Sains Malaysiana 52(7)(2023): 2069-2093
http://doi.org/10.17576/jsm-2023-5207-14

Advances in the Bioregulation of Mesenchymal Stem Cells by Low-Level Laser Therapy during Bone Formation: A Narrative Review

(Kemajuan dalam Kawal Atur Sel Stem Mesenkima oleh Terapi Laser Tahap Rendah semasa Pembentukan Tulang: Kajian Naratif)

Wang Yan Pei[1], Mohamad Arif Awang Nawi[1] & Norma Ab Rahman[2,*]

[1]*School of Dental Sciences, Universiti Sains Malaysia, Health Campus,16150 Kubang Kerian, Kelantan, Malaysia*
[2]*Orthodontic Unit, School of Dental Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia*

Received: 11 November 2022/Accepted: 5 July 2023

ABSTRACT

This article reviews the progress of research on the modulation of the biological effects of low-level laser therapy (LLLT) on Mesenchymal stem cells (MSC) and the influence of the basic LLLT parameters and irradiation scenarios. A thorough search of the literature in the PubMed, Web of Science and Embase databases, including articles published in peer-reviewed journals, was conducted to collect relevant information on LLLT and MSCs. A comprehensive search of PubMed, Web of Science, and Embase databases was performed using the keywords "Low level laser," "mesenchymal stem cells," " cell proliferation," and "osteogenic differentiation." The search was limited to studies published in English between 2009 and 2022, including in vitro and in vivo studies. LLLT has the potential to promote MSC proliferation and osteogenic differentiation, with significant applications in bone tissue engineering. Factors that influence the biological effects of LLLT on MSC include cell type, culture medium, duration of irradiation, the frequency of laser irradiation, irradiation spot size, and type of light flux distribution. The limitations of this review include heterogeneous experimental conditions and the inability to design experiments that consider all influencing factors simultaneously.

Keywords: Cell proliferation; low level laser; mesenchymal stem cells; osteogenic differentiation

ABSTRAK

Artikel ini mengkaji kemajuan penyelidikan mengenai modulasi kesan biologi terapi laser peringkat rendah (LLLT) pada sel stem mesenkima (MSC) dan pengaruh parameter LLLT asas dan senario penyinaran. Pencarian teliti kepustakaan dalam pangkalan data PubMed, Web of Science dan Embase, termasuk artikel yang diterbitkan dalam jurnal semakan rakan sebaya, telah dijalankan untuk mengumpul maklumat yang berkaitan tentang LLLT dan MSC. Carian komprehensif pangkalan data PubMed, Web of Science dan Embase telah dilakukan menggunakan kata kunci 'laser tahap rendah', 'sel stem mesenkima', 'percambahan sel' dan 'pembezaan osteogenik'. Pencarian terhad kepada kajian yang diterbitkan dalam bahasa Inggeris antara 2009 dan 2022, termasuk kajian *in vitro* dan *in vivo*. LLLT berpotensi untuk menggalakkan percambahan MSC dan pembezaan osteogenik dengan aplikasi penting dalam kejuruteraan tisu tulang. Faktor yang mempengaruhi kesan biologi LLLT pada MSC termasuk jenis sel, medium kultur, tempoh penyinaran, kekerapan penyinaran laser, saiz tempat penyinaran dan jenis taburan fluks cahaya. Kekangan dalam ulasan ini termasuk keadaan uji kaji heterogen dan ketidakupayaan untuk mereka bentuk uji kaji yang mempertimbangkan semua faktor yang mempengaruhi secara serentak.

Kata kunci: Laser tahap rendah; pembezaan osteogenik; percambahan sel; sel stem mesenkima

INTRODUCTION

This narrative review discusses the current knowledge of the role of LLLT in MSC regulation and its potential applications in regenerative medicine. This review focuses on different types of MSCs, LLLT parameters, cell culture conditions, and biological effects. In 1967, Endre Mester (Mester, Szende & Gärtner 1968), a

Hungarian physician, first suggested the potential of low-level lasers on biological tissues, and since then, interest in low-level laser therapy (LLLT) applied to mesenchymal stem cells has been aroused. Mesenchymal stem cells (MSC) exhibit 2 fundamental characteristics: self-renewal and differentiation, which are considered to be important factors influencing the cellular therapy and process of inducing osteogenic differentiation (Han et al. 2019; Marques et al. 2016). It was because MSC could be separated from the tissue of teeth that this event was a major advancement not just for regenerative stomatology but also for the whole field of regenerative medicine (Fageeh 2021). LLLT refers to the regulation of biological functions of tissues or cells through specific photoelectric effects after exposure to low light (Migliario et al. 2018). LLLT has been demonstrated to affect MSC biological effects *in vitro* and *in vivo* in several aspects, including proliferation rates, cell migration movements, and cytoskeletal alterations (Yin et al. 2017). However, the different LLLT parameters chosen in various studies have led to inconsistent conclusions about the modulation of LLLT on the biological effects of MSC, either positively or negatively (Min et al. 2015; Yuan et al. 2017). In particular, the positive regulatory effect of LLLT on the MSC is gaining more and more attention in the field of oral regenerative medicine. The available research material is unconvincing and hinders the standardization of LLLT applications to MSC.

Methods

LITERATURE SEARCH STRATEGY

A comprehensive literature search was conducted in PubMed, Web of Science and Embase databases to identify relevant articles published from 2009 to 2022. The search strategy employed combinations of the following keywords: 'low-level laser therapy', 'low-level light therapy', 'photobiomodulation', 'mesenchymal stem cells', 'mesenchymal stromal cells', 'MSCs', 'bone marrow-derived mesenchymal stem cells', 'adipose-derived stem cells', 'dental pulp stem cells', 'proliferation', 'differentiation', 'osteogenesis'.

INCLUSION AND EXCLUSION CRITERIA

Studies were included if they met the following criteria: (1) Original research articles published in English; (2) Studies that examined the interaction between LLLT and MSCs *in vitro* or *in vivo*; (3) Studies that reported LLLT parameters, MSC types, and culture conditions.

Exclusion criteria were: (1) Review articles, case reports, and conference abstracts; (2) Studies that did not provide detailed LLLT parameters or MSC information; (3) Studies that focused only on the effects of LLLT on other cell types or only on the effects of MSCs without LLLT treatment.

DATA EXTRACTION AND ANALYSIS

Two independent reviewers extracted data from the included studies using a standardized form. The disagreements between the 2 reviewers have been discussed and negotiated, and the third reviewer makes the final decision. The extracted data included the following information: study type, LLLT parameters, MSC type, culture conditions, and main outcomes. The extracted data were then analyzed and synthesized to provide a comprehensive review of the LLLT-MSC interaction.

REVIEW-DISCUSSION

EFFECT ON THE BIOLOGICAL EFFECTS OF MSC

CELLULAR PROLIFERATION

The results of the experimental studies showed that LLLT could promote the proliferation of MSC. In experiments with animal cells, some scholars (Min et al. 2015) treated MSCs cultured in vitro with LLLT and subsequently transplanted them into rats and found that LLLT promoted MSC proliferation not only *in vitro* but also *in vivo.* In experiments with human cells, it was noteworthy that LLLT promoted MSC proliferation in association with cell cycle alterations (Cavalcanti et al. 2015). LLLT induced a transition from G0/G1 phase cells to the G2/M phase, leading to rapid cell proliferation of MSC, and the level of transition increased with increasing LLLT energy density. However, some studies have shown that inappropriate LLLT has adverse effects on MSC, such as inhibition of cell proliferation and induction of apoptosis, which may be related to increased reactive oxygen species (ROS) production and DNA damage in MSC (Yuan et al. 2017). In conclusion, the effect of LLLT on MSC proliferation is influenced by a variety of factors.

OSTEOGENIC DIFFERENTIATION AND MINERALIZATION

The differentiation of MSC into osteoblasts is an important guarantee of osteogenesis for bone regeneration. The mineralized nodule is an important biologic marker of the later stages of MSC osteogenesis. In experiments

with animal cells, it is well known that osteogenesis is impaired in MSCs obtained from the bone marrow of ovariectomized rats (OVX-BMSC). However, Fallahnezhad et al. (2020) reported that LLLT also still promoted the formation of mineralized nodules in OVX-BMSC during osteogenesis induction. In experiments with human cells, the results of Zhang et al. (2018) showed that LLLT on the osteogenic induction base promoted osteogenic differentiation of MSC and produced mineralized nodules; alizarin red staining showed that the number of mineralized nodules produced by MSC in the LLLT group was significantly higher than that in the osteogenic inducer group alone.

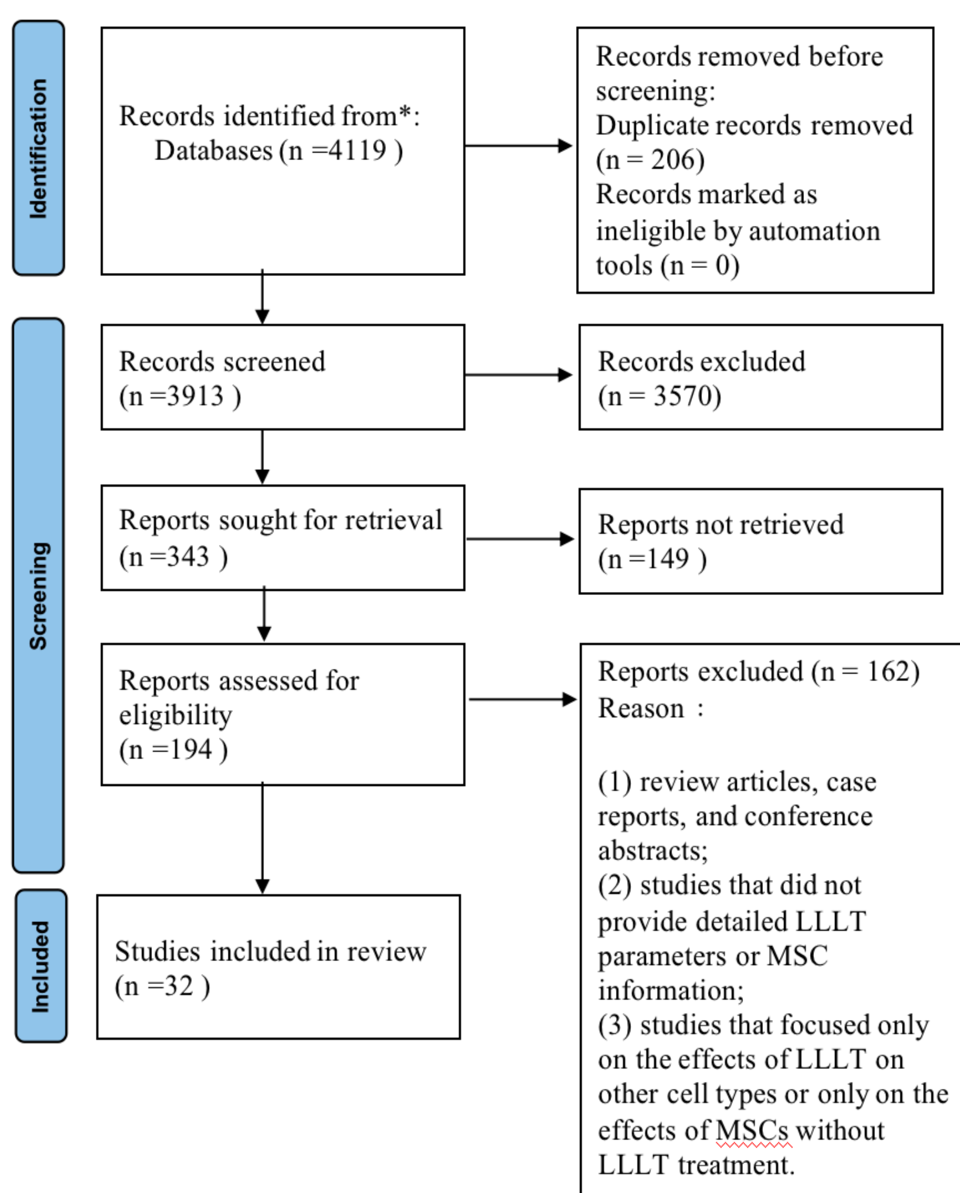

FIGURE 1. PRISMA flow diagram on the outcomes of the search strategy

TABLE 1. Effect factors of LLLT on mesenchymal stem cells

Author, Year	Cell type	*In vivo*/ *in vitro* experiments	Wavelength (nm)	Fluence (J/cm^2)	Irradiance	Cell culture conditions	Duration of irradiation	Frequency of laser irradiation	Irradiation spot size	Type of light flux distribution	Main outcomes
Yuan et al. (2017)	Bone marrow-derived mesenchymal stem cells (BMSCs)	*In vitro*	470 nm	12 J/cm^2	20 mW/cm^2	DMEM supplemented with 10% FBS and antibiotics, maintained at 37 °C in a humidified atmosphere of 5% CO2	1 min, 5 min, 10 min, 30 min, and 60 min	Not specified	Not specified	Not specified	Study demonstrated that blue LED light inhibited cell proliferation, inhibited osteogenic differentiation, and induced apoptosis in BMSCs, which are associated with increased ROS production and DNA damage. These findings may provide important insights for the application of LEDs in future BMSCbased therapies
Cavalcanti et al. (2015)	Dog bone marrow stem cells (DBMSC)	*In vitro* experiments	660 nm	1, 2, 4, 6, 8, 10, and 12 J/cm^2	50 mW	Cells were plated in columns of six wells in each column in flat microtiter plates at a density of 10^4 cells/well and incubated for one day at 37 °C in a humidified incubator containing 5% CO2	Total exposure time varied from 0 s (control group) to 90 s (12 J/cm^2)	Not specified	Spot area was 0.38 cm^2, and the laser stick cross-section area was 0.028 cm^2	Continuous beam, visible light, red laser	Low-level red laser positively affected the viability and proliferation of mesenchymal stem cells. The study evaluated the effect of laser irradiation on dog bone marrow stem cells and found that low doses of low-level red laser increased proliferation

Min et al. (2015)	Human adipose-derived stem cells (ADSCs)	Both *in vitro* and *in vivo* studies were conducted	830 nm	In the *in vitro* study, the fluence was 0.5 J/cm^2. In the *in vivo* study, the fluence was not specified	In the *in vitro* study, the irradiance was 50 mW/cm^2. In the *in vivo* study, the irradiance was not specified	The ADSCs were cultured in Dulbecco's modified Eagle's medium supplemented with fetal bovine serum and antibiotics	In the *in vitro* study, cells were irradiated for 1 min per day for three consecutive days. In the *in vivo* study, rats were irradiated for 10 min immediately after transplantation of ADSCs or before transplantation of ADSCs	In the *in vitro* study, cells were irradiated once a day for three consecutive days. In the *in vivo* study, rats were irradiated once immediately after transplantation of ADSCs or before transplantation of ADSCs	The total surface area of a 96-well plate was irradiated with a laser beam with a diameter of 1 cm	Not specified	LLLT enhanced cell viability and proliferation of grafted human ADSCs both *in vitro* and *in vivo*
Fallahnezhad et al. (2019)	Bone marrow mesenchymal stem cells (BMMSCs) from healthy and osteoporotic rats	*In vitro* experiments	810 nm	2 J/cm^2	100 mW/cm^2	BMMSCs were cultured in osteogenic induction medium (OIM)	60 s per day for 14 days	Once daily for 14 days	0.5 cm^2	Continuous wave	The combined application of photobiomodulation therapy and oxytocin improved the viability and mineralization of osteoporotic BMMSCs, as well as increased the gene expression levels of Osteocalcin, Osteoprotegerin, and Runt-related transcription factor 2
Zhang et al. (2018)	Bone marrow stromal cells (BMSCs)	*In vitro* experiments	Not specified	Not specified	Not specified	BMSCs were cultured in DMEM/F12 medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37 °C in a humidified atmosphere of 5% CO2	Not specified	Not specified	Not specified	Not specified	Low-level laser irradiation promoted the differentiation of BMSCs into osteoblasts through the APN/Wnt/β-catenin pathway

Zungu, Hawkins Evans & Abrahamse (2009)	Normal and injured human skin fibroblast cells	*In vitro* experiments	632.8 nm (He-Ne laser)	5 or 16 J/cm²	3 mW/cm²	Modified to simulate conditions of hypoxia and acidosis, central scratch model used to simulate a wound	45 min (5 J/cm²) or 150 min (16 J/cm²)	Two exposures on days 1 and 4	9.08 cm²	Continuous wave emission (CW)	The study aimed to establish if laser irradiation had an effect on hypoxic and acidotic cells by assessing changes in mitochondrial membrane potential, cyclic AMP, intracellular Ca^{2+} and adenosine triphosphate (ATP) cell viability. The results showed that low-level laser irradiation had a stimulatory effect on hypoxic and acidotic cells by improving mitochondrial function
Wu et al. (2013)	Human periodontal ligament cells	*In vitro* experiments	660 nm	Doses of 0, 1, 2, or 4 J/cm² were used	The power density was 15.17 mW/cm²	Cells were irradiated daily at room temperature on a clean bench	Cells were exposed to laser irradiation for 66, 132 and 264 s to apply energy densities of 1, 2 and 4 J/cm², respectively	Cells were irradiated daily	Not specified	Not specified	LPLI at a dose of 2 J/cm² significantly promoted hPDL cell proliferation at days 3 and 5. Osteogenic differentiation was assessed by Alizarin Red S staining and alkaline phosphatase (ALP) activity. Additionally, osteogenic marker gene expression was confirmed by real-time reverse transcription-polymerase chain reaction (RT-PCR)

Zhu et al. (2019)	Human gingival mesenchymal stem cells (hGMSCs)	*In vitro* experiments	420-480 nm	0, 1, 2, 4 or 6 J/cm^2	100 mW/cm^2 at the cell-layer level	Non-irradiated cells were cultured under the same conditions as irradiated cells	Cells were irradiated consecutively for 10 s, 20 s, 40 s and 60 s every other day for a total of 28 days	Every other day	The diameter of the light spot on the irradiated cultures was 3.5 cm	Continuous output	Blue light irradiation enhanced osteogenic differentiation in hGMSCs and increased expression of osteogenic genes. This may have potential applications in regenerative medicine for restoring alveolar bone tissue. However, further study is needed to explore the use of blue LEDs combined with hGMSCs in periodontal tissue regeneration
Merigo et al. (2015)	Osteogenic cells	*In vitro* experiments	532 nm	4 J/cm^2	Not specified	Cells were cultured in complete medium and irradiated at confluence	Not specified	Not specified	4 cm diameter defocalizing lens	Not specified	results highlight that this LLLT experimental protocol with green light (KTP, 532 nm) at 4 J/cm^2 has a positive effect on the osteogenic differentiation of murine bone marrow stromal cells. These preliminary results could be used as a basis to further investigate the effect of this KTP laser protocol on bone tissue engineering models *in vivo* and *in vitro*

Wang et al. (2017a)	Human adipose-derived stem cells (hASCs)	*In vitro* experiments	415, 540, 660, and 810 nm	3 J/cm^2 for all wavelengths	16 mW/cm^2 for all wavelengths	Proliferation medium	188 s for all wavelengths	Not specified	4 cm^2 for all wavelengths	Continuous-wave (CW) for all wavelengths	Blue and green light (415 and 540 nm) inhibited proliferation of hASCs, while red and near-infrared light (660 and 810 nm) stimulated proliferation. Intracellular calcium was higher after blue/green light, and could be inhibited by the ion channel blocker capsazepine
Fekrazad et al. (2018)	Mesenchymal stem cells derived from rabbit iliac bone marrow	*In vitro* experiments	635, 660, 810, and 980 nm (single lasers); combinations of 635/660 nm and 810/980 nm (dual lasers)	Ranged from 0.5 to 10 J/cm^2 depending on the wavelength and combination used	Ranged from 5 to 100 mW/cm^2 depending on the wavelength and combination used	Cells were cultured in alpha-minimum essential medium supplemented with fetal bovine serum and antibiotics	Ranged from 30 s to 3 min depending on the wavelength and combination used	Three times a week for up to two weeks	Approximately 1 cm^2	Continuous wave mode	The study found that certain combinations of laser wavelengths resulted in increased proliferation and differentiation of mesenchymal stem cells into bone or cartilage compared to single wavelengths or control groups. However, the optimal combination varied depending on the specific outcome being measured
Zare et al. (2019)	Human adipose stem cells and human bone marrow-derived stem cells	*In vitro* experiments	630 plus 810 nm wavelengths	Not specified	Not specified	Not specified	Not specified	Not specified	Not specified	Not specified	The study found that 630 plus 810 nm wavelengths induced more *in vitro* cell viability of human adipose stem cells than human bone marrow-derived stem cells

Yang et al. (2020)	Stem cells from the apical papilla (SCAPs)	*In vitro* experiments	420-480 nm	1 J/cm^2, 2 J/cm^2, 3 J/cm^2, 4 J/cm^2	100 mW/cm^2	Osteogenic induction medium	10 s, 20 s, 30 s, and 40 s for different fluences	Not specified	The spot diameter at a distance of 1 cm from the light source was measured to be 3.5 cm	Not specified	The study aimed to evaluate the effects of low-energy blue LED irradiation on the osteogenic differentiation of SCAPs. The main outcomes were that blue LED irradiation enhanced osteogenic differentiation and proliferation of SCAPs in a dose-dependent manner
Wang et al. (2019)	Bone marrow mesenchymal stem cells (BMSCs	*In vitro* experiments	1064 nm	0-16 J/cm^2	0.25 W output power for 20 s	BMSCs were cultured in α-MEM (10% FBS) until they reached 80% confluence	Not specified	Not specified	Single-probe laser handpiece perpendicular to the surface, scanning the cells with a beam angle of 90°	Not specified	LLLT at 2-4 J/cm^2 significantly promoted proliferation and osteogenesis of BMSCs
Horvat-Karajz et al. (2009)	Murine mesenchymal stem cells (mMSCs)	*In vitro* experiments	660 nm	Range of energy density: 1.9 -11.7 J/cm^2	76-156 mW/cm^2; output power was constantly 60 mW	Not specified	Not specified	Not specified	Diameter of the laser beam ranged from 7 to 10 mm depending on the energy density and power density used	Not specified	The study investigated the effect of four cytostatics and low-power laser irradiation on mMSCs. The results showed that cytostatics can decrease, but low-power laser irradiation can increase cell growth. The interaction between LPLI, MSCs, and cytostatics was also explored

Tani et al. (2020)	Human osteoblasts and mesenchymal stromal cells	*In vitro* experiments	Red (635 nm), near-infrared (808 nm), and violet-blue (405 nm)	1.5 J/cm^2 for red and violet-blue light, 3 J/cm^2 for near-infrared light	50 mW/cm^2 for red and violet-blue light, 100 mW/cm^2 for near-infrared light	Cells were cultured in DMEM supplemented with 10% FBS, penicillin, and streptomycin	24 h	Once a day for three consecutive days	0.5 cm^2	Continuous wave mode	The study found that red light promoted osteoblast proliferation and differentiation through Akt signaling activation, while violet-blue light had no significant effect on the cells. Near-infrared light induced changes in cytoskeleton assembly, Runx-2 expression, and mineralization pattern. The study suggests that photobiomodulation with a 635 nm laser may be an effective option for promoting bone regeneration
Cardoso et al. (2020)	rat calvaria osteoblasts	*In vitro* experiments	The study used red laser (AlGaInP - 660 nm), infrared laser (GaAlAs - 808 nm), and LED (637 ± 15 nm) for PBM	ranged from 0.12 J/cm^2 to 1.91 J/cm^2	ranged from 0.024 mW/cm^2 to 1660 mW/cm^2	Before irradiation, the cells were put in a quiescent state with 1% FBS for 24 h	The duration of irradiation varied depending on the experiment, ranging from 5 seconds to once a week	The frequency of laser irradiation also varied depending on the experiment, ranging from triple per day to once a week	ranged from 0.32 cm^2 to 1.91 cm^2	Not specified	One of the main outcomes was that PBM had an osteogenic induction effect on rat calvaria osteoblasts even without osteogenic medium. Red laser and LED showed the best effects on increasing cell viability and bone nodule formation. A sum of effects was observed when cells were cultured in osteogenic medium and irradiated with red laser, LED, and infrared laser

Ruan et al. (2020)	Human bone marrow mesenchymal stem cells (BMSCs)	*In vitro* experiments	660 nm	0-8 J/cm^2	50 mW/cm^2	BMSCs were cultured in α-MEM supplemented with 10% FBS and 1% penicillin-streptomycin at 37 °C in a humidified atmosphere of 5% CO2	0-40 s	Not specified	4 cm^2	Not specified	The study found that high-intensity red LED irradiation enhanced the osteogenic differentiation and mineralization of BMSCs through the Wnt/β-catenin signaling pathway. The LED irradiations did not alter BMSC proliferation after 72 h
Blatt et al. (2016)	Stem cells in the bone marrow	*In vivo* experiments were conducted on farm pigs	Not specified	Not specified	Not specified	Not applicable as *in vivo* experiments were conducted	Laser was applied to the tibia and iliac bones 30 min, and 2 and 7 days post-induction of MI	Not specified	Not specified	Not specified	The number of c-kit + cells (stem cells) in the circulating blood of the laser-treated (LT) pigs was 2.62- and 2.4-fold higher than in the non-laser-treated (NLT) pigs 24 and 48 h post-MI, respectively. The extent of scarring was analyzed by histology and MRI, and heart function was analyzed by echocardiography. The study showed promising results for reducing scarring and improving heart function after a myocardial infarction

Nurković et al. (2016)	Human adipose tissue-derived mesenchymal stem cells (hAT-MSCs)	*In vitro* experiments	808	3	0.2 W/cm^2	hAT-MSCs were isolated from subcutaneous adipose tissue of six persons ranging in age from 21 to 56 years	EMF was applied for a period of 7 days, once a day for 30 min; LLL was applied also for 7 days, once a day for 5 min	Not specified	1 cm^2	Continuous wave	The combined effects of electromagnetic field and low-level laser increased proliferation and altered the morphology of hAT-MSCs
Fallahnezhad et al. (2016)	Osteoporotic bone marrow-derived mesenchymal stem cells from ovariectomy-induced osteoporotic rats	*In vitro* experiments	Helium-neon laser with a wavelength of 632.8 nm	1 J/cm^2	5 mW/cm^2	DMEM supplemented with 10% FBS and antibiotics, incubated at 37 °C in a humidified atmosphere of 5% CO2	60 s per day for three consecutive days	Once daily for three consecutive days	0.5 cm^2	Gaussian beam profile	Low-level laser therapy with helium-neon laser improved the viability of osteoporotic bone marrow-derived mesenchymal stem cells from ovariectomy-induced osteoporotic rats
Zare et al. (2019)	Rat diabetic mesenchymal stem cells (DMSCs)	*In vitro* experiments	632.8 nm	0.5, 1, and 2 J/cm^2	Not specified	Not specified	Not specified	Once, twice, and thrice	Not specified	Not specified	Photobiomodulation therapy using a helium-neon laser at an energy density of 1 J/cm^2 and once or twice frequency improved cell viability and proliferation while decreasing apoptosis in DMSCs. The impact of PBM therapy on DMSCs was found to be dose- and time-dependent

Peng et al. (2012)	Bone marrow mesenchymal stem cells (MSCs)	*In vitro* experiments	620 nm	1 J/cm^2, 2 J/cm^2, and 4 J/cm^2	6.67 mW/cm^2	MSCs cultured in normal medium or osteogenic differentiation medium (ODM)	150 s for 1 J/cm^2, 300 s for 2 J/cm^2, and 600 s for 4 J/cm^2	Every other day since the medium had been changed to ODM	Diameter of the light spot was 10 cm	Noncoherent red light irradiation emitted from a light-emitting diode (LED)	Red LED light at different energy densities promoted proliferation but did not induce osteogenic differentiation of MSCs in normal medium, while it enhanced osteogenic differentiation and decreased the rate of proliferation of MSCs in ODM. Red light can effectively increase the rate of osteodifferentiation despite its inability to induce osteodifferentiation alone
Yang et al. (2016)	Human umbilical cord mesenchymal stem cells (hUMSCs)	*In vitro* experiments	620 nm	2 J/cm^2	Not specified	hUMSCs were cultured in osteogenic differentiation medium	Not specified	Not specified	Not specified	Not specified	The study found that LED light at a wavelength of 620 nm enhanced osteogenic differentiation activity of hUMSCs cultured in osteogenic differentiation medium

Amaroli et al. (2018)	Bone marrow stromal cells (MSCs)	*In vitro* experiments	808 nm diode laser	64 J/cm^2	1 W/cm^2	MSCs were cultured in osteogenic medium for 21 days prior to laser irradiation	60 s per irradiation exposure time	Laser irradiation was repeated every 24 h over different periods of time (Day 0, T0; 5 days, T1; 10 days, T2; and 15 days, T3)	Not specified	Not specified	The study found that photobiomodulation with an 808 nm diode laser at a higher fluence of 64 J/cm^2 significantly enhanced the osteogenic differentiation of MSCs *in vitro*. Specifically, the researchers observed increased alkaline phosphatase activity and mineralization in the treated cells compared to control cultures
Yin et al. (2017)	Mesenchymal stem cells (hMSCs)	*In vitro* experiments	660 nm	0.5 J/cm^2, 1 J/cm^2, and 2 J/cm^2	5 mW/cm^2	Stem cell culture solution	30 s, 60 s, and 120 s	Once a day for three consecutive days	0.785 cm^2	Single point light output	Low level laser irradiation at appropriate parameters can significantly promote the proliferation and migration of hMSCs, as well as inhibit apoptosis

Almeida-Jr. et al. (2019)	Stem cells from human exfoliated deciduous teeth (SHED)	*In vitro* experiments	660 nm	0.4 J/cm^2	10 mW output power, 0.04-cm^2 laser tip area, and 0.225-cm tip diameter	SHED were cultured in α-MEM supplemented with 15% FBS and antibiotics	Multiple irradiation groups (groups 4 and 5) received laser every 6 h for a total of three times, while single irradiation groups (groups 1 to 3) received only one laser treatment	Every 6 h for multiple irradiation groups	The laser tip had a diameter of 0.225 cm	Not specified	The study aimed to evaluate whether low-level laser therapy (LLL) stimulates regeneration-associated events in SHED cells. The main outcomes were the viability and proliferation of SHED cells after single or multiple doses of LLL therapy. The results showed that both single and multiple doses of LLL therapy significantly increased cell viability and proliferation compared to non-irradiated control groups
Li, Chen & Huang (2013)	rat bone marrow mesenchymal stem cells (rbMSCs)	*in vitro*	Red (630 nm) and near infrared (NIR, 850 nm) light emitting diodes (LEDs) were used for LLLI	4 J/cm^2	The light source used was a home-made LED array designed to fit over a standard 96-well microplate for cell culture. LED lamps were purchased from Lenoo Electronic Co. Ltd. (New Taipei City, Taiwan)	The rbMSCs were cultured *in vitro* under standard conditions	not provided	not provided	The LED array was designed to fit over a standard 96-well microplate for cell culture	not provided	LLLI using red and NIR light significantly increased rbMSC mobility and transmembrane migration up to 292.9% and 263.6% respectively. This effect was accompanied by enhanced enzymatic activities of MMP-2 and MMP-9, as well as F-actin accumulation and distribution correlated to increased migration in light-irradiated MSCs

de Villiers, Houreld & Abrahamse (2011)	Human adipose derived stem cells (hADSCs)	*In vitro* experiments	636 nm	5 J/cm^2	8.59 mW/cm^2	Not specified	Up to 72 h	Not specified	Not specified	Not specified	Low intensity laser irradiation positively affects isolated hADSCs *in vitro* by increasing cellular proliferation and viability, and by maintaining stem cell morphology and protein expression for up to 72 h. Laser irradiation at this fluence and wavelength does not appear to induce differentiation into another cell type, even after an extended period of time. The study also showed that stem cells isolated from human adipose tissue can be differentiated into smooth muscle cells using retinoic acid
de Andrade et al. (2018)	dipose tissue (hASCs)	*in vitro*	660 nm	0.56 J/cm^2, 1.96 J/cm^2, and 5.04 J/cm^2	40 mW	The hASCs were cultured in media in the 24-well plates	The hASCs received PBM irradiation at three different time points - 24 h, 48 h, and 72 h after seeding - with each group receiving a different energy level	not mentioned	not mentioned	not mentioned	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

Ferreira et al. (2018)	Stem cells from human exfoliated deciduous teeth (SHEDs)	*In vitro* experiments	660 nm	Six different average energy densities were applied according to the experimental groups (Table 1)	Not specified	SHEDs were cultured in alpha-minimum essential medium supplemented with 20% fetal bovine serum and antibiotics	every other day during 1 week	Every other day during 1 week	For the optically clear 96-well plates, each well was irradiated in a single point; for 6-well plates, each well was irradiated in five equidistant points	Not specified	The study aimed to analyze the effect of photobiomodulation therapy (PBMT) on the proliferation and undifferentiating status of SHEDs. The results showed that PBMT did not significantly affect the proliferation or undifferentiated status of SHED
Hanna et al. (2019)	Osteoblasts	*In vitro* experiments	980 nm	60 J/cm^2 for FT hand-piece irradiations and 66 J/cm^2 for ST hand-piece irradiations	1 W/cm^2 for FT hand-piece irradiations and 1.1 W/cm^2 for ST hand-piece irradiations	Not specified	60 s in continuous wave (CW)	Not specified	Not specified	Hand-pieces with Gaussian vs. Flat-Top profiles were compared	The study found that both Gaussian and Flat-Top profiles were effective in promoting osteoblast maturation, but there was no significant difference between them. Photobiomodulation increased cell viability, proliferation, and expression of Bcl-2 protein while decreasing expression of Bax protein

Bölükbaşı Ateş et al. (2020)	Adipose-derived stem cells (ADSC)	*In vitro* experiments	635 and 809 nm	0.5, 1, and 2 J/cm^2	50 mW/cm^2	Osteogenic differentiation medium (ODM)	10, 20, or 40 s	Not specified	600 µm fiber for the diode laser with a wavelength of 635 nm and a spot size of 400 µm fiber for the diode laser with a wavelength of 809 nm	Non-contact continuous irradiation mode	The study investigated the effects of photobiomodulation at two different wavelengths and three different energy densities on the osteogenic differentiation of ADSCs. The outcomes were evaluated through cell viability and proliferation assays, alkaline phosphatase activity, Alizarin red staining, and gene expression analysis. The results showed that PBM can enhance stem cell proliferation and differentiation into osteoblasts at certain energy densities and wavelengths

CYCLIC ADENOSINE MONOPHOSPHATE (CAMP) FACTOR

CAMP is a critical signaling molecule in numerous cell procedures, including differentiation, cell proliferation, inflammation, and apoptosis (Gether 2000). According to a related study reported, LLLT increases intracellular cAMP levels (Zungu et al. 2009). Wu et al. (2013) discovered that adenylate lyase inhibitor (SQ22536) hindered LLLT enhancement of MSC osteogenic differentiation and cell proliferation (SQ22536). This study also indicated that cAMP is a major regulator of LLLT-mediated gene expression that stimulates MSC proliferation and osteogenic differentiation. Notably, without LLLT, adenylate cyclase inhibitor (SQ22536) did not affect the expression of genes for MSC differentiation into osteogenesis and cell proliferation.

BASIC PARAMETERS

The biological effects of LLLT on MSC are influenced by several basic parameters.

WAVELENGTHS

The biological effect of LLLT on MSC is affected by wavelength. The main wavelengths used for LLLT are 400 ~ 1100 nm and can be used to reduce inflammation, and promote healing and analgesia (Serrage et al. 2019). Two wavelengths are most used to promote osteogenesis in MSCs. One is blue-green light (414 - 543 nm) and the other is red and near-infrared light (662 - 809 nm), especially as the latter can also promote MSC proliferation.

Investigators used blue LLLT at a wavelength of 420-480 nm (blue-green light) to regulate MSC and found that LLLT reduced cell proliferation in MSC, but significantly promoted the expression of osteogenic genes such as type I collagen, RUNT-related transcription factor 2 (Runx2) and osteocalcin, increased the activity of ALP, a marker of osteogenic differentiation, and increased the number of mineralized nodules (Zhu et al. 2019). Recent research (Merigo et al. 2016) has found that green LLLT at a wavelength of 532 nm (green light) promoted osteogenic differentiation of MSC and mineralization of its extracellular matrix, but had less effect on the proliferation of MSC.

Wang et al. (2017b) compared the effects of four different wavelengths of LLLT (415 nm, 540 nm, 660 nm, 810 nm) on MSC proliferation and osteogenic differentiation and found that blue (415 nm) and green (540 nm) wavelengths were more effective than near-infrared (NIR, 810 nm) and red (660 nm) wavelengths in stimulating osteoblast differentiation, but inhibited MSC proliferation. However, Fekrazad et al. (2019), who studied the effects of different wavelengths of LLLT on MSC, came to the opposite conclusion, finding that LLLT at near-infrared (NIR, 810 nm) wavelengths had the greatest influence on boosting MSC proliferation and osteogenic differentiation, followed by LLLT at red (660 nm) wavelengths. The only similarity between Fekrazad and Wang is that Fekrazad et al. (2019) concluded that LLLT at green (532 nm) wavelength had the strongest inhibitory effect on cell proliferation.

In conclusion, LLLT at 414-543 nm can promote MSC differentiation towards osteogenesis but inhibit MSC proliferation. LLLT at 662-809 nm can promote both MSC proliferation and osteogenic differentiation, which is a more ideal wavelength for LLLT to regulate the biological effects of MSC.

The wavelength of LLLT follows the 'Arndt-Schultz law', also called biphasic dose-response, which means that too weak a stimulus does not induce any biological response, while too strong a stimulus has an inhibitory effect (Zein, Selting & Hamblin 2018). The effect of two wavelengths (810 and 980 nm) on MSC has been studied (Wang et al. 2017a). There was a biphasic dose-response for both wavelengths, but 810 nm stimulated proliferation with a peak dosage of 3 J/cm² over 24 h, while 980 nm had a peak dosage of 0.03 or 0.3 J/cm² 10 to 100 times lower (Wang et al. 2017a). In addition, 980 nm blocked temperature-gated calcium channels, while 810 nm mainly inhibited mitochondrial cytochrome c oxidase, both of which affect the biological effects of LLLT on MSC at different levels (Wang et al. 2017a).

In addition, the combination of different wavelengths of LLLT promoted MSC proliferation better than a single wavelength (and was not affected by biphasic dose effects). Pasternak-Mnich et al. (2019) found that simultaneous emission of LLLT at 2 wavelengths (808 nm and 905 nm) using a multi-wavelength locking system could significantly promote MSC proliferation. In recent years, it has been reported that dual-wavelength (630 nm and 810 nm) LLLT can significantly promote MSC proliferation, which supports this view (Zare et al. 2019).

ENERGY DENSITY

Energy density is an important fundamental parameter in LLLT that affects the biological effect of LLLT on MSC and also follows the biphasic dose effect of LLLT (Zein, Selting & Hamblin 2018). In the literature, it has

been reported that energy densities of 0.3 to 12 J/cm² are favorable for LLLT to modulate the biological effects of MSC. According to Yang et al. (2020)'s findings, low levels of bluish LED laser at 1, 2, 3 and 4 J/cm² could prevent MSC from proliferating and stimulate MSC differentiation into osteoblasts. Wang et al. (2019) used LLLT with energy densities of 2, 4, 8, and 16 J/cm² to irradiate MSC in normal or inflammatory environments once at 1 day intervals, and examined the proliferation and osteogenic differentiation of the cells after 7 days. They found that LLLT with energy densities of 4 J/cm² had a positive effect on the proliferation of MSC. They found that LLLT with an energy density of 4 J/cm² significantly promoted MSC proliferation and osteogenic differentiation; however, in the inflammatory microenvironment, LLLT with an energy density of 8 J/cm² was required to significantly promote MSC proliferation and osteogenic differentiation. In contrast, LLLT at higher energy densities (16 J/cm²) significantly inhibited MSC proliferation and osteogenic differentiation in both normal and inflammatory environments. Cavalcanti et al. (2015) used LLLT with energy densities of 1-12 J/cm² to irradiate MSC for 3 consecutive days and showed that energy density was positively correlated with the proliferative effect of MSC, and LLLT promoted the MSC transition to G2/M phase, thus promoting the proliferation of MSC.

In addition, the energy density has a superimposed effect. LLLT at energy densities of 1.9 J/cm² and 3.8 J/cm² both promoted MSC proliferation, and the biological effects of one exposure to 3.8 J/cm² and two exposures to 1.9 J/cm² within 24 h were consistent in promoting MSC proliferation (Horvát-Karajz et al. 2009). Last but not least, the investigators showed that the optimal energy density for LLLT at 980 nm to stimulate MSC proliferation was 0.3 J/cm² and 3 J/cm² at 810 nm (Wang et al. 2017a). Therefore, it was indicated that the optimal energy density of LLLT to modulate the physical effects of MSC creation is also related to other experimental conditions.

TYPES

LLLT technologies that have been widely documented have included helium-neon (HeNe) gas lasers, indium gallium aluminum phosphide (InGaAlP) diode lasers, gallium-arsenide (GaAs), neodymium-doped yttrium aluminum garnet (Nd: YAG), gallium aluminum arsenide (GaAlAs), non-ablative, non-thermal carbon dioxide (CO2) lasers, LED arrays, and visible light (Zecha et al. 2016). Therefore, the diversity of LLLT types can sometimes lead to conflicting experimental results. What is controversial is whether LLLT coherence affects the biological effects of MSC. Because LEDs are non-coherent, diverging LLLTs, the energy of the LED is not concentrated, and special optics may be required to focus the energy on the target area. Tani et al. (2018) compared the effects of different types of LLLT (405 ± 5 nm) on the biological effects of MSC and found that the LED type of LLLT did not affect the biological effects of MSC compared to the control group, whereas the use of the same energy density of GaAlAs type of LLLT promoted MSC proliferation and osteogenic differentiation. However, Cardoso et al. (2021) demonstrated that LLLT with a red laser and a 637 nm LED was fully capable of inducing its mineralization in conventional media without osteogenic promoting factors, whereas no mineralization was observed under the same conditions with the infrared laser. Furthermore, another study showed that although LLLT of the LED type had no significant effect on MSC proliferation, LED could promote osteogenic differentiation of MSC by boosting the entry of β-catenin into the nucleus and activating the Wnt pathway (Ruan et al. 2021). Therefore, whether the coherence of LLLT is a key factor affecting MSC and whether coherence is associated with other factors needs further elaboration and research.

MSC ENVIRONMENT AND CELLS ACTING CELLS

LLLT can modulate the biological effects of many types of MSC. MSCs were first discovered in the bone marrow, but are not unique in their origin; in terms of cell culture passages, primary, third and fourth generation cells are usually used (Ahrabi et al. 2019). Stem cells of the bone mesenchymal type (BMSC), those from the dental pulp (DPSC), and those from the adipose tissue (ASC) are some of the most common types.

BMSCs can be used to treat myocardial infarction, Parkinson's disease, and diabetes mellitus (Blatt et al. 2016; Nandy et al. 2014; Tsai et al. 2014). However, the activity and tolerance of BMSC are weak, and transplantation of BMSC into the host microenvironment is severely limited even at very high cellular concentrations, so clinically large amounts of BMSC are required for therapeutic purposes.

DPSCs have been tested *in vitro* and DPSCs have been shown to be induced to differentiate into osteoblasts, keratinocytes, and smooth muscle cells (Syed-Picard et

al. 2015). Undeniably, DPSCs have made remarkable progress in the field of regenerative stomatology, but still face many challenges, such as their ability to repair tissues decreases with age and their directed differentiation efficiency is still not very high (Ballini et al. 2015).

ASC characteristics include self-renewal ability, multidirectional differentiation potential, easy availability, wide supply sources, and low adverse effects. In surgery, ASC can accelerate wound healing, inhibit scar formation and improve skin aging (Nurković et al. 2017).

In terms of cell physiological characteristics, MSC can be healthy or pathological (Fallahnezhad et al. 2016), and the viability and proliferation of pathological MSC (e.g., MSC in a high glucose microenvironment) are significantly impaired. Zare et al. (2020) used LLLT irradiation at a wavelength of 632.8 nm, an energy density of 1 J/cm², and a frequency of one to two times to improve the proliferation of MSC in a high glucose microenvironment.

CELL CULTURE MEDIUM

Cell culture media also affect the biological effects of LLLT on MSC. The commonly used cell culture media are modified Eagle's medium (DMEM) and osteogenic induction medium (ODM). Peng et al. (2012) compared the effects of LLLT on MSC in ODM and DMEM media and showed that different biological effects could be exhibited under different cultural conditions. In DMEM, LLLT promoted MSC proliferation but did not induce osteogenic differentiation, while in ODM LLLT promoted MSC osteogenic differentiation but inhibited its proliferation. Another research study found that LLLT had no effect on the biological effects of MSC in DMEM but significantly promoted the proliferation and osteogenic differentiation of MSC in ODM, significantly upregulated the expression of bone bridge protein gene, an important marker of MSC osteogenic differentiation, and promoted the formation of mineralized nodules (Yang et al. 2016).

LASER IRRADIATION

DURATION OF IRRADIATION

Irradiation time can affect the biological effect of LLLT on MSC, and the irradiation time was not positively correlated with the biological effect of MSC. Amaroli et al. (2018) used LLLT at 808 nm to irradiate MSC for 5, 10 and 15 days. They found that the expression of Runx2, an important early marker of osteogenic differentiation, started to increase at 5 days, reached its maximum at 10 days, and decreased at 15 days. In another study, MSCs exposed to LLLT were divided into three groups and irradiated for 1 h, 2 h, and 3 h, respectively, and then the researchers measured the effect of cell proliferation and found that 1 h had the greatest effect on cell proliferation; and all three groups showed different degrees of promotion and increase in cell proliferation (Yin et al. 2017). Therefore, when LLLT regulates the biological effects of MSC, the duration of irradiation must be controlled in order to achieve the desired results.

FREQUENCY OF LASER IRRADIATION

The frequency of LLLT irradiation had a large effect on the biological effects of MSC. Almeida-Jr. et al. (2019) compared the effect of single and multiple LLLT irradiation on MSCs, with an interval of 6h between multiple LLLT irradiations, and showed that 3 times LLLT irradiations were more effective in promoting MSC proliferation than single irradiation under the same conditions. Considering that the frequency of LLLT irradiation may cause different energy received by MSC, they also compared the effect of applying one LLLT irradiation with that of three LLLT irradiations at the same energy level on MSC, and the results showed that LLLT irradiation in three sessions promoted MSC proliferation better when the total energy received by the two groups was the same. Researchers compared the effect of multiple versus single application of LLLT on MSC, and irradiated cultured MSC with multiple or single LLLT irradiation within 13 days (every two days as an irradiation cycle), and found that the number of MSC was higher than the control group only 2 days after irradiation with single LLLT irradiation, while it was consistently higher than the control group with multiple LLLT irradiation. They (40) further found that multiple LLLT irradiation further improved the osteogenic differentiation of MSC (Li et al. 2013).

IRRADIATION SPOT SIZE

The irradiated area of LLLT is closely related to the energy density. de Villiers, Houreld and Abrahamse (2011) used a spot with an irradiated area of 9.1 cm² and an energy density of 5 J/cm² to irradiate MSC, which covered the entire surface of the culture wells, and found that the number of MSC in the LLLT group was significantly higher than in the control group within 3 days after irradiation. de Andrade et al. (2019) used a

very small spot (0.028 cm²) to irradiate MSC and found that it also promoted MSC proliferation, however, they did not describe how the very small spot irradiated the MSC in the entire well. Usually, a reliable method is to irradiate the entire MSC in the culture well by scanning (Pasternak-Mnich et al. 2019). However, it has also been shown that LLLT irradiation can have a beneficial biological effect on MSC even if the area irradiated by LLLT is less than 1/10 of the culture well area (Ferreira et al. 2019).

LIGHT FLUX DISTRIBUTION FORM

LLLT beam delivery systems can be divided into two types based on the light flux distribution: the 'standard' beam delivery system (Gaussian light flux distribution) and the 'flat-top' beam delivery system (uniform light flux distribution). Hanna et al. (2019) showed that the 'flat-top' beam delivery system is more beneficial for osteogenic differentiation of cells due to the uniform distribution of energy at various points on the cross-section of the emitted light spot, which helps to increase the biological activity of the cells. On the contrary, the 'standard' beam delivery system emits light with a high center and low peripheral energy distribution, which may have less impact on the biological effects of MSC (Bölükbaşı Ateş et al. 2020).

LIMITATIONS

Heterogeneity of experimental conditions Differences in LLLT parameters, MSC types, and culture conditions make it difficult to directly compare results across studies.

Lack of standardization There is no standardized method for studying the interaction between LLLT and MSCs, which can make it difficult to interpret and reproduce results.

Poor understanding of molecular mechanisms The molecular mechanisms by which LLLT regulates MSCs are not fully understood, limiting the ability to optimize treatment protocols for specific clinical applications.

Limited Clinical Trials Most of the research on LLLT-MSC interactions has been *in vitro* or in animal models, with limited clinical trials to evaluate safety and efficacy in humans.

CONCLUSIONS

LLLT as a physical method to regulate MSC has the advantages of being simple, cheap, and efficient, and can be combined with other chemicals to regulate the biological effects of MSC. The proper use of LLLT can promote the proliferation and osteogenic differentiation of MSC, which has important applications in bone tissue engineering. Given the limitations of the experimental conditions and the heterogeneity of the methods, it is often difficult to design experiments that can include all the influencing factors at the same time.

ACKNOWLEDGEMENTS

This review was made possible thanks to the guidance and assistance of my supervisor, Dr. Norma. Authors declare no conflict of interest.

REFERENCES

Ahrabi, B., Rezaei Tavirani, M., Khoramgah, M. S., Noroozian, M., Darabi, S., Khoshsirat, S., & Abbaszadeh, H.A. 2019. The effect of photobiomodulation therapy on the differentiation, proliferation, and migration of the mesenchymal stem cell: A review. *Journal of Lasers in Medical Sciences* 10(Suppl. 1): S96-S103. https://doi.org/10.15171/jlms.2019.S17

Almeida-Jr., L.A., Marques, N.C.T., Prado, M.T.d.O., Oliveira, T.M. & Sakai, V.T. 2019. Effect of single and multiple doses of low-level laser therapy on viability and proliferation of stem cells from human exfoliated deciduous teeth (SHED). *Lasers in Medical Science*, *34*(9), 1917–1924. https://doi.org/10.1007/s10103-019-02836-y

Amaroli, A., Agas, D., Laus, F., Cuteri, V., Hanna, R., Sabbieti, M.G. & Benedicenti, S. 2018. The effects of photobiomodulation of 808 nm diode laser therapy at higher fluence on the *in vitro* osteogenic differentiation of bone marrow stromal cells. *Frontiers in Physiology* 9: 123. https://doi.org/10.3389/fphys.2018.00123

Ballini, A., Mastrangelo, F., Gastaldi, G., Tettamanti, L., Bukvic, N., Cantore, S., Cocco, T., Saini, R., Desiate, A., Gherlone, E. & Scacco, S. 2015. Osteogenic differentiation and gene expression of dental pulp stem cells under low-level laser irradiation: A good promise for tissue engineering. *J. Biol. Regul. Homeost. Agents* 29(4): 813-822. https://europepmc.org/article/med/26753641

Blatt, A., Elbaz-Greener, G.A., Tuby, H., Maltz, L., Siman-Tov, Y., Ben-Aharon, G., Copel, L., Eisenberg, I., Efrati, S., Jonas, M., Vered, Z., Tal, S., Goitein, O. & Oron, U. 2016. Low-level laser therapy to the bone marrow reduces scarring and improves heart function post-acute myocardial infarction in the pig. *Photomedicine and Laser Surgery* 34(11): 516-524. https://doi.org/10.1089/pho.2015.3988

Bölükbaşı Ateş, G., Ak, A., Garipcan, B. & Gülsoy, M. 2020. Photobiomodulation effects on osteogenic differentiation of adipose-derived stem cells. *Cytotechnology* 72(2): 247-258. https://doi.org/10.1007/s10616-020-00374-y

Cardoso, M.V., do Vale Placa, R., Sant'Ana, A.C.P., Greghi, S.L.A., Zangrando, M.S.R., de Rezende, M.L.R., Oliveira, R.C. & Damante, C.A. 2021. Laser and LED photobiomodulation effects in osteogenic or regular medium on rat calvaria osteoblasts obtained by newly forming bone technique. *Lasers in Medical Science* 36(3): 541-553. https://doi.org/10.1007/s10103-020-03056-5

Cavalcanti, M.F.X.B., Maria, D.A., de Isla, N., Leal-Junior, E.C.P., Joensen, J., Bjordal, J.M., Lopes-Martins, R.A.M.B., Diomede, F., Trubiani, O. & Frigo, L. 2015. Evaluation of the proliferative effects induced by low-level laser therapy in bone marrow stem cell culture. *Photomedicine and Laser Surgery* 33(12): 610-616. https://doi.org/10.1089/pho.2014.3864

de Andrade, A.L.M., Luna, G.F., Brassolatti, P., Leite, M.N., Parisi, J.R., de Oliveira Leal, Â.M., Frade, M.A.C., de Freitas Anibal, F. & Parizotto, N.A. 2019. Photobiomodulation effect on the proliferation of adipose tissue mesenchymal stem cells. *Lasers in Medical Science* 34(4): 677-683. https://doi.org/10.1007/s10103-018-2642-2

de Villiers, J.A., Houreld, N.N. & 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 Reviews and Reports* 7(4): 869-882. https://doi.org/10.1007/s12015-011-9244-8

Fageeh, H.N. 2021. Preliminary evaluation of proliferation, wound healing properties, osteogenic and chondrogenic potential of dental pulp stem cells obtained from healthy and periodontitis affected teeth. *Cells* 10(8): 2118. https://doi.org/10.3390/cells10082118

Fallahnezhad, S., Jajarmi, V., Shahnavaz, S., Amini, A., Ghoreishi, S.K., Kazemi, M., Chien, S. & Bayat, M. 2020. Improvement in viability and mineralization of osteoporotic bone marrow mesenchymal stem cell through combined application of photobiomodulation therapy and oxytocin. *Lasers in Medical Science* 35(3): 557-566. https://doi.org/10.1007/s10103-019-02848-8

Fallahnezhad, S., Piryaei, A., Tabeie, F., Nazarian, H., Darbandi, H., Amini, A., Mostafavinia, A., Ghorishi, S.K., Jalalifirouzkouhi, A. & Bayat, M. 2016. Low-level laser therapy with helium-neon laser improved viability of osteoporotic bone marrow-derived mesenchymal stem cells from ovariectomy-induced osteoporotic rats. *Journal of Biomedical Optics* 21(9): 98002. https://doi.org/10.1117/1.JBO.21.9.098002

Fekrazad, R., Asefi, S., Eslaminejad, M.B., Taghiar, L., Bordbar, S. & Hamblin, M.R. 2019. Photobiomodulation with single and combination laser wavelengths on bone marrow mesenchymal stem cells: Proliferation and differentiation to bone or cartilage. *Lasers in Medical Science* 34(1): 115-126. https://doi.org/10.1007/s10103-018-2620-8

Ferreira, L.S., Diniz, I.M.A., Maranduba, C.M.S., Miyagi, S.P.H., Rodrigues, M.F.S.D., Moura-Netto, C. & Marques, M.M. 2019. Short-term evaluation of photobiomodulation therapy on the proliferation and undifferentiated status of dental pulp stem cells. *Lasers in Medical Science* 34(4): 659-666. https://doi.org/10.1007/s10103-018-2637-z

Gether, U. 2000. Uncovering molecular mechanisms involved in activation of G protein-coupled receptors. *Endocrine Reviews* 21(1): 90-113. https://doi.org/10.1210/edrv.21.1.0390

Han, Y., Li, X., Zhang, Y., Han, Y., Chang, F. & Ding, J. 2019. Mesenchymal stem cells for regenerative medicine. *Cells* 8(8): 886. https://doi.org/10.3390/cells8080886

Hanna, R., Agas, D., Benedicenti, S., Ferrando, S., Laus, F., Cuteri, V., Lacava, G., Sabbieti, M.G. & Amaroli, A. 2019. A comparative study between the effectiveness of 980 nm photobiomodulation delivered by hand-piece with Gaussian vs. flat-top profiles on osteoblasts maturation. *Frontiers in Endocrinology* 10: 92. https://doi.org/10.3389/fendo.2019.00092

Horvát-Karajz, K., Balogh, Z., Kovács, V., Drrernat, A.H., Sréter, L. & Uher, F. 2009. *In vitro* effect of carboplatin, cytarabine, paclitaxel, vincristine, and low-power laser irradiation on murine mesenchymal stem cells. *Lasers in Surgery and Medicine* 41(6): 463-469. https://doi.org/10.1002/lsm.20791

Li, W.T., Chen, C.W. & Huang, P.Y. 2013. Effects of low level light irradiation on the migration of mesenchymal stem cells derived from rat bone marrow. *Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE Engineering in Medicine and Biology Society.* pp. 4121-4124. https://doi.org/10.1109/EMBC.2013.6610452

Marques, M.M., Diniz, I.M.A., de Cara, S.P.H.M., Pedroni, A.C.F., Abe, G.L., D›Almeida-Couto, R.S., Lima, P.L.V., Tedesco, T.K. & Moreira, M.S. 2016. Photobiomodulation of dental derived mesenchymal stem cells: A systematic review. *Photomedicine and aser Surgery* 34(11): 500-508. https://doi.org/10.1089/pho.2015.4038

Merigo, E., Bouvet-Gerbettaz, S., Boukhechba, F., Rocca, J.P., Fornaini, C. & Rochet, N. 2016. Green laser light irradiation enhances differentiation and matrix mineralization of osteogenic cells. *Journal of Photochemistry and Photobiology. B, Biology* 155: 130-136. https://doi.org/10.1016/j.jphotobiol.2015.12.005

Mester, E., Szende, B. & Gärtner, P. 1968. The effect of laser beams on the growth of hair in mice. *Radiobiologia, Radiotherapia* 9(5): 621-626. https://europepmc.org/article/med/5732466

Migliario, M., Sabbatini, M., Mortellaro, C. & Renò, F. 2018. Near infrared low-level laser therapy and cell proliferation: The emerging role of redox sensitive signal transduction pathways. *Journal of Biophotonics* 11(11): e201800025. https://doi.org/10.1002/jbio.201800025

Min, K.H., Byun, J.H., Heo, C.Y., Kim, E.H., Choi, H.Y. & Pak, C.S. 2015. Effect of low-level laser therapy on human adipose-derived stem cells: *in vitro* and *in vivo* studies. *Aesthetic Plastic Surgery* 39(5): 778-782. https://doi.org/10.1007/s00266-015-0524-6

Nandy, S.B., Mohanty, S., Singh, M., Behari, M. & Airan, B. 2014. Fibroblast growth factor-2 alone as an efficient inducer for differentiation of human bone marrow mesenchymal stem cells into dopaminergic neurons. *Journal of Biomedical Science* 21(1): 83. https://doi.org/10.1186/s12929-014-0083-1

Nurković, J., Zaletel, I., Nurković, S., Hajrović, Š., Mustafić, F., Isma, J., Škevin, A.J., Grbović, V., Filipović, M.K. & Dolićanin, Z. 2017. Combined effects of electromagnetic field and low-level laser increase proliferation and alter the morphology of human adipose tissue-derived mesenchymal stem cells. *Lasers in Medical Science* 32(1): 151-160. https://doi.org/10.1007/s10103-016-2097-2

Pasternak-Mnich, K., Ziemba, B., Szwed, A., Kopacz, K., Synder, M., Bryszewska, M. & Kujawa, J. 2019. Effect of photobiomodulation therapy on the increase of viability and proliferation of human mesenchymal stem cells. *Lasers in Surgery and Medicine* 51(9): 824-833. https://doi.org/10.1002/lsm.23107

Peng, F., Wu, H., Zheng, Y., Xu, X. & Yu, J. 2012. The effect of noncoherent red light irradiation on proliferation and osteogenic differentiation of bone marrow mesenchymal stem cells. *Lasers in Medical Science* 27(3): 645-653. https://doi.org/10.1007/s10103-011-1005-z

Ruan, Y., Kato, H., Taguchi, Y., Yamauchi, N. & Umeda, M. 2021. Irradiation by high-intensity red light-emitting diode enhances human bone marrow mesenchymal stem cells osteogenic differentiation and mineralization through Wnt/β-catenin signaling pathway. *Lasers in Medical Science* 36(1): 55-65. https://doi.org/10.1007/s10103-020-03002-5

Serrage, H., Heiskanen, V., Palin, W.M., Cooper, P.R., Milward, M.R., Hadis, M. & Hamblin, M.R. 2019. Under the spotlight: Mechanisms of photobiomodulation concentrating on blue and green light. *Photochemical & Photobiological Sciences* 18(8): 1877-1909. https://doi.org/10.1039/c9pp00089e

Syed-Picard, F.N., Du, Y., Lathrop, K.L., Mann, M.M., Funderburgh, M.L. & Funderburgh, J.L. 2015. Dental pulp stem cells: A new cellular resource for corneal stromal regeneration. *Stem Cells Translational Medicine* 4(3): 276-285. https://doi.org/10.5966/sctm.2014-0115

Tani, A., Chellini, F., Giannelli, M., Nosi, D., Zecchi-Orlandini, S. & Sassoli, C. 2018. Red (635 nm), near-infrared (808 nm) and violet-blue (405 nm) photobiomodulation potentiality on human osteoblasts and mesenchymal stromal cells: A morphological and molecular *in vitro* study. *International Journal of Molecular Sciences* 19(7): 1946. https://doi.org/10.3390/ijms19071946

Tsai, P.J., Wang, H.S., Lin, C.H., Weng, Z.C., Chen, T.H. & Shyu, J.F. 2014. Intraportal injection of insulin-producing cells generated from human bone marrow mesenchymal stem cells decreases blood glucose level in diabetic rats. *Endocrine Research* 39(1): 26-33. https://doi.org/10.3109/07435800.2013.797432

Wang, L., Wu, F., Liu, C., Song, Y., Guo, J., Yang, Y. & Qiu, Y. 2019. Low-level laser irradiation modulates the proliferation and the osteogenic differentiation of bone marrow mesenchymal stem cells under healthy and inflammatory condition. *Lasers in Medical Science* 34(1): 169-178. https://doi.org/10.1007/s10103-018-2673-8

Wang, Y., Huang, Y.Y., Wang, Y., Lyu, P. & Hamblin, M.R. 2017a. Photobiomodulation of human adipose-derived stem cells using 810nm and 980nm lasers operates via different mechanisms of action. *Biochimica Et Biophysica Acta. General Subjects* 1861(2): 441-449. https://doi.org/10.1016/j.bbagen.2016.10.008

Wang, Y., Huang, Y.Y., Wang, Y., Lyu, P. & Hamblin, M.R. 2017b. Red (660 nm) or near-infrared (810 nm) photobiomodulation stimulates, while blue (415 nm), green (540 nm) light inhibits proliferation in human adipose-derived stem cells. *Scientific Reports* 7(1): 7781. https://doi.org/10.1038/s41598-017-07525-w

Wu, J.Y., Chen, C.H., Yeh, L.Y., Yeh, M.L., Ting, C.C. & Wang, Y.H. 2013. Low-power laser irradiation promotes the proliferation and osteogenic differentiation of human periodontal ligament cells via cyclic adenosine monophosphate. *International Journal of Oral Science* 5(2): 85-91. https://doi.org/10.1038/ijos.2013.38

Yang, D., Yi, W., Wang, E. & Wang, M. 2016. Effects of light-emitting diode irradiation on the osteogenesis of human umbilical cord mesenchymal stem cells *in vitro. Scientific Reports* 6: 37370. https://doi.org/10.1038/srep37370

Yang, Y., Zhu, T., Wu, Y., Shu, C., Chen, Q., Yang, J., Luo, X. & Wang, Y. 2020. Irradiation with blue light-emitting diode enhances osteogenic differentiation of stem cells from the apical papilla. *Lasers in Medical Science* 35(9): 1981-1988. https://doi.org/10.1007/s10103-020-02995-3

Yin, K., Zhu, R., Wang, S. & Zhao, R.C. 2017. Low-level laser effect on proliferation, migration, and antiapoptosis of mesenchymal stem cells. *Stem Cells and Development* 26(10): 762-775. https://doi.org/10.1089/scd.2016.0332

Yuan, Y., Yan, G., Gong, R., Zhang, L., Liu, T., Feng, C., Du, W., Wang, Y., Yang, F., Li, Y., Guo, S., Ding, F., Ma, W., Idiiatullina, E., Pavlov, V., Han, Z., Cai, B. & Yang, L. 2017. Effects of blue light emitting diode irradiation on the proliferation, apoptosis and differentiation of bone marrow-derived mesenchymal stem cells. *Cellular Physiology and Biochemistry* 43(1): 237-246. https://doi.org/10.1159/000480344

Zare, F., Bayat, M., Aliaghaei, A. & Piryaei, A. 2020. Photobiomodulation therapy compensate the impairments of diabetic bone marrow mesenchymal stem cells. *Lasers in Medical Science* 35(3): 547-556. https://doi.org/10.1007/s10103-019-02844-y

Zare, F., Moradi, A., Fallahnezhad, S., Ghoreishi, S.K., Amini, A., Chien, S. & Bayat, M. 2019. Photobiomodulation with 630 plus 810 nm wavelengths induce more *in vitro* cell viability of human adipose stem cells than human bone marrow-derived stem cells. *Journal of Photochemistry and Photobiology B: Biology* 201: 111658. https://doi.org/10.1016/j.jphotobiol.2019.111658

Zecha, J.A.E.M., Raber-Durlacher, J.E., Nair, R.G., Epstein, J.B., Sonis, S.T., Elad, S., Hamblin, M.R., Barasch, A., Migliorati, C.A., Milstein, D.M.J., Genot, M.T., Lansaat, L., van der Brink, R., Arnabat-Dominguez, J., van der Molen, L., Jacobi, I., van Diessen, J., de Lange, J., Smeele, L.E., Schubert, M.M. & Bensadoun, R.J. 2016. Low level laser therapy/photobiomodulation in the management of side effects of chemoradiation therapy in head and neck cancer: Part 1: Mechanisms of action, dosimetric, and safety considerations. *Supportive Care in Cancer: Official Journal of the Multinational Association of Supportive Care in Cancer* 24(6): 2781-2792. https://doi.org/10.1007/s00520-016-3152-z

Zein, R., Selting, W. & Hamblin, M.R. 2018. Review of light parameters and photobiomodulation efficacy: Dive into complexity. *Journal of Biomedical Optics* 23(12): 1-17. https://doi.org/10.1117/1.JBO.23.12.120901

Zhang, R.F., Wang, Q., Zhang, A.A., Xu, J.G., Zhai, L.D., Yang, X.M. & Liu, X.T. 2018. Low-level laser irradiation promotes the differentiation of bone marrow stromal cells into osteoblasts through the APN/Wnt/β-catenin pathway. *European Review for Medical and Pharmacological Sciences* 22(9): 2860-2868. https://doi.org/10.26355/eurrev_201805_14988

Zhu, T., Wu, Y., Zhou, X., Yang, Y. & Wang, Y. 2019. Irradiation by blue light-emitting diode enhances osteogenic differentiation in gingival mesenchymal stem cells *in vitro. Lasers in Medical Science* 34(7): 1473-1481. https://doi.org/10.1007/s10103-019-02750-3

Zungu, I.L., Hawkins Evans, D. & Abrahamse, H. 2009. Mitochondrial responses of normal and injured human skin fibroblasts following low level laser irradiation--an *in vitro* study. *Photochemistry and Photobiology* 85(4): 987-996. https://doi.org/10.1111/j.1751-1097.2008.00523.x

*Corresponding author; email: drnorma@usm.my