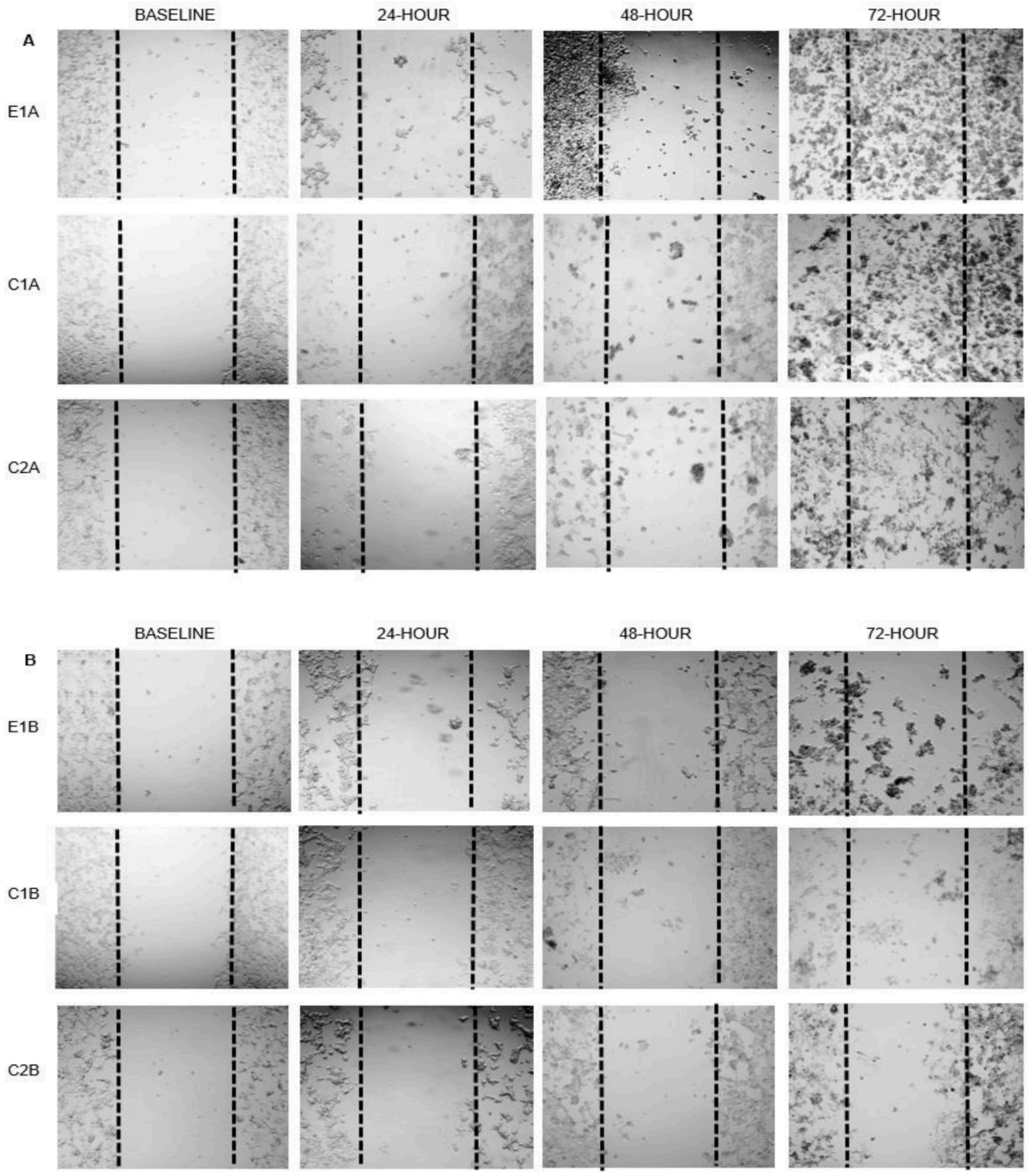



**Fig. 3.** Images of the Scratch Wounds of HDF (A) and SR-SY5Y (B) Cells at Baseline (Before Light Irradiation) and 24, 48 and 72 h After Light Irradiation. The width of the baseline wounds is taken as reference and in all images this reference range is shown between two dashed lines.

cells in the blue LED light group with geometrical tool increased more than in the other groups after 72 h of light irradiation ($p < 0.05$). In the SR-SY5Y cells, the wound closure rate of the non-light group was higher than the light-exposed groups after 72 h ($p > 0.05$). Other interesting results included that HDF cells in the blue LED light group with geometrical tool migrated through the direction of light in the well after 48 h of light exposure and the groups with light exposure mostly migrated though the centers of the wells in the SR-SY5Y cells after 72 h of light irradiation. In the literature, it was reported that necrosis can occur at the center of the well due to lack of nutrition and oxygen [22, 23]. We could say that blue LED light has an effect on the migration of cells with a different pattern than non-light cells.

Each color, shape, surface, and direction of light creates different electric and magnetic fields. We assumed that light has major effects on human cells with combinations of these factors. In this study, we used a spiral geometrical tool with blue LED color that could make a quantum effect of electrons in spin orbit. We were, therefore, able to observe the different effects of optically guided blue LED light on fibroblasts, as well as on cancer cells, compared to direct light.

### 4.1. Strengths and limitations

This study was planned as an experimental study with an innovative content. Because we could not locate a similar study in the literature, this study provided new and original contribution based on evidence. We designed a spiral tool and placed the LED and cooler inside of the

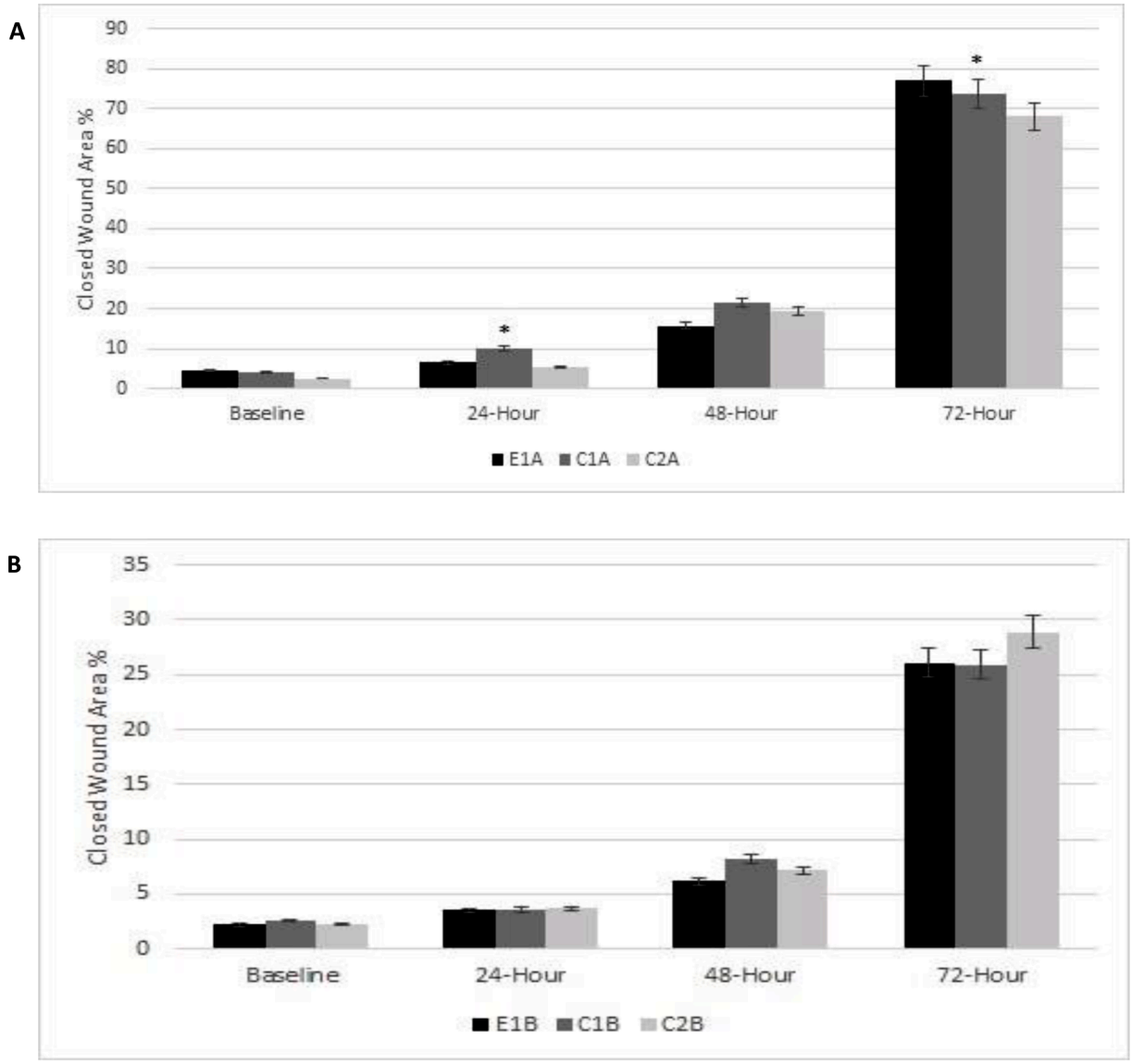



**Fig. 4.** Closed wound area percentage of HDF (A) and SR-SY5Y (B) Cells Before and 24, 48, and 72 h After Light Irradiation. In order to determine the percentage of scratch closure, area of cell free space according to the baseline reference was measured using ImageJ 1.52v programme. Data was shown mean ± SD for each group. $^{*}p < 0.05$

spiral. One problem was that we had little space to insert all the apparatuses inside of the spiral. We searched the LEDs that were convenient to use in the spiral (i.e., products on the market that could be reached were searched one by one according to our parametres, such as LED size, LED power, resilience to heat, brightness of light at the other end of the spiral). Therefore, as we had limited options, only photometric measurements (photometric quantity-lux) were performed, and we could not measure the radiometric quantity and spectral quantity.

The guided (spiral) LED had optical losses due to optical guide coupling and path. To compensate for these losses and to achieve the same illumination level for both guided and direct illumination, the forward current value of direct illumination LED was decreased until the two lux-metres provided the same value. For this, two lux-metres were placed in similar places for both plates. The use of adjustable current sources and the direct measurement of illumination by photo radiometres resulted in the achievement of similar illumination values at the surface of the plates during the entire experiment time. We did not measure the fluence value directly, but both the direct and guided illumination paths emitted the same fluence value at the surface of the plates. In addition, we did not use an optical filter for the photometric measurements, so the overall spectral response of the system, including the entrance optic and photodetector, could not match the V-lambda curve. We aimed in this study, however, to apply the same light intensity and we used the same LEDs and photometres on the light-exposed groups.

## 5. Conclusion

It is a known fact that the energy level of light on different surfaces, colors and geometric shapes changes. Therefore, we need more information on the effect of this change on human health. We searched the effects of blue LED light applied through an optical path with a special geometry on the activation of dermal fibroblasts and cancer cells in this study.

The number of cancerous cells was found to be lower and the rate of necrosis was found to be higher in the geometrical light-treated group compared to the direct light and non-light groups. The migration of HDF cells in blue LED light group with a geometrical tool increased more than the other groups after 72 h of light irradiation. In cancer cells, the wound closure rate of the non-light group was higher than the light- exposed

groups after 72 h. The results of this study provided new information and guidance on new treatment methods. It is recommended to investigate the effects of LED light with a geometrical tool on cell proliferation, apoptosis, migration, and cell cycle in different time zones, colors, and light densities for future studies.

## Availability of data and materials

All data generated in this study are included in this article.

## Consent for publication

All of authors listed consented to publication.

## CRediT authorship contribution statement

**Ebru Kilicarslan Toruner:** Formal analysis, Writing – original draft, Writing – review & editing. **Handan Kayhan:** Formal analysis, Writing – original draft, Writing – review & editing. **Fatih Suheyl Ezgu:** Writing – original draft, Supervision, Writing – review & editing.

## CRediT authorship contribution statement

**Ebru Kilicarslan Toruner:** Formal analysis, Writing – original draft, Writing – review & editing. **Handan Kayhan:** Formal analysis, Writing – original draft, Writing – review & editing. **Fatih Suheyl Ezgu:** Writing – original draft, Supervision, Writing – review & editing.

## Declaration of Competing Interest

The authors declare that they have no financial or personel competing interests. No conflict of interest exits in the submission of this manuscript, and the manuscript is approved by all authors for publication. The manuscript has not been previously published, is not currently submitted for review to any other journal, and will not be submitted elsewhere before one decision is made.

## Acknowledgments

This research was supported by Projects of Scientic Investigation Departmant of Gazi University, Turkey under grant: 47/2019-04, and with fellowships to EKT, HK, and FSE. We wish to thank two electrical electronics engineers, Behcet Kilicarslan, MsC EE and Okan Demirel, MsC EE who were made the geometrical tool for the research.

Also, we want to thank Assoc. Prof. Naime Altay, Sanem Gokcen, PhD, Halil Dundar, PhD, and Prof. A. Munci Yagci for helpful scientific comments and Samet Ece from Diagen Biotechnology Systems for his support at this project. The fund provider played no part in study design, data collection, analysis, decision to publish or manuscript preparation.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jpap.2021.100087.

## References

[1] J.A. Maville, C.G. Huerta, Health Promotion in Nursing, 2nd ed., Thomson Delmar Learning, NewYork, 2008, pp. 400–413.
[2] T. Dai, A. Gupta, C.K. Murray, M.S. Vrahas, G.P. Tegos, M.R. Hamblin, Blue light for infectious diseases: propionibacterium acnes, helicobacter pylori, and beyond, Drug Resist. Updates 15 (2012) 223–236.
[3] J.S. Guffey, J. Wilborn, *In vitro* bactericidal effects of 405-nm and 470-nm blue light, Photomed. Laser Surg. 24 (2006) 684–688.
[4] M. Maclean, S.J. MacGregor, J.G. Anderson, G. Woolsey, Inactivation of bacterial pathogens following exposure to light from a 405-nanometer light-emitting diode array, Appl. Environ. Microbiol. 75 (2009) 1932–1937.
[5] R. McDonald, S.J. MacGregor, J.G. Anderson, M. Maclean, H. Granta, Effect of 405-nm high-intensity narrow-spectrum light on fibroblast-populated collagen lattices: an *in vitro* model of wound healing, J. Biomed. Opt. 16 (2011), 048003.
[6] P. Babilas, S. Schreml, M. Landthaler, R.M. Szeimies, Photodynamic therapy in dermatology: state-of-the-art, Photodermatol. Photoimmunol. Photomed. 26 (2010) 118–132.
[7] P.S. Oh, H.J. Jeong, Therapeutic application of light emitting diode: photo-oncomic approach, J. Photochem. Photobiol. B 192 (2019) 1–7.
[8] V. Nesi-Reis, D.S. Lera-Nonose, J. Oyama, M.P. Silva-Lalucci, I.G. Demarchi, S. M. Aristides, J.J. Teixeira, T.G. Silveira, M.V. Lonardoni, Contribution of photodynamic therapy in wound healing: a systematic review, Photodiagn. Photodyn. Ther. 21 (2018) 294–305.
[9] H. Lev-Tov, N. Brody, D. Siegel, J. Jagdeo, Inhibition of fibroblast proliferation *in vitro* using low-level infrared light-emitting diodes, Dermatol. Surg. 39 (2013) 422–425.
[10] A. Mamalis, J. Jagdeo, Light-emitting diode–generated red light inhibits keloid fibroblast proliferation, Dermatol. Surg. 41 (2015) 35–39.
[11] G. Magni, M. Banchelli, F. Cherchi, E. Coppi, M. Fraccalvieri, M. Rossi, F. Tatini, A. M. Pugliese, D.R. Degl'Innocenti, D. Alfieri, P. Matteini, R. Pini, F.S. Pavone, F. Rossi, Experimental study on blue light interaction with human keloid-derived fibroblasts, Biomedicines 8 (2020) 1–19.
[12] S.L. Hopkins, B. Siewert, S. Askes, P. Veldhuizen, R. Zwier, M. Heger, S. Bonnet, *In vitro* cell irradiation protocol for testing photopharmaceuticals and the effect of blue, green, and red light on human cancer cell lines, Photochem. Photobiol. Sci. 15 (2016) 644–653.
[13] S.W. Choe, K. Park, C. Park, J. Ryu, H. Choi, Combinational light emitting diode-high frequency focused ultrasound treatment for HeLa cell, Comput. Assist. Surg. 22 (2017) 79–85.
[14] T. Yoshimoto, Y. Morine, C. Takasu, R. Feng, T. Ikemoto, K. Yoshikawa, S. Iwahashi, Y. Saito, H. Kashihara, M. Akutagawa, T. Emoto, Y. Kinouchi, M. Shimada, Blue light-emitting diodes induce autophagy in colon cancer cells by Opsin 3, Ann. Gastroenterol. Surg. 2 (2018) 154–161.
[15] P.S. Oh, H. Hwang, H.S. Jeong, J. Kwon, H.S. Kim, M. Kim, S.T. Lim, M.H. Sohn, H. J. Jeong, Blue light emitting diode induces apoptosis in lymphoid cellsby stimulating autophagy, Int. J. Biochem. Cell Biol. 70 (2016) 13–22.
[16] G. Yan, L. Zhang, C. Feng, R. Gong, E. Idiiatullina, Q. Huang, M. He, S. Guo, F. Yang, Y. Li, F. Ding, W. Ma, V. Pavlov, Z. Han, Z. Wang, C. Xu, B. Cai, Y. Yuan, L. Yang, Blue light emitting diodes irradiation causes cell death in colorectal cancer by inducing ROS production and DNA damage, Int. J. Biochem. Cell Biol. 103 (2018) 81–88.
[17] M. He, G. Li, X. He, Y. Wang, H. Lei, Q. Wang, G. Yan, R. Gong, G. Liu, T. Li, B. Cai, L. Li, Y. Yuan, Blue LED causes cell death in human hepatoma by inducing DNA damage, 2020, doi: 10.21203/rs.3.rs-54193/v1. Available at: https://www.researchgate.net/publication/343707479_Blue_LED_causes_cell_death_in_human_hepatoma_by_inducing_DNA_damage, Retrieved October 14, 2021.
[18] O. Hosten, P. Kwiat, Observation of the spin hall effect of light via weak measurements, Science 319 (2008) 787–790.
[19] C. Emary, Scientists discover fundamental property of light –150 years after, Maxwell, 2015. Available at: http://theconversation.com/scientists-discover-fundamental-property-of-light-150-years-after-maxwell-43928, Retrieved Agust 13, 2020.
[20] A. Mamalis, M. Garcha, J. Jagdeo, Light emitting diode-generated blue light modulates fibrosis characteristics: fibroblast proliferation, migration speed, and reactive oxygen species generation, Lasers Surg. Med. 47 (2015) 210–215.
[21] P.S. Oh, H.S. Kim, E.M. Kim, H. Hwang, H.H. Ryu, S. Lim, M.H. Sohn, H.J. Jeong, Inhibitory effect of blue light emitting diode on migration and invasion of cancer cells, J. Cell Physiol. 232 (2017) 3444–3453.
[22] S. Breslin, L. O'Driscoll, Three-dimensional cell culture: the missing link in drug discovery, Drug Discov. Today 18 (2013) 240–249.
[23] P. Neuzi, S. Giselbrecht, K. Lange, T.J. Huang, A. Manz, Revisiting lab-on-a-chip technology for drug discovery, Nat. Rev. Drug Discov. 11 (2012) 620–632.