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Original Research

Biphasic Dose/Response of Photobiomodulation Therapy on Culture of Human Fibroblasts

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Abstract

***Objective*:** The objective of this study was to evaluate the effects of application of different fluences and energies of laser in the 24-, 48-, and 72-h periods in fibroblasts originating from human skin (HFF-1).
***Methods*:** The cell used as a template for cell proliferation was HFF-1. For the photobiomodulation (PBM) application, a 660 nm laser with a power of 40 mW and energies of 0.84, 1.40, 5.88, and 6.72 J was used. Five experimental groups were studied: one control group (CG) with simulated PBM and four groups that received PBM in different doses. The changes observed after laser irradiation were evaluated by cell viability (trypan blue) and proliferation [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)] tests. Intergroup comparisons were performed using two-way analysis of variance and the Tukey post hoc test (software GraphPad Prism 7.0).
***Results*:** In the trypan blue test, the total number of cells was significantly different between the irradiated groups and the CG at all times studied. The total number of cells increased in laser group (LG)1 (0.84 J) and LG2 (1.40 J) and decreased in LG4 (6.72 J). The mitochondrial activity increased significantly in LG1 and LG2 at 48 and 72 h and decreased in LG3 (5.88 J) and LG4 (6.72 J) compared with CG.
Conclusions: The results indicate that the lower doses (0.45 and 0.75 J/cm^2) of PBM induce the highest mitochondrial activity and cellular viability.

Keywords: dose/response curve, photobiomodulation, red laser, fibroblasts, *in vitro* study

Introduction

Laser photobiomodulation (PBM), also known as low-level laser therapy, is a widely used alternative for clinical treatments with emphasis on regeneration of several tissues, especially the cutaneous.[1–4] PBM is thought to act in the tissue repair process through mechanisms modulating inflammatory processes, in addition to proliferation of endothelial cells and fibroblasts, thus forming skin tissue with characteristics closer to healthy tissue, and acting to accelerate the process resolution.[5–7]

The literature indicates that fibroblast proliferation occurs more rapidly when they are exposed to PBM, especially with red light at appropriate doses.[2,5,8] It is believed that PBM has biostimulatory action, converting light energy into metabolic and thus triggering several factors that contribute to the process of cell proliferation, such as the increase of reactive oxygen species and nitric oxide, besides a higher production of adenosine triphosphate (ATP).[9,10] However, it is important to note that the effects are only possible within an ideal therapeutic window characterized by a specific answer for each tissue and/or cell type, and in this regard, the literature still presents a large discrepancy in the parameterization, generating conflicts over the therapeutic effects provided by the technique, as well as incomplete information on what molecular mechanisms are actually involved in achieving the benefits reported.[11]

Nowadays, there is already pre-established knowledge that PBM therapy may present stimulatory and inhibitory results, as well as not producing effects depending on the dose used, resulting in the so-called biphasic dose/response.[6] The principle of the biphasic response states that a very low dose of light has no effect and a slightly higher dose generates a positive effect until a threshold is reached. If the

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dose of light is increased beyond that point, the benefit progressively decreases until the baseline (no effect) is reached, and further increases will begin to have detrimental effects on the tissue.[5,6]

The fact that it is a ''dose-dependent'' technique raises great discussions about its efficacy and about the molecular mechanisms of action that are activated with certain parameters, which often makes it difficult to compare the results between the protocols presented in the current literature. It is essential to mention that the biphasic response is able to positively and safely direct the choice of the parameters to be applied, and such knowledge favors and facilitates the application of this therapy in the clinical area, as the tendency to standardize the different protocols grows.[6,9]

Thus, the present study aimed to evaluate different doses of laser energy to establish a ''dose/response curve'' in human fibroblasts to determine which doses provide positive stimuli related to the activation of cell proliferation and which do not produce effects or have inhibitory effects in this cell type.

Methodology

The present study was approved by the Ethics Committee of the Federal University of São Carlos under the protocol no. 9715040416. The cells used in the study were from the human fibroblast HFF-1 line provided by the National Institute of Optics and Photonics Science and Technology—INCT - INOF. under the coordination of Prof. Dr. Vanderlei Bagnato from the Institute of Physics of the University of São Paulo (USP), São Carlos campus.

Cell culture

HFF-1 cells (human skin fibroblasts) were cultured in 75 cm^2 T-bottles (Greiner) containing DMEM (Sigma-Aldrich), supplemented with 10% fetal bovine serum (Hyclone), 1% penicillin/streptomycin antibiotic (Gibco), L-glutamine, HEPES, and bicarbonate (Sigma-Aldrich). The T vials were maintained at 37°C with 5% CO_2 in an incubator (Thermo Scientific). After reaching the appropriate number of cells, the cell contents were transferred to 24-well plates, with 2×10^4 cells (Kasvi, Brazil) being inoculated into each well. After inoculation, the plates were maintained with culture medium under the conditions mentioned above. To perform irradiation with PBM, the plates were removed from the incubator and then returned to the controlled environment. The same procedure was applied to the control group (CG) to guarantee the reproducibility of the data. The whole experiment was performed in triplicate in three independent experiments.

Irradiation with PBM

A red laser was used with a wavelength of 660 nm, a power of 40 mW, and a diode of aluminum gallium indium phosphorus (InGaAIP) (Photon Laser III, DMC, São Carlos, Brazil), with energies of 0.84 J, 1.4 J, 5.88 J, and 6.72 J (Table 1).

The plates were irradiated in three distinct periods (24, 48, and 72 h after inoculum). Thus, plate #1 received only one irradiation 24 h after inoculation, plate #2 received two irradiations, 24 and 48 h after inoculation, and plate #3 received three irradiations, 24, 48, and 72 h after inoculation. The laser tip was directed perpendicular to each well and remaining fixed by a holder with a distance of 3.34 cm from the tip of the laser probe to the bottom of the well to allow the irradiation to cover the full diameter of the well of interest. The distance was calculated using a light diffuser to adjust the parameters to ensure their delivery, described in Table 1. It is important to note that the cells were seeded so that a well, among the seeded, was left empty to prevent the intentional dispersion of light between the wells.[2]

Table 1. InGaIP Laser Parameters Used

Manufacturer	DMC®
Model identifier	Photon Laser III
Year produced	2010
Number and type of emitters (laser or LED)	Laser
Wavelength and bandwidth (nm)	660
Pulse mode (CW or Hz, duty cycle)	CW
Irradiance at target (mW/cm^2)	21.5
If pulsed peak irradiance (mW/cm^2)	—
Exposure duration (sec)	21; 35; 147; 168
Radiant exposure (J/cm^2)	0.45; 0.75; 3.16; 3.61
Radiant energy (J)	0.84; 1.4; 5.88; 6.72
Number of points irradiated	1
Area irradiated (cm^2)	1.86
Application technique	Punctual
Number and frequency of treatment sessions	Three sessions (each 24 h)
Total radiant energy per group at each time (J)	24 h: 0.84 (LG1); 1.4; 5.88; 6.72 48 h: 1.68; 2.8; 11.76; 13.44 72 h: 2.52; 4.2; 17.64; 20.16

CW, continuous wave; LED, light-emitting diode; LG, laser group.

Experimental groups

The study was divided into four groups, three groups being irradiated by PBM and one simulated group:

CG: HFF-1 with simulation of PBM therapy.

Laser group 0.45 J/cm^2 (LG1): HFF-1 irradiated by PBM (660 nm) with 0.84 J of energy.

Laser group 0.75 J/cm^2 (LG2): HFF-1 irradiated by PBM (660 nm) with 1.4 J of energy.

Laser group 3.16 J/cm^2 (LG3): HFF-1 irradiated by PBM (660 nm) with 5.88 J of energy.

Laser group 3.61 J/cm^2 (LG4): HFF-1 irradiated by PBM (660 nm) with 6.72 J of energy.

Proliferation and cell viability

For the evaluation of proliferation, the tetrazolium 3- colorimetric method was performed [3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT); Sigma-Aldrich]. After 4 h of incubation, the absorbance was read on a Multiskan FC microplate reader (Thermo Scientific®) with a wavelength of 570 nm.

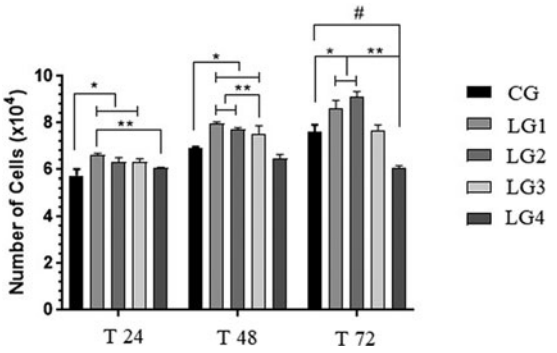

FIG. 1. Evaluation of cell growth through the trypan blue assay at different times in all groups. *Significant difference compared with the control ($p < 0.05$). **Significant difference compared with the LG3 and LG4. #Significant difference when compared with CG×LG4. CG, control group; LG, laser group.

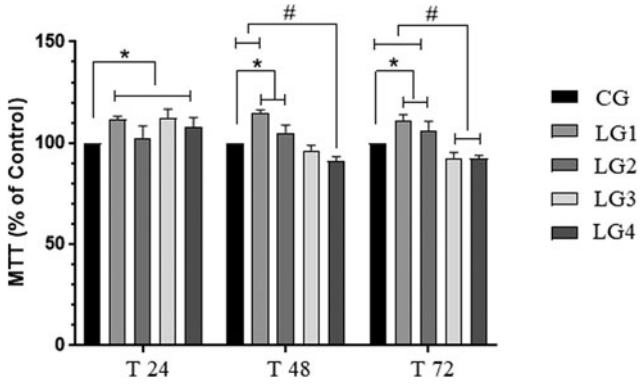

FIG. 2. Percentage of proliferation of all groups at different times when compared with the control. *Significant difference ($p < 0.05$). #Significant difference between irradiated groups ($p < 0.05$).

For the analysis of viability, the trypan blue dye exclusion method (0.4% solution; Gibco) was used. Cell density was determined by hemocytometer counting. Medium from each inoculated well was removed, and immediately these wells were washed with PBS and then incubated with TrypLE™ cell dissociation enzyme (Gibco). After this process, 20 μL aliquots were taken, where 20 μL of a 0.4% (m/v) previously prepared trypan blue solution was added. After stirring, the mixture was deposited on the hemocytometer for counting using a model BX51 microscope (Olympus, Japan). Independent triplicates were performed by analyzing six wells per group in each experiment. Cell counting was performed by two previously calibrated blinded examiners. All evaluations were performed 24 h after PBM irradiation, respecting the experimental times of 24, 48, and 72 h, totalizing three PBM applications. Microscopic images of cells adhered to the plates were obtained throughout the experiment (24, 48, and 72 h) (Fig. 3B).

Statistical analysis

Homogeneity and normality analyses were performed by the Levene's and Shapiro–Wilk's tests, respectively. To determine the differences between the experimental groups, the two-way analysis of variance test and Tukey's post hoc test were used. The values presented were considered significant when $p \leq 0.05$. For this purpose, the GraphPad Prism software was used (version 7.04 for Windows).

Results

Figure 1 shows the cell growth results evaluated by the trypan blue exclusion test at different times and groups. When comparing the means of the number of cells, we can observe that there are significant differences between the irradiated groups and the CG at all times. After the first 24 h of irradiation, LG1 ($6.6 \times 10^4 \pm 0.08$), LG2 ($6.3 \times 10^4 \pm 0.2$), and LG3 ($6.3 \times 10^4 \pm 0.1$) presented higher cell growth than the CG ($5.7 \times 10^4 \pm 0.2$). In addition, lower LG4 cell growth ($6 \times 10^4 \pm 0.03$) was also observed compared with LG1. At 48 h after irradiation, the highest cell growth was maintained for the same groups (LG1, LG2, and LG3) in comparison with the CG, noting that LG1 ($7.9 \times 10^4 \pm 0.06$) and LG2 ($7.7 \times 10^4 \pm 0.07$) presented higher results than LG4 ($6.4 \times 10^4 \pm 0.1$). At the end of the experimental period (72 h), only LG1 ($8.6 \times 10^4 \pm 0.3$) and LG2 ($9.1 \times 10^4 \pm 0.2$) had significantly higher cell growth than the CG ($7.6 \times 10^4 \pm 0.3$). In LG4 ($6 \times 10^4 \pm 0.1$), there was a significant reduction in the number of cells when compared with the other groups.

Still, the trypan blue exclusion test was able to calculate cell viability, and the data are shown in Table 2. The results showed that all irradiated groups, with the exception of LG4, showed cellular viability similar to the CG, demonstrating that PBM in these parameters did not alter the cellular characteristics, while LG4 presented a significant reduction of viability in relation to the CG at 48 and 72 h.

The LG1 (111% ± 1.2%), LG3 (112% ± 4.2%), and LG4 (108% ± 4.3%) in the 24-h period presented a significant improvement in the proliferation percentage obtained through the MTT test (Fig. 2), significantly higher than CG. At 48 and 72 h, it was observed that LG1 (115% ± 1.2% and 111% ± 2.8%) and LG2 (105% ± 3.5% and 106% ± 4.4%) showed a significant increase than the CG. The LG3 (96% ± 2.3%) and LG4 (91% ± 1.9%) showed a significant

Table 2. Mean Percentage of Viability of Human Fibroblasts from the HFF-1 Lineage Assessed by the Trypan Blue Method After Photobiomodulation at the Times and Groups Evaluated

	CG	*LG1*	*LG2*	*LG3*	*LG4*
T 24	99.6 ± 0.63	99.7 ± 0.58	99.7 ± 0.58	97.6 ± 2.28	98.6 ± 1.57
T 48	99.0 ± 0.99	99.3 ± 1.15	99.3 ± 1.17	96.1 ± 2.08	91.5 ± 2.70*
T 72	99.7 ± 0.58	100 ± 0.00	99.7 ± 0.42	91.7 ± 1.76*	87.9 ± 3.1*†

*Significant difference compared with control ($p < 0.0001$).
†Significant difference compared with LG3 ($p < 0.039$).
CG, control group.

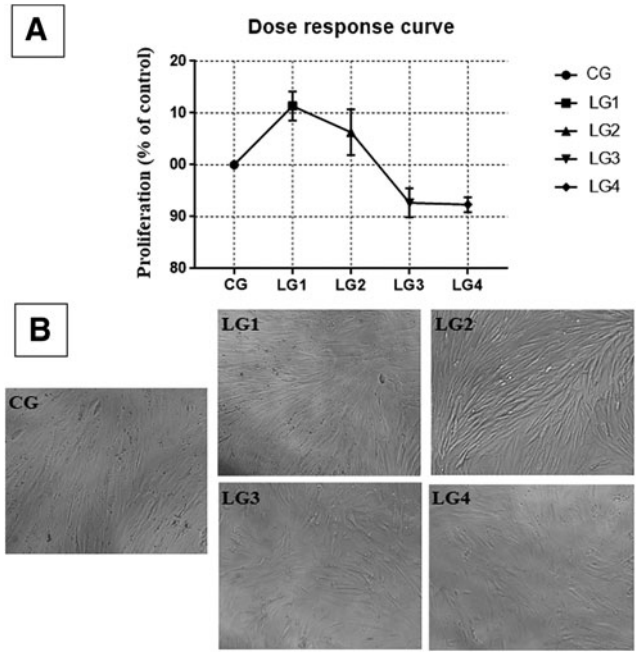

FIG. 3. **(A)** Dose/response curve. Proliferation of HFF-1 lineage fibroblasts expressed as a percentage compared with CG after 72 h of irradiation at different fluences. **(B)** Photomicrographs representative of the experimental groups regarding cell proliferation at 72 h after irradiation.

decrease in proliferation 72 h after the first irradiation compared with the CG.

Figure 3A shows the results of the proliferation at the end of the experimental period, and a dose/response curve was elaborated, demonstrating that the energy accumulation of LG1 and LG2 was stimulatory for the HFF-1, the energies of LG3 and LG4 may have been detrimental to cell activity. In Fig. 3B, photomicrographs of the cell cultures are also shown at 72 h, showing higher cell growth in the CG, LG1, and LG2 compared with LG3 and LG4.

Discussion

PBM is a term intended for the use of light sources or nonionizing and nonthermal biophotonic irradiation with low potencies that are capable of interacting with the biological tissue environment and triggering interesting therapeutic responses such as analgesia, inflammation modulation, scarring, and/or stimulation regeneration in different tissues.[1,4,7,10,12–15]

The objective of the present study was to evaluate the cellular behavior (human fibroblast lineage HFF-1) against the exposure of different doses of fluence and consequently energy. The literature indicates that the use of PBM with laser light is capable of stimulating intrinsic biological processes related to the cell cycle, such as proliferation, differentiation, and growth. In addition, it demonstrates a wide range of investigations with expressive results mainly related to the process of regeneration and/or tissue healing in the most diverse pathologies.[13,16–19] Similarly, they also present the action of phototherapy in in vitro models with different cell types such as fibroblasts, keratinocytes, endothelial cells, skeletal cells, and myofibroblasts, among others.[7,20–22] In contrast, despite the diversity of studies and reports currently found in the scientific field, few authors explore the biphasic response or dose/response curve related to cellular behavior versus the application of different doses of energy at the same wavelength.

When we mention the application of laser light in a therapeutic way, we induce the understanding of the use of light in the spectrum comprising red and near infrared, that is, between 600 and 1100 nm. This type of irradiation can be either continuous or pulsed and consists of a low and constant energy density (0.04–50 J/cm^2) directed to a specific tissue or cell monolayer using powers measured in mW.[4] Still within this range of wavelengths, Karu[10] describes the occurrence of some biostimulatory effects. Such effects are closely related to the absorption of light by specific photoacceptors, considered the main cytochrome C oxidase, located inside the mitochondria. After this absorption, the electron transport chain is stimulated, inducing an increase in the rate of cellular respiration and cellular metabolism, resulting in increased ATP production and DNA and RNA synthesis, providing both the improvement of nutrition and oxygenation activity as a whole.

Hawkins et al.[6] present in detail the mechanisms induced by phototherapy and expose the importance of the dose/response curve. To better understand the biphasic effects provided by cell exposure to laser light, Arndt-Schulz's law is applied, where a fundamental principle includes the relationship between applied dose and observed effects. Thus, the law states that weak stimuli are able to slightly accelerate cellular vital activity, and moderate stimuli increase activity, but at a given moment a peak is reached and thereafter, if stimuli increase, the process is suppressed until an inhibitory response is achieved.

The results found in the present study fit exactly into the effects described by the Arndt-Schulz law. At doses with lower creep (30 and 50 J/cm^2), it was possible to observe positive effects such as stimulation and cell proliferation, and at the highest dose (240 J/cm^2), the cell inhibition process was observed. Interestingly, the study by Maldaner et al.[20] also presents the use of laser (660 nm) with a power close to that used in the present work, but with much lower rates of fluids (3, 4, 5, 6, and 8 J/cm^2) in pulsed mode applied in both normal fibroblasts and in senescent fibroblasts. The authors reported that cytogenetic, anti-inflammatory, and proliferative effects were found in both cell types, and that the laser light in these parameters was able to partially or totally reverse the deleterious effects caused by H_2O_2 in the senescent cells.

Also in this sense, Ayuk et al.[23] in a study using normal fibroblasts and fibroblasts representing the conditions of diabetic hypoxia treated with laser at two different wavelengths, 660 and 830 nm (108 and 94 mW, respectively, and 5 J/cm^2), pointed out that both lasers were able to reduce the proteolytic environment by increasing the expression of the tissue inhibitor, metalloproteinase 1 (TIMP-1), and reducing the expression of matrix metalloproteinases (MMP-3 and MMP-9), further increasing collagen expression levels of type I. Similarly to this, Hawkins et al.[6] also concluded in their study with culture of human skin fibroblasts that a single dose of 5 J/cm^2 or multiple exposures with 2.5 J/cm^2

promote stimulation of wound healing and stimulate mitochondrial activity with fibroblast proliferation without damaging the cells.

Faced with such information it becomes indispensable to compare the doses used and also the effects found. To facilitate this correlation of parameters, we direct attention to the applied energy values, since all studies above present analyses in fibroblast lineage. In the present study, we investigated the energies of 0.84, 1.4, 5.88, and 6.72 J. On the contrary, Maldaner et al.[20] also evaluated the relatively lower energies in the same cell type (HFF-1) compared with the present study (0.35–0.98), concluding that lower energy provides beneficial effects related to cell proliferation, while higher energy may induce the increase of factors related to apoptosis. In contrast, the study by Moore et al.[24] points out that wavelengths within the red spectrum, especially close to the range of 665 nm, are effective in stimulating fibroblast activity, unlike the wavelength of 810 nm (infrared), which showed inhibitory characteristics in the cell cycle.

Thus, within the presented, it is possible to emphasize that the studies of Maldanar et al.[20] partially corroborate our findings if we direct the vision for the amount of deliberate energy in the culture of fibroblasts. It is also known that the literature presents in general the dose in values of fluency and that this often confuses the real comparison, because the values of fluency are always linked to the laser models used, and each company has a type of design showing differences in the area of the light beam. However, when the comparison is directed to the total energy applied, it facilitates the understanding, because despite the different powers and energies presented by the protocols, the majority at the end has an energy value in a near range.[4]

Despite this difficulty in standardizing both the protocols and the expression of values by the authors in the literature, it is essential to emphasize that dose/response (light and cellular response) is the primary factor and should be taken into account when choosing the treatment protocol to be used in both the scientific and clinical settings. Huang et al.[11] point out that the biochemical mechanisms underlying the positive effects of the use of PBM are still incompletely understood, and the diversity in the choice of parameters for a single application protocol led to the publication of scientific articles with both positive and negative effects, making eligibility difficult to choose. In addition, the authors emphasize the importance of understanding the biological mechanisms involved and the orientation of choice in the biphasic dose/response curve (low doses are beneficial and high doses promote deleterious effects); however, the high dose that they refer to is in the range between 50 and 100 J/cm^2, and due to this, it contrasts with the results found by our research group, since doses up to 50 J/cm^2 were beneficial for the cellular environment, being only deleterious from 210 J/cm^2.

In view of this, it is prioritized that the knowledge of cellular behavior related to its viability and proliferation is indispensable for the formation of new protocols of studies and applications. It is hoped that in the near future, the information related to the molecular mechanisms involved in this dose/response will be further explored so that the union of acquired information benefits both the basic research and the standardization of the protocols used in the clinical environment.

Conclusions

Based on the results found in the present study, it was possible to conclude that the evaluation and knowledge of the dose/response curve can be of great value in the choice of protocols to be proposed and can be used in the scientific and clinical area in different types of pathology. The isolated evaluation of a cell type and its behavior at different doses contributes to the understanding of how the application of the therapy could benefit the cellular environment when in a specific tissue. Thus, the present study confirms the information found in the literature and describes the principles of the Arndt-Schulz law, in which smaller doses provide positive stimuli to the cellular environment and expressively higher doses cause deleterious effects inhibiting cellular activity.

Author Disclosure Statement

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References

1. Otterço AN, Andrade AL, Brassolatti P, Pinto KNZ, Araújo HSS, Parizotto NA. Photobiomodulation mechanisms in the kinetics of the wound healing process in rats. J Photochem Photobiol B Biol 2018;183:22–29.
2. de Andrade ALM, Luna GF, Brassolatti P, et al. Photobiomodulation effect on the proliferation of adipose tissue mesenchymal stem cells. Lasers Med Sci 2018;32:1–7.
3. Brassolatti P, Bossini PS, Oliveira MCD, et al. Comparative effects of two different doses of low-level laser therapy on wound healing third-degree burns in rats. Microsc Res Tech 2016;79:313–320.
4. Avci P, Gupta A, Sadasivam M, et al. Low-level laser (light) therapy (LLLT) in skin: stimulating, healing, restoring. Semin Cutan Med Surg 2013;32:41–52.
5. Mignon C, Uzunbajakava NE, Castellano-Pellicena I, Botchkareva NV, Tobin DJ. Differential response of human dermal fibroblast subpopulations to visible and near-infrared light: potential of photobiomodulation for addressing cutaneous conditions. Lasers Surg Med 2018; 50:859–882.
6. Hawkins D, Houreld N, Abrahamse H. Low level laser therapy (LLLT) as an effective therapeutic modality for delayed wound healing. Ann N Y Acad Sci 2005;1056: 486–493.
7. Engel KW, Khan I, Arany PR. Cell lineage responses to photobiomodulation therapy. J Biophotonics 2016;9:1148–1156.
8. Kreisler M, Christoffers AB, Al-haj H, Willershausen B, Hoedt B. Low level 809-nm diode laser-induced in vitro stimulation of the proliferation of human gingival fibroblasts. Lasers Surg Med 2002;369:365–369.
9. Hamblin MR. Mechanisms and mitochondrial redox signaling in photobiomodulation. Photochem Photobiol 2018; 94:199–212.
10. Karu T. Mitochondrial mechanisms of photobiomodulation in context of new data about multiple roles of ATP. Photomed Laser Surg 2010;28:159–160.

11. Huang Y, Sharma SK, Carroll J, Hamblin MR. Biphasic dose response in low level light therapy—an update. Dose Responde 2011;9:602–618.
12. Karu T, Pyatibrat L, Kalendo G. Irradiation with HeNe laser increases ATP level in cells cultivated in vitro. J Photochem Photobiol B Biol 1995;27:219–223.
13. Bossini PS, Rennó ACM, Ribeiro DA, et al. Low level laser therapy (830nm) improves bone repair in osteoporotic rats: similar outcomes at two different dosages. Exp Gerontol 2012;47:136–142.
14. Renno ACM, Iwama AM, Shima P, et al. Effect of low-level laser therapy (660 nm) on the healing of second-degree skin burns in rats. J Cosmet Laser Ther 2011;13: 237–242.
15. Chung T, Sc BM, Baxter GD, Phil D. Laser photobiomodulation of proliferation of cells in culture: a review of human and animal studies. Photomed Laser Surg 2010; 28 Suppl 1:S3–S40.
16. Liebert MA, Claudia ANA, Renno M, et al. Effects of 830-nm laser, used in two doses, on biomechanical properties of osteopenic rat femora. Photomed Laser Surg 2006;24:202–206.
17. Oliveira PC, Meireles GCS, dos Santos NR, et al. The use of light photobiomodulation on the treatment of second-degree burns: a histological study of a rodent model. Photomed Laser Surg 2008;26:289–299.
18. Choi K, Kang B-J, Kim H, et al. Low-level laser therapy promotes the osteogenic potential of adipose-derived mesenchymal stem cells seeded on an acellular dermal matrix. J Biomed Mater Res Part B Appl Biomater 2013;101:919–928.
19. Kelly S, Szezerbaty F, De Oliveira RF, et al. The effect of low-level laser therapy (660 nm) on the gene expression involved in tissue repair. Lasers Med Sci 2018;33: 315–321.
20. Maldaner DR, Azzolin VF, Barbisan F, et al. In vitro effect of low-level laser therapy on the proliferative, apoptosis modulation, and oxi-inflammatory markers of premature-senescent hydrogen peroxide-induced dermal fibroblasts. Lasers Med Sci 2019;34:1333–1343.
21. Mamalis A, Siegel D, Jagdeo J. Visible red light emitting diode photobiomodulation for skin fibrosis: key molecular pathways. Curr Dermatol Rep 2016;5:121–128.
22. Alghamdi KM, Kumar A. Low-level laser therapy: a useful technique for enhancing the proliferation of various cultured cells. Lasers Med Sci 2012;27:237–249.
23. Ayuk SM, Abrahamse H, Houreld NN. Photobiomodulation alters matrix protein activity in stressed fibroblast cells in vitro. J Biophotonics 2018;11. DOI: 10.1002/jbio.201700127.
24. Moore P, Ridgway TD, Higbee RG, Howard EW, Lucroy MD. Effect of wavelength on low-intensity laser irradiation-stimulated cell proliferation in vitro. Lasers Surg Med 2005; 36:8–12.

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