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ORIGINAL ARTICLE

Photobiomodulation effect on the proliferation of adipose tissue mesenchymal stem cells

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Abstract

The use of mesenchymal stem cells (MSCs) in tissue engineering has been extensively investigated. The greater the proliferation of this cellular group, the greater the regenerative and healing capacity of the tissue to which they belong. In this context, photobiomodulation (PBM) is an efficient technique in proliferation of distinct cell types. However, its parameters and mode of action are still unclear and require further investigation. This study aimed to evaluate the PBM action with different energies in MSCs of adipose tissue (hASCs). We used hASCs, seeded in 24-well plates, with 3×10^4 cells per well, in culture media. We used a total of four experimental groups, one with hASCs and simulated PBM and three other groups, which received PBM irradiation at 24, 48, and 72 h, with a 660-nm laser and power of 40 mW and energy of 0.56, 1.96, and 5.04 J. We performed analyses of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromidefor) and trypan blue to evaluate cell proliferation and viability, 1 h after PBM irradiation. Software Graph PadPrism 7.0 was used. Intergroup comparisons were performed with ANOVA two-way and we used the Tukey post hoc test. Mitochondrial activity evaluated by MTT revealed the statistical difference in the first 24 h for group with more high energy when compared to control group; and in the 72 h for two irradiated groups when compared to the control group. The trypan blue test showed significant differences at the end of the experiment for two irradiated groups LG1 ($4.52 \times 10^4 \pm 0.2$) and LG2 ($4.85 \times 104 \pm 0.8$), when compared to the control group ($1.87 \times 10^4 \pm 0.7$). Both tests failed to be statistically different at the end of the experiment for groups LG1 and LG2 and observed a reduction in cellular mitochondrial growth and activity for group LG3. We conclude that PBM with energy close to 0.56 and 1.96 J promote proliferation of hASCs, and higher energy, such as 5.04 J, can be harmful.

Keywords Photobiomodulation · Mesenchymal stem cells

Introduction

The use of mesenchymal stem cells (MSCs) has been widely investigated in different areas of tissue engineering and regenerative medicine as an alternative to conventional therapeutic methods. Their multipotentiality offers applications in cell therapy, in addition to their ability to self-renewal, proliferation, and differentiation into several specialized cell types like adipocytes, chondrocytes, and osteoblasts [1]. These cells are easily isolated, expanded, and cultivated in vitro and can be obtained from several tissues, such as bone marrow, dental pulp, periodontal ligament, umbilical cord, and adipose tissue [2–5].

Adult stem cells can be isolated from adipose tissue liposuctioned in significant numbers and show stable growth and proliferative kinetic in culture [2, 6, 7]. MSCs derived from

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adipose tissue (hASCs) have differentiation potential similar to those derived from bone marrow (BMSCs), and their regenerative potential is due mainly to this multipotentiality and they are capable to originate distinct cell types, such as mesodermal tissues like bone, cartilage, muscle, tendon/ligament, fat, and derm. They are, therefore, a potential source for future application in tissue engineering in clinical context [7].

Despite of its high differentiation potential, MSCs have low proliferative rate; however, since this cellular group is highly applied in regenerative medicine, the establishment of methods able to accelerate their proliferation process is a crucial step to keep cells viable.

Literature shows that the greater the proliferation of this cellular group, the greater the regenerative and healing capacity of the tissue to which they belong. In this context, we can highlight the photobiomodulation (PBM) therapy, which is an efficient therapy to promote proliferation of several cell types such as fibroblasts [8, 9], endothelial cells [8], osteoblasts [10], epithelial cells [11], lymphocytes [12], and MSCs [3].

PBM therapy has gained field due to its use of low fluency and wavelength, easy penetration, resulting in biomodulators effects in cells and tissues, with the ability to stimulate higher cell proliferation through absorption of light by the intracellular chromophores, with this light being converted into metabolic energy [2, 13].

Regarding MSCs proliferation, PBM has shown positive and promising effects; however, the biologic mechanisms of these effects remain partially unclear. PBM with wavelengths of 600 to 700 nm stimulates higher proliferation of MSCs, in addition to their differentiation [7, 9, 14]. However, due to the variety of factors that influence the process, biostimulation is not always observed, which means that the interaction of the laser light with the cells and tissues can stimulate or inhibit cell proliferation [15]. Therefore, in vitro biostimulation depends on the PBM parameters, such as wavelength, energy, power and time of irradiation [16], type of irradiated cell [9], and cellular physiological features [17]. It is hypothesized that the knowledge of both the behavior of the cells against the exposition of PBM and of the best protocol of application in the in vitro model can assist in understanding the real biological mechanisms triggered by the light/cellular environment interaction and with this, potentiate the events, which comprise cell proliferation. Thus, the aim of this study was to investigate the action of PBM in different energies applied in adipose tissue MSCs (in vitro), in order to choose which of the parameters employed contribute positively to the stimulus of cell proliferation.

Methodology

The present study was approved by the Ethics Committee of the Federal University of São Carlos under protocol No.6224231115. We obtained hASCs from abdominal surgeries performed in the Hospital and Clinics of the College of Medicine of Ribeirão Preto; samples were donated by the patients upon signing of consent form. Cells were isolated through digestion of extracellular matrix (ECM) in 0.075% collagenase (Sigma-Aldrich, St. Louis, USA) for 30 min at 37 °C, according to protocol previously established [6]. The digestion was neutralized with Dulbecco's Modified Eagle Medium (DMEM, Invitrogen, Carlsbad, CA, USA), with 10% fetal bovine serum (FBS, Invitrogen, Carlsbad, CA, USA).

The adipose tissue was centrifuged and then plated on tissue culture plate. The hASCs were cultivated and expanded at 37 °C, with 5% CO_2, using culture medium (α-MEM from Gibco, Tastrup, Copenhagen, Denmark, 10% fetal bovine serum, 1% antibiotic-antimycotic from Gibco, Tastrup, Copenhagen, Denmark, and L-Glutamine 200 mM). The medium was changed twice a week, and cells were transferred at 80% confluence, using 0.25% trypsin-EDTA (Invitrogen, Carlsbad, CA, USA). The study used only the 4th and 5th transfers.

Characterization was performed and reported by Caetano et al. (2015) [18], and flow cytometry reported hASCs positivity for CD29, CD44, CD73, CD90, and CD105 (BD Biosciences, San Jose, CA, USA), which are stem cell markers. Cells were then cultivated in osteogenic and adipogenic differentiation media (differentiation kits, StemPro®, Invitrogen Corp., USA) for 21 to 28 days, and their multipotent nature was confirmed with optical microscopy.

hASCs culture

Each well received 1×10^4 cells, using culture plates with 24 wells (TPP, Trasadingen, Switzerland), which were kept in culture medium composed of low glucose Dulbecco's Modified Eagle Medium (Invitrogen), supplemented with 10% fetal bovine serum (SFB)—both supplied by Nutricell®, São Paulo, Brazil—and antibiotic-antimycotic (A/A) (Gibco, Tastrup, Copenhagen, Denmark). After seeding, cultures were incubated at 37 °C, 5% CO_2, until removed for irradiation. The same procedure was adopted for the control group (CG). The experiment was performed in triplicate to ensure reproducibility.

PBM irradiation

We used red laser, diode aluminum gallium indium phosphorus (InGaAIP) (Photon Laser III, DMC São Carlos/SP, Brazil) with wavelength of 660 nm, power of 40 mW, beam transverse area of 0.028 cm^2, and energy of 0.56 J, 1.96 J, and 5.04 J (Table 1).

It is important to highlight that the parameters of power, wavelength, and energy were based on a study by Ginani et al. (2015) [16], and that the choice of wavelength was related to the interest of studying the cellular behavior before the red light stimulus to be implemented in skin lesions, since Avci et al. (2013) [18] report that such wavelength is capable to penetrate 1–4 when applied to the tissue and that this would benefit the treatment of cutaneous tissue.

Plates were irradiated after 24, 48, and 72 h after inoculation. We evaluated cell viability and proliferation for each established time. The laser equipment was fixed on a bench stand in a perpendicular direction so that all the irradiations performed obtained the same distance between the laser emitting tip and the well of interest. The distance was previously calculated and determined in order to allow the irradiation to cover the full diameter of the well of interest and therefore it was established that 3.34 cm measured between the laser emission tip and the bottom of the well (Fig. 1). Cells were seeded every other well to avoid intentional dispersion of light between the wells.

Design

The study was divided in four groups, three of them irradiated with PBM and one simulated.

Control Group (CG): hASCs with simulated PBM. Plates from this group were withdrawn from the culture conditions (37 °C, 5% CO_2) and positioned below the PBM equipment for 63 s (mean treatment time of the other groups); however, the equipment was switched off.

Laser Group 1 (LG1): hASCs irradiated with PBM (660 nm) and 0.56 J energy

Laser Group 2 (LG2): hASCs irradiated with PBM (660 nm) and 1.96 J energy

Laser Group 3 (LG3): hASCs irradiated with PBM (660 nm) and 5.04 J energy

Cytotoxicity and cell viability

We used the MTT cytotoxicity assay to analyze mitochondrial activity in order to evaluate the cell proliferation. The assay involves the conversion of 3-[4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich, St. Louis, USA) into an insoluble formazan. Absorbance reading was performed after 4 h of incubation, in a *Multiskan FC* (Thermo Scientific®, USA) microplate reader, with 570 nm.

We evaluated cell viability and growth with trypan blue test (Gibco, Tastrup, Copenhagen, Denmark), in which cells are marked with trypan blue in each well and are counted in a Neubauer chamber. Cell count was performed by two blind (biochemical) examiners, with respect to the groups, and previously calibrated.

Table 1 InGalP laser parameters used

Parameters	Values
Power (mW)	40
Wavelength (nm)	660
Mode of action	Continuous
Beam transverse area (cm^2)	0.028
Energy density (J/cm^2)	20, 70, 180
Time (s)	14, 49, 126
Energy (J)	0.56, 1.96, 5.04

All evaluations were performed 1 h after PBM irradiation, in a total of three applications.

Statistical analyses

The results were expressed as average ± standard deviation. The result analysis was performed with the Software Graph PadPrism 7.0 (San Diego, CA, USA). We performed the Saphiro Wilk test to assess the data normality, and the data presented normal distribution, thus was used for comparisons between groups, the parametric test of the ANOVA two-way. For multiple comparisons, we used the Bonferroni post hoc test with significance level of $p < 0.05$.

The effect size was calculated considering the effect strength as small (r value of 0.10 to 0.29), mean (r value of 0.30 to 0.49), and large (value of $r \geq 0.50$).

Results

Results of the trypan blue assay revealed no significant differences in cell growth between the groups after inoculation, except for LG3. Group LG2 ($2.79 \times 10^4 \pm 0.3$) showed significant difference when compared to the CG ($1.79 \times 10^4 \pm 0.4$) after 48 h. This difference was maintained after 72 h (LG2: $4.85 \times 10^4 \pm 0.8$; CG: $1.87 \times 10^4 \pm 0.7$). We also observed a

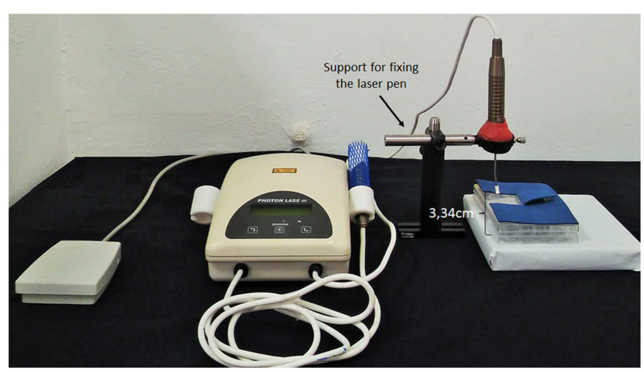

Fig. 1 PBM irradiation. InGalP laser (Photon Laser III, DMC®) fixed to a bracket for maintenance of the equipment fixing. A 24-well plate, 3.34 cm distance from the tip of the pen to the bottom of the well

significant growth of group LG1 ($4.52 \times 10^4 \pm 0.2$) in this same period, compared to group CG ($1.87 \times 10^4 \pm 0.7$) (Fig. 2).

It is important to highlight that LG3 ($2.21 \times 10^4 \pm 0.4$) presented, in the first 24 h, cell growth superior to the CG ($1.05 \times 10^4 \pm 0.2$); however, after 48 h, we observed inhibition (LG3: $1.45 \times 10^4 \pm 0.4$), which persisted to the end of the experimental period (LG3: $1.87 \times 10^4 \pm 0.4$) (Fig. 2).

Trypan blue assay did not reveal significant differences regarding cell viability, not even with intergroup comparison nor among the time points studied (Table 2).

Mitochondrial activity evaluation was performed with MTT test and showed significant difference in the first 24 h between the group LG3 and CG; however, we did not observe difference among the remaining groups. We observed an increased proliferative cells rate in groups LG1, LG2, and LG3, 48 h after inoculation, when compared to CG, without difference among the irradiated groups. At the end of the experiment, we observed that both groups LG1 and LG2 showed proliferative rates higher than the other groups, with no difference between the two of them. However, group LG2 showed significant increase of the proliferative rate when compared to groups CG and LG3, showing that energy of 1.96 J was more efficient than the remaining doses (Fig. 3).

We believe that at the end of 72 h, group LG3 showed inhibitory action due to the applied energy dose, while group LG1 showed accumulation of energy, with more promising results.

Regarding the size of the effect, for the MTT variable, all groups when compared to the control had $r > 0.50$, with the results: LG1 = 0.89, LG2 = 0.912, and LG3 = 0.61 (Fig. 4).

Discussion

Current literature has highlighted the importance of accelerating the cell proliferation process for a better efficacy of its application in tissue engineering. In the recent years, the use of photobiomodulation therapy has been investigated in different fields as an option for pain relief, tissue repair, and proliferation and viability of several cell types, for example, mesenchymal stem cells (MSTs). However, its efficient use depends on a variety of factors, including adequate parameterization [17–20]. Even though the PBM mechanism of action is still not fully clear [19], the applied irradiation in different cell types is followed by an increase of nitric oxide (NO), growth factors, and reactive oxygen species (ROS) [20].

The present study sought to observe the behavior of hASCs when submitted to PBM with different energies, reported in the literature [16], in order to demonstrate that different energy doses are able to generate distinct responses, in different periods.

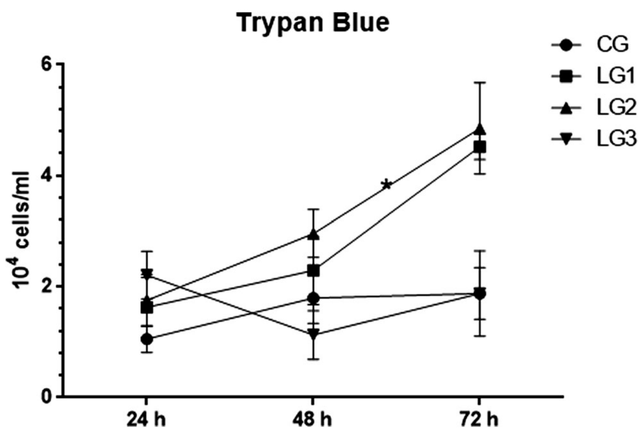

Fig. 2 Cell growth evaluation using trypan blue assay in different time points. An asterisk indicates significant difference ($p < 0.05$)

Our study showed that regardless of the energy applied by PBM with visible light spectrum laser (red), all irradiated groups revealed improved proliferation when compared to the CG.

The literature remains controversial regarding adequate parameterization; however, most studies highlight that the use of PBM with red laser improves proliferation and cell viability. Eduardo et al. (2008) [13] used PBM to evaluate cell proliferation in the periodontal ligament trunk (hPDLSC), with laser wavelength of 660 nm and power close to the one used in our study (20 mW and 40 mW), and showed that PBM stimulated the proliferation of this type of cell, in addition to maintaining the viability, similarly to our study.

A study performed by Soares et al. (2015) [3] evaluated the PBM action in hPDLSC cells, in time 0, 24, 48, and 72 h with 660-nm laser, power of 30 mW, and energies of 0.48 and 1 J. Results were promising with the higher energy, suggesting that lower energy has little impact on proliferation rates immediately, corroborating with our data in similar times and parameters. However, it is noteworthy that there is a dose-response threshold for the use of much higher energies; our study showed that the higher energy used inhibited the proliferation rates after 72 h.

Karu (1987) [15] showed that the application of high doses of PBM can damage photoreceptors and lead to metabolic inhibition and cell death. This idea was supported by several studies, including Kreisler et al. (2002) [21], which observed that the use of high energies has negative effect in fibroblasts,

Table 2 Average percentage of viability of stem cells (hASCs), evaluated by the blue trypan method, at different times after PBM in the groups

	GC	GL1	GL2	GL3
T24	100%	154%	166%	210%
T48	100%	127%	155%	81%
T72	100%	241%	259%	99%

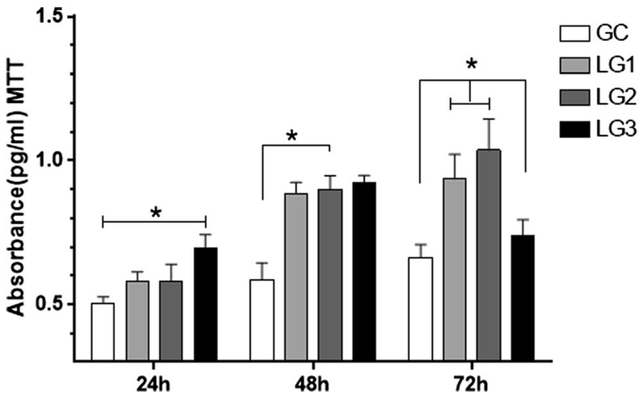

Fig. 3 Mitochondrial activity of cell cultures, submitted or not to PBM, with different energies, in different times. An asterisk indicates statistical difference ($p < 0.05$)

in agreement with Huang et al. (2011) [22], who revised the possibility of BPM biphasic responses when applied to different cell types.

Huang et al. (2011) [22] described the PBM action with biphasic responses in animal and in vitro models, stating that little light cannot create response and too much light can be harmful. The authors also hypothesized that the BPM biphasic response can be explained through the ROS action, and highlight that there are two types, one being beneficial and the other injurious. Thus, lower doses may create beneficial ROS, stimulating electrons transport and ATP production, while high doses can produce harmful ROS and affect mitochondrial activity.

Karu (1988) [23] reports that the accumulative effect of high doses are inhibitory and are characterized by decrease in the cell viability and proliferation, with harmful effects to the cell membrane and DNA. On the other hand, the study also shows that the cumulative effect with low energy can lead to significant results.

According to Huang et al. (2011) [22] and Karu (1988) [23], we can observe that the results of the present study illustrate the authors' idea of the "dose response" of "Arndt-Schulz Law" being that the different energies employed were able to generate distinct responses regarding cell proliferation and viability of hASCs. Our study showed that energies considered "low," such as 0.56 J, may show positive results when applied several times, illustrating the efficacy of low doses when accumulated in cells. On the other hand, the accumulation of high doses of energy can generate negative responses, as in our study in that the energy of 5.04 J when applied in a single session presented positive results, but when accumulated the dose resulted in a cellular inhibition.

There is a lacking and divergent information in the literature regarding the cell type investigated in our study. Data are still divergent, not only regarding parameterization and ideal number of applications. In addition, we should consider the different irradiation conditions, with laser beam and spreading, output power, equipment calibration, among other factors.

A review by Ginani et al. [16] showed that only four studies correlated phototherapy and human and animal ASCs stem cells. Three out of these studies used red laser with power between 50 and 110 mW, a variable number of applications and energy values that were not always revealed, which shows a notable divergence of parameterization. This review also presents results from PBM action in BMSCs stem cells, in which the parameterization described show smaller

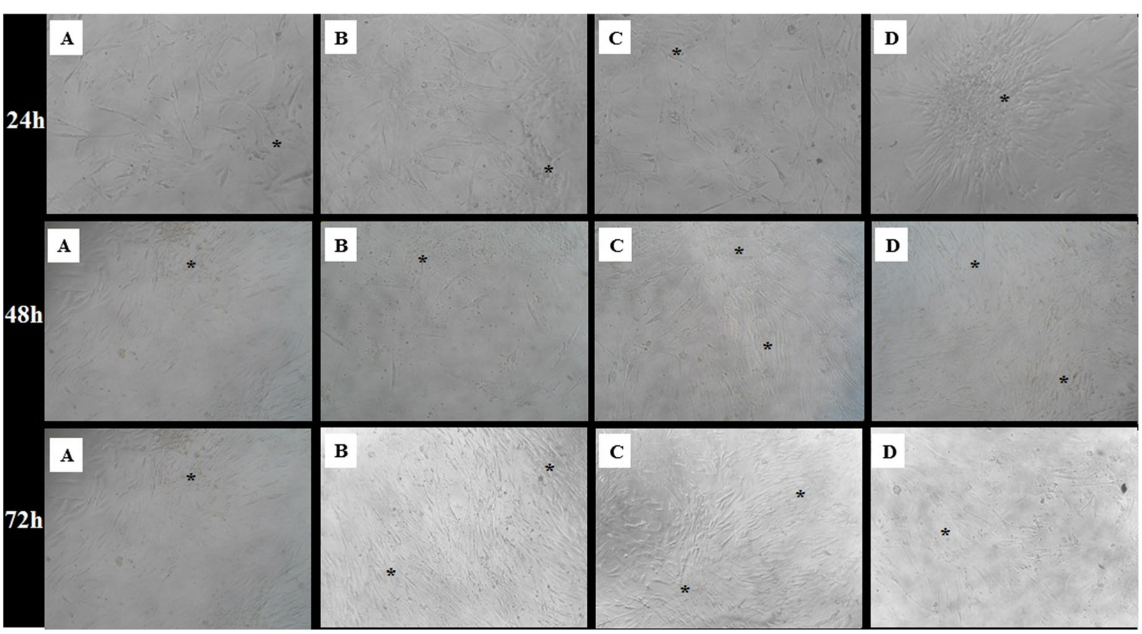

Fig. 4 Photomicrographs representative of the experimental groups regarding the cell proliferation in the times 24 h, 48 h, and 72 h. An asterisk indicates more cells (× 100)

discrepancy, with 76% of the studies using red laser and power of approximately 60 mW. The applied energy, however, presented wide variation, from 0.15 to 45 J.

Our choice to perform this study was based on the studies presented by the review and the wide discrepancy of parameterization. We aimed to highlight the difference among distinct application parameters and thus suggest more efficient doses for the application of PBM to hASCs cells.

Due to the great divergence of parameters and results in literature, we face great difficulty in comparing studies involving PBM in the proliferation of diverse cell groups, since most studies do not detail the experimental conditions and parameters used. Thus, we suggest the need for studies with increased scientific rigor to facilitate the comparison between them, in order to establish more efficient protocols for each cell type.

Conclusion

According to the presented data, we conclude that PBM (660 nm) stimulates the proliferation of hASCs stem cells when using energy between 0.56 and 1.96 J, even with varying times. On the other hand, higher energies like 5.04 J were initially efficient but presented inhibitory action when applied repeatedly.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The present study was approved by the Ethics Committee of the Federal University of São Carlos under protocol No.6224231115.

Informed consent The hASCs were obtained through donations of lipoaspirated tissues from patients at the Hospital and Clinics of the College of Medicine of Ribeirão Preto, after signing the “consent form” established by the internal regulations of this hospital.

References

1. Horvát-Karajz K, Balogh Z, Kovács V, Drrernat AH, Sréter L, Uher F (2009) In vitro effect of carboplatin, cytarabine, paclitaxel, vincristine, and low-power laser irradiation on murine mesenchymal stem cells. Lasers Surg Med 41:463–469
2. Mvula B, Mathope T, Moore T, Abrahamse H (2008) The effect of low level laser irradiation on adult human adipose derived stem cells. Lasers Med Sci 23:277–282
3. Soares DM, Ginani F, Henriques ÁG, Barboza G (2015) Effects of laser therapy on the proliferation of human periodontal ligament stem cells. Lasers Med Sci 30:1171–1174
4. Soleimani M, Abbasnia E, Fathi M, Sahraei H, Fathi Y, Kaka G (2012) The effects of low-level laser irradiation on differentiation and proliferation of human bone marrow mesenchymal stem cells into neurons and osteoblasts — an in vitro study. Lasers Med Sci 27:423–430
5. Vasconcelos RG, Ribeiro RA, Vasconcelos MG, Lima KC, Barboza CA (2012) In vitro comparative analysis of cryopreservation of undifferentiated mesenchymal cells derived from human periodontal ligament. Cell Tissue Bank 13:461–469
6. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH (2001) Multilineage cells from human adipose tissue : implications for cell-based therapies. Tissue Eng 7: 211–228
7. de Villiers JA, Houreld NN, Abrahamse H (2011) Influence of low intensity laser irradiation on isolated human adipose derived stem cells over 72 hours and their differentiation potential into smooth muscle cells using retinoic acid. Stem Cell Rev 7:869–882
8. Hawkins D, Abrahamse H (2006) Effect of multiple exposures of low-level laser therapy on the cellular responses of wounded human skin fibroblasts. Photomed Laser Surg 24:705–714
9. Moore P, Ridgway TD, Higbee RG, Howard EW, Lucroy MD (2005) Effect of wavelength on low-intensity laser irradiation-stimulated cell proliferation in vitro. Lasers Surg Med 36:8–12
10. Fujihara NA, Hiraki KR, Marques MM (2006) Irradiation at 780 nm increases proliferation rate of osteoblasts independently of dexamethasone presence. Lasers Surg Med 38:332–336
11. Eduardo FP, Mehnert DU, Monezi TA, Zezell DM, Schubert MM, Eduardo CP, Marques MM (2007) Cultured epithelial cells response to phototherapy with low intensity laser. Lasers Surg Med 39:365–372
12. Benedicenti S, Pepe IM, Angiero F, Benedicenti A (2008) Intracellular ATP level increases in lymphocytes irradiated with infrared laser light of wavelength 904 nm. Photomed Laser Surg 26:451–453
13. Eduardo Fde P, Bueno DF, de Freitas PM, Marques MM, Passos-Bueno MR, Eduardo Cde P, Zatz M (2008) Stem cell proliferation under low intensity laser irradiation: a preliminary study. Lasers Surg Med 40:433–438
14. Stein E, Koehn J, Sutter W, Wendtlandt G, Wanschitz F, Thurnher D et al (2008) Initial effects of low-level laser therapy on growth and differentiation of human osteoblast-like cells. Wien Klin Wochenschr 120:112–117
15. Karu TI (1987) Photobiological fundamentals of low-power laser therapy. J Quantum Electron J Quantum Electron 23:1703–1717
16. Ginani F, Soares DM, Barreto MP, Barboza CA (2015) Effect of low-level laser therapy on mesenchymal stem cell proliferation: a systematic review. Lasers Med Sci 30:2189–2194
17. de Oliveira TS, Serra AJ, Manchini MT (2014) Effects of low level laser therapy on attachment, proliferation, and gene expression of VEGF and VEGF receptor 2 of adipocyte-derived mesenchymal stem cells cultivated under nutritional deficiency. Lasers Med Sci 30:217–223
18. Avci P, Gupta A, Sadasivam M, Vecchio D, Pam Z, Pam N et al (2013) Low-level laser (light) therapy (LLLT) in skin: stimulating, healing, restoring. Semin Cutan Med Surg 32:41–52
19. Caetano GF, Bártolo PJ, Domingos M, Oliveira CC, Leite MN, Frade MAC (2015) Osteogenic differentiation of adipose-derived mesenchymal stem cells into polycaprolactone (PCL) scaffold. Procedia Engineering 110:59–66
20. Fekrazad R, Asefi S, Allahdadi M, Kalhori KA (2016) Effect of photobiomodulation on mesenchymal stem cells. Photomed Laser Surg 34:533–542
21. Kreisler M, Christoffers AB, Al-haj H, Willershausen B, d'Hoedt B (2002) Low level 809-nm diode laser-induced in vitro stimulation of the proliferation of human gingival fibroblasts. Lasers Surg Med 30:365–369

22. Huang YY, Sharma SK, Carroll J, Hamblin MR (2011) Biphasic dose response in low level light therapy – an update. Dose-Response 9:602–618
23. Karu TI (1988) Molecular mechanisms of therapeutic effect of low-intensity laser irradiation. Laser Life Sci 2:53–74