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Molecular impacts of photobiomodulation on bone regeneration: A systematic review

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ABSTRACT

Photobiomodulation (PBM) encompasses a light application aimed to increase healing process, tissue regeneration, and reducing inflammation and pain. PBM is specifically aimed to modify the expression of cellular molecules; however, PBM impacts on cellular and molecular pathways especially in bone regenerative medicine have been investigated in scattered different studies. The purpose of the current study is to systematically review evidence on molecular impact of PBM on bone regeneration. A comprehensive electronic search in Medline, Scopus, EMBASE, EBSCO, Cochrane library, web of science, and google scholar was conducted from January 1975 to October 2018 limited to English language publications on administrations of photobiomodulation for bone regeneration which evaluated biological factors. In addition, hand search of selected journals was done to retrieve all articles. This systematic review was performed based on PRISMA guideline. Among these studies, five articles reported *in vitro* results, twelve articles were *in vivo*, and three of them were clinical trials. The data tabulated according to the type of markers (osteogenic markers, angiogenic markers, growth factors, and inflammation mediators). PBM's effects depend on many parameters which energy density is more important than the others. PBM can significantly enhance expression of osteocalcin, collagen, RUNX-2, vascular endothelial growth factor, bone morphogenic proteins, and COX-2. Although since the heterogeneity of the studies and their limitations, an evidence-based decision for definite therapeutic application of PBM is still unattainable, the findings of our review can help other researchers to ameliorate their study design and elect more efficient approach for their investigation.

Contents

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1. Introduction

Photobiomodulation (PBM) encompasses a light application aimed to increase healing process (Posten et al., 2005), tissue regeneration (da Silva et al., 2010), reducing inflammation and pain (Woodruff et al., 2004), and alteration in immunologic activity (Dostalova et al., 2017). Nowadays, PBM as a noninvasive therapeutic approach applied for wide medical purposes from nerve injuries (Sene et al., 2013) and wound healing (Ustaoglu et al., 2017) to osteoarthritis treatment (Huang et al., 2015). PBM commonly consist of low level laser therapy, red/infrared coherent or LED non coherent light which provides lower (1–500 mW) output power in comparison to other laser treatment methods (AlGhamdi et al., 2012). Hence, not only this treatment modality is not harmful due to lack of any thermal effects (Basford, 1995), but also it can stimulate some beneficial photochemical reactions that affect cellular metabolism.

In vivo investigations have shown that PBM can stimulate mesenchymal stem cells (MSCs) proliferation by potential changes in gene expressions and activations (Peat et al., 2018; Schneede et al., 1988; Wu et al., 2012). Funk et al. represented that cytokine release will alter after PBM via He – Ne laser (Funk et al., 1992). In addition, there are many studies reported beneficial impacts of PBM on wound healing and tissue repair which have been suggested to cause by modulating of inflammation mediators like interleukin (IL) −1, interferon−γ (Deng et al.), and tumor necrosis factor (TNF) −α, and increasing of growth factor secretion such as fibroblast growth factor (FGF), platelet derived growth factor (PDGF), and transforming growth factor (TGF) (Al-Watban and Zhang, 2004; Enwemeka et al., 2004; Neiburger, 1999; Safavi et al., 2008). PBM induces new bone formation with enhancing osteoblastic activity and reducing osteoclastic activity (Ninomiya et al., 2007). It has been shown that laser therapy can increase bone regeneration via affecting regulatory factors in bone matrix (Ueda and Shimizu, 2003). Moreover, PBM is able to increase the expression of bone morphogenic protein-4 (BMP-4) and activation of transcription factors such as Runt-related transcription factor −2 (RUNX-2) genes during bone healing process in animal model (Fávaro–Pípi et al., 2011). There are several proposed molecular mechanism for explaining PBM's effects such as affecting beta catenin pathway, cyclinD1 pathway, and mitogen activated protein kinase pathway (de Freitas and Hamblin, 2016). PBM can increase intracellular adenosine triphosphate (ATP) which plays a major role in cellular signaling (Farivar et al., 2014). It has been seen that Calcium concentration increased after PBM application (Santulli and R Marks, 2015). Thereby, Calcium-sensitive signaling pathways can be triggered by laser irradiation (Sharma et al., 2011; Wu et al., 2010).

Although one of the goals of PBM is to modify the expression of cellular molecules, the molecular mechanisms especially in bone regenerative medicine have been investigated in various scattered studies and are not precisely understood. The current study aims to systematically review the available literature on molecular impact of PBM on bone regeneration.

2. Material and methods

2.1. Protocol

This review is organized and followed PRISMA guidelines (Moher et al., 2015) for systematic reviews.

2.2. Eligibility criteria

Types of studies: All clinical investigations (clinical trials, case-controls, and case series), *in vitro* and *in vivo* investigations, which administrated level laser therapy, red/infrared coherent or LED non-coherent light (photobiomodulation) for bone regenerative investigations on cell lines, humans and animal models were included. Case reports, systematic and literature reviews, letter to editors, and thesis were omitted.

Types of participants: Humans and any type of animal models (such as rats and primates), photobiomodulation (such as red lasers, and infrared lasers) were included. Animal models with other systemic variables (such as diabetic rats) were excluded.

Types of interventions: Studies investigated photobiomodulation on osteoprogenitor stem cells, osteoblast like cell lines, and bone regeneration in human beings and animal models which assessed osteogenic, angiogenic, growth factors, and inflammation mediators were included and others were excluded. In addition, studies which did not aim to evaluate the molecular impact of photobiomodulation application on osteogenic, angiogenic, growth factors, and inflammation mediators and only assessed the amount of bone formation were excluded.

Types of outcome measures: Level of biological factors reported by any of following tests; Alizarin Red staining and gene analysis. Bone regeneration reported by any of the following tests; Histological and immunohistochemistry analysis, enzyme-linked immunosorbent assays, and real-time polymerase chain reaction (RT-PCR).

2.3. Information sources

MEDLINE (NCBI PubMed and PMC), EMBASE, EBSCO, Scopus, Cochrane library, web of science, and google scholar were the information sources. Moreover, a hand search was done from January 2007 to October 2018 specifically in the following journals; Lasers in Medical Science, Journal of Photochemistry & Photobiology B, Photomedicine and Laser Surgery, Tissue Engineering: Part A, Lasers in Surgery, and Medicine and Journal of Dental Research.

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2.4. Search strategy

An electronic search was conducted in PubMed from January 1975 to October 2018 limited to English language publications. Published papers on photobiomodulation in bone regeneration were found using the following keywords alone or ensemble: ("Laser Therapy"[Mesh] OR "Low-Level light Therapy"[Mesh] OR "Laser Phototherapy"[Mesh] OR "Photobiomodulation Therapy"[Mesh] OR "Low-intensity laser therapy"[Mesh] OR "Low-Level Laser Irradiation"[Mesh]) AND ("Bone regeneration"[Mesh] OR "Inflammation Mediators"[Mesh] OR "Bony Defects"[Mesh] OR "Biological Factors"[Mesh] OR "Biomarkers"[Mesh]). In addition, manually search of the articles in the selected journals has been done.

2.5. Study selection and data extraction

Two independent reviewers retrieved studies according to the defined keywords. They also performed initial screening on titles and abstracts of the selected articles according to the pre-defined eligibility criteria. Disagreement between reviewers was resolved by discussion and if still remain, a third person consulting will use. After all, we reviewed all full texts in which all the authors selected and confirmed. Data extraction were performed according to PRISMA statement.

2.6. Data items

Data were summarized according to followings: 1) Author and year of publication; 2) laser properties and treatment protocol; 3) Type of study (clinical investigations and *in* vivo/*in vitro*); 4) types of animal models; 5) types of cells for *in vitro* investigations; 6) defects characteristics; 7) Method of assessment; 8) type of evaluated marker(s); 9) duration of follow ups; and 10) outcomes of assessments.

3. Results

3.1. Study selection

Fig. 1 illustrated the PRISMA flow diagram of search strategy used in this study. 2873 articles were retrieved from the databases and one article from other sources and two studies from hand search. A total of 2876 articles were initially screened by two independent reviewers and 2781 studies were excluded due to

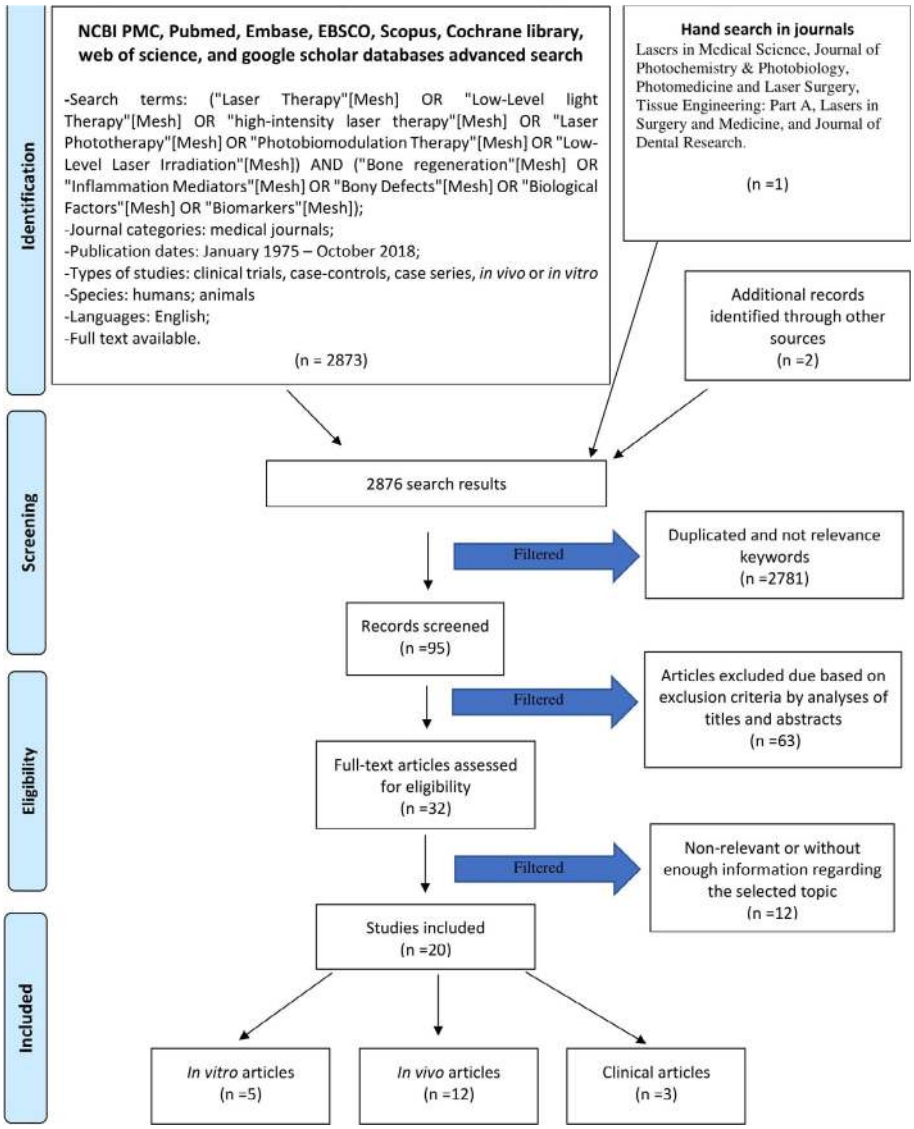

Fig. 1. PRISMA flow diagram of search strategy used in this study.

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duplication and un related keywords according to our study design. After exclusion of non-relevant articles, the records of 95 studies were evaluated. Within these studies, four of them were excluded since they only investigated pre-osteoblast cell proliferation after laser therapy (Aihara et al., 2006; El-Maghraby et al., 2013; Garcia et al., 2013; Huertas et al., 2014). Three studies were excluded due to the fact that they evaluated differentiation by alkaline activity measurement in their study (Coombe et al., 2001; Jawad et al., 2013; Korany et al., 2012). Seven other studies excluded because of utilization of diabetic (Mostafavinia et al., 2017a; Patrocínio-Silva et al., 2014) and osteoprotic (Bossini et al., 2011; Fredoni et al., 2017; Mohsenifar et al., 2016; Mostafavinia et al., 2017b; Scalize et al., 2015) rats as their animal models. Five studies assessed bone formation or osteointegration just by radiographic scans and histological analysis were also excluded (de Vasconcellos et al., 2016; Fekrazad et al., 2015; Gerbi et al., 2005; Gomes et al., 2015; Mandić et al., 2015). In addition, there were many clinical investigations that only assessed clinical parameters and did not report any molecular or cellular findings. Finally, out of 32 related full texts, 20 studies were included and analyzed in this systematic review. Among these studies, five articles were *in vitro*, twelve studies were *in vivo*, and three articles were clinical trials.

3.2. Osteogenic markers, angiogenic markers, and growth factors

Nineteen articles investigated osteogenic & angiogenic markers, and growth factors that consist of five *in vitro* (Ateş et al., 2017; Chen et al., 2018; Peat et al., 2017; Saracino et al., 2009; Wang et al., 2016), and eleven *in vivo* studies (Table 1) (Fallahnezhad et al., 2018; Kesler et al., 2011; Magri et al., 2015; Omasa et al., 2012; Park et al., 2015; Patrocínio-Silva et al., 2014; Sella et al., 2015; Tim et al., 2014, 2015, 2016; Wang et al., 2018), and three clinical trials (Table 2) (Domínguez et al., 2015; Mozzati et al., 2011, 2012). In two studies, neodymium-doped yttrium aluminum garnet (Nd:YAG) laser were applied (Kesler et al., 2011; Peat et al., 2018), and one study used He–Ne laser (Fallahnezhad et al., 2018); In other studies gallium-aluminum-arsenide (Ga–Al–As) laser used (Ateş et al., 2017; Magri et al., 2015; Omasa et al., 2012; Park et al., 2015; Saracino et al., 2009; Sella et al., 2015; Tim et al., 2014, 2015, 2016; Wang et al., 2016). In addition, one investigation utilized LED array and filtered lamp for photobiomodulation (Wang et al., 2016). Although wavelength of the laser irradiation include a wide spectrum from 420 nm (Wang et al., 2016) to 2940 nm (Kesler et al., 2011), most of the studies applied 800–830 nm (Ateş et al., 2017; Magri et al., 2015; Omasa et al., 2012; Sella et al., 2015; Tim et al., 2014, 2015, 2016; Wang et al., 2016). Three article used pulsed mode of irradiation (Kesler et al., 2011; Peat et al., 2018; Saracino et al., 2009) and others applied continuous wave mode. Energy density also encompass a wide range from 1.5 J/cm2 (Ateş et al., 2017) to 1000 J/cm2 (Tim et al., 2015); However, in most of articles its amount was less than 5 J/cm2 (Ateş et al., 2017; Magri et al., 2015; Saracino et al., 2009; Tim et al., 2016; Wang et al., 2016). *In vitro* studies used pre-osteoblasts, osteoblasts, mesenchymal and adipose derived stem cells (Ateş et al., 2017; Fallahnezhad et al., 2018; Peat et al., 2018; Saracino et al., 2009; Wang et al., 2016). All *in vivo* investigations used rats as their animal model (Chen et al., 2018; Kesler et al., 2011; Magri et al., 2015; Omasa et al., 2012; Park et al., 2015; Sella et al., 2015; Tim et al., 2014, 2015, 2016; Wang et al., 2018) and bone defect at tibia bone was the most common one (Kesler et al., 2011; Magri et al., 2015; Tim et al., 2014, 2015, 2016). Real time polymerase chain reaction (RT-PCR) was the most frequent type of assessment. Seven studies used immunohistochemistry (IHC) analysis. One article applied Western blot (Park et al., 2015) and two used enzyme-linked immunosorbent assay (Fallahnezhad et al., 2018; Peat et al., 2018) and one study used micro array hybridization as their assessment method (Tim et al., 2015). Angiopoietin 2 (ANGPT-2), bone morphogenetic protein (BMP) −1, −2, −4, −7, Collagen type I (COL1), fibroblast growth factor (FGF) −2, −3, −5, −7, −14, −15, −17, −22, osterix (OSX), osteocalcin (OCN), osteopontin (OPN), osteonectin (Chang et al., 2010, peroxisome proliferators-activated receptor (PPAR), receptor activator of nuclear factor kappa-B ligand (RANKL), runt-related transcription factor (RUNX − 2), transforming growth factor beta (TGF b), insulin-like growth factor (IGF), von Willebrand factor (vWF), and vasculoendothelial growth factor (VEGF) were studied in these articles (Ateş et al., 2017; Chen et al., 2018; Kesler et al., 2011; Magri et al., 2015; Omasa et al., 2012; Park et al., 2015; Peat et al., 2018; Saracino et al., 2009; Sella et al., 2015; Tim et al., 2014, 2015, 2016; Wang et al., 2016, 2018). Within these studies, one study indicated only qualitative findings (Tim et al., 2015). All of the remaining articles, found at least one positive result with significant difference between laser treated and control groups ($p < 0.05$) (Ateş et al., 2017; Kesler et al., 2011; Magri et al., 2015; Omasa et al., 2012; Park et al., 2015; Patrocínio-Silva et al., 2014; Peat et al., 2018; Saracino et al., 2009; Sella et al., 2015; Tim et al., 2014, 2016; Wang et al., 2016). Clinical trials demonstrated that PBM application (continuous or super pulsed) at 670 nm and 910 nm can up regulate RANKL (Domínguez et al., 2015), Col I and III (Mozzati et al., 2011).

3.3. Inflammation mediators

Six articles investigated inflammatory markers that consist of one *in vitro* (Peat et al., 2018), three *in vivo* (Matsumoto et al., 2009; Tim et al., 2014, 2016), and two clinical studies (Mozzati et al., 2011, 2012). All extracted data were summarized in Table 3. Within these studies, Nd:YAG laser with pulsed wave mode was applied in one article (Peat et al., 2018), and Ga–Al–As laser with continuous wave used in others (Matsumoto et al., 2009; Tim et al., 2014, 2016). Two studies applied 830 nm wavelength (Tim et al., 2014, 2016), one study utilized 1064 nm irradiation (Peat et al., 2018), and another one used 735 nm laser (Matsumoto et al., 2009). Energy densities and time of irradiation were varied among studies (9.77 J/cm2 for 10 s (Peat et al., 2018), 2.8 J/cm2 for 94 s (Tim et al., 2016), 120 J/cm2 for 34 s (Tim et al., 2014), and 16 J/cm2 for 60 s (Matsumoto et al., 2009)). Wistar rat was the only assessed animal models (Matsumoto et al., 2009; Tim et al., 2014, 2016) among these articles. All defects were created in tiabia bone (Matsumoto et al., 2009; Tim et al., 2014, 2016). Human bone marrow mesenchymal stem cells (BMMSCs) was investigated *in vitro* (Peat et al., 2018). IHC was the most frequent type of assessment (Matsumoto et al., 2009; Tim et al., 2014, 2016) followed by RT-PCR (Peat et al., 2018). Cyclooxygenase-2 (COX-2), Heat Shock Protein 90 (HSP90), interferons gamma (IFN-c), interleukin (IL) −1, −4, −6, −8, −10, −13, −17, macrophage differentiation-associated gene (MMD), prostaglandin I2 receptor (PTGIR), prostaglandin-endoperoxide synthase (PTGS-2), and tumor necrosis factor (TNF) were studied as the inflammation mediators (Matsumoto et al., 2009; Peat et al., 2018; Tim et al., 2014, 2016). Within these studies, only two studies reported significant differences in COX-2 expression (Matsumoto et al., 2009; Tim et al., 2016). Among clinical trials, one study has shown down regulated level of IL-6 and -10 after laser therapy in comparison to control groups (Mozzati et al., 2011); whereas the other one indicated up regulation at the same laser therapeutic protocol (Mozzati et al., 2012). However, both of them represented a significant down regulation of IL-1 beta expression in PBM groups compared to controls ($p < 0.05$) (Mozzati et al., 2011, 2012).

4. Discussion

PBM has been applied in various field of medicine such as promoting tissue healing (Posten et al., 2005) and repair (da Silva et al., 2010), analgesic and anti-inflammatory effects(Woodruff

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Table 1
Impact of photobiomodulation on osteogenic, angiogenic, and growth factors' expression according to *in vitro*/*in vivo* studies.

Authors & year	Laser properties and treatment protocol	Type of study	Animal model	Defect type	Cell type	Method	Marker	Follow-up period	Results
Wang YH et al. (Wang et al., 2018), 2018	660 nm, Ga–Al–As continuous laser,13.3 J/cm2, 70 mW, 540 s Irritated 24 h after surgery, and once daily for 16 weeks.	*In vivo*	24 Sprague-Dawley rats	Critical-sized calvarial – 7 mm	hADSCs	IHC	vWF, BMP-2, Col I, OCN	16 weeks	1. Up-regulation of vWF in hADSCs and hADSCs + Laser groups in comparison to control ($p < 0.05$). 2. No significant difference of BMP-2, Col I, and OCN levels ($p > 0.05$).
Fallahnezhad S et al. (Fallahnezhad et al., 2018), 2018	632.8 nm, He–Ne laser, 1.2 J/cm2, 3 mW, 378 s Irritation 0, 3, and 7 days after surgery.	*In vitro*	12 Wistar rats	–	Rat BMMSCs	ELISA	TGF-beta, IGF-I	7 days	Up-regulation of TGF-beta and IGF-I in laser group compared to control ($p < 0.05$).
Chen CH et al. (Chen et al., 2018), 2018	660 nm, Ga–Al–As continuous laser,13.3 J/cm2, 70 mW, 540 s Irritated 24 h after surgery, and once daily for 16 weeks.	*In vivo*	24 Sprague-Dawley rats	Critical-sized calvarial – 7 mm	hADSCs	IHC	vWF, BMP-2, Col I, OCN	16 weeks	Up-regulation of vWF in hADSCs and hADSCs + Laser groups in comparison to control ($p < 0.05$).
Ates GB et al. (Ateş et al., 2017), 2017	Ga–Al–As, diode red, 1) 635 nm, 0.5 J/cm2, 10 s 2) 635 nm, 1 J/cm2, 20 s 3) 635 nm, 2 J/cm2, 40 s 4) 809 nm, 0.5 J/cm2, 10 s 5) 809 nm, 1 J/cm2, 20 s 6) 809 nm, 2 J/cm2, 40 s Irritated one dosage instantly after surgery.	*In vitro*	–	–	Human osteoblasts	RT-PCR	COL1A, BGLAP	14 days	1. A statistically significant down-regulation of BGLAP was observed with a laser dose of 0.5 J/cm2 and to a lesser extent in 1 and 2 J/cm2 irradiations at both wavelength ($p < 0.05$). 2.635 nm laser at 2 J/cm2 dose caused up-regulation of COL1A gene. 3.809 nm laser at 0.5 J/cm2 irradiation did not altered none of these gene expressions. 4.809 nm laser at 1 and 2 J/cm2 showed a significant decrease in BGLAP expression at the 14th day ($p < 0.05$). 5.809 nm laser at 1 and 2 J/cm2 significantly increased COL1A expression ($p < 0.05$).
Peat FJ et al. (Peat et al., 2018), 2017	1) 1064 nm, Nd:YAG laser, pulsed, 9.77 J/cm2, 10 s One dosage and one-time exposure.	*In vitro*	–	–	Human BMSCs	ELISA	VEGF, TGFb	24 h	1. An increase in VEGF production was seen in irradiated cells, compared with control cells, at 24 h postirradiation ($p < 0.05$). The mean increase in VEGF expression by irradiated BMSCs was 19.43U greater than VEGF expression by control BMSCs ($p = 0.03241$). 2. No significant difference in TGFb production between control and irradiated BMSCs at 24 h postirradiation ($p > 0.05$).
Wang Y et al. (Wang et al., 2016), 2016	1) 420 nm, LED array, 16 mW, 3 J/cm2, 188 s 2) 540 nm, Filtered lamp, 16 mW, 3 J/cm2, 188 s 3) 660 nm, Diode laser, 16 mW, 3 J/cm2, 188 s 4) 810 nm, Diode laser, 16 mW, 3 J/cm2, 188 s Irritated five times over three weeks.	*In vitro*	–	–	Human adipose-derived stem cells	RT-PCR	RUNX-2, OSX, OCN	7, 14, and 21 days	1. RUNX-2 level of 540 nm light group at all three time points were higher than 660 nm, 810 nm and control ($p < 0.05$). 2. RUNX-2 level of 410 nm light group was higher than 660 nm, 810 nm and control at 7 days ($p < 0.05$). 3. For gene expression, 540 nm and 410 nm groups had better effects than 660 nm, 810 nm and control at 21 days ($p < 0.05$). 4. For OCN gene expression, at 21 days, 540 group was better than 660 nm and control groups, and 410 nm group was better than control group ($p < 0.05$).
Tim CR et al. (Tim et al., 2016), 2016	830 nm, Ga–Al–As continuous laser,2.8 J, 30 mW, 94 s Irritation 0, 3, and 7 days after surgery.	*In vivo*	60 male Wistar rats	Tibia bone defect – 3 mm	–	IHC, RT-PCR	VEGF, FGF-14, FGF-2, FGFBP-1, ANGPT-2	36 h, 3, and 7 days	IHC VEGF expression was predominantly observed in the capillary walls and granulation tissue for both experimental groups 36 h after the surgery. On days 3 and 7 after surgery, the immunoexpression of VEGF was identified in the

(*continued on next page*)

Table 1 (*continued*)

Authors & year	Laser properties and treatment protocol	Type of study	Animal model	Defect type	Cell type	Method	Marker	Follow-up period	Results
									capillary walls, granulation tissue and osteoblastic cells for experimental and control groups ($p < 0.05$). RT-PCR The inflammatory and angiogenic genes were significantly up- regulated at 36 h and 3 days after surgery, followed by a down regulation of the genes on day 7 ($p < 0.05$).
Tim CR et al. (Tim et al., 2015), 2015	830 nm, Ga−Al−As continuous laser, 30 mW, 1000 J/cm2, 94 s Irritated 0, 3, 5, and 7 days after surgery.	*In vivo*	100 male Wistar rats	Tibia bone defect − 3 mm	–	MAH, RT-PCR	MAH BMP1,BMP2,BMP3, BMP4,BMP7,FGF2, FGF3,FGF4,FGF5, FGF7,FGF14,FGF15, FGF16,FGF22, RUNX-2, TGFb2,TGFbi, OCN qRT-PCR RPS18, PTGER2, IL1,ANGPT4,PDGFD, FGF2	12 h, 36 h, 3 days, 5 days, 7 days	MAH 1. Upregulation of TGF were at 12 and 36 h. 2. Stimulating the expression of BMPs, FGF, and RUNX-2 in all set points evaluated. 3. OCN was significantly up-regulated on day 3, 5, and 7. RT-PCR The expression profiles of the genes tested confirmed the microarray results.
Sella VRG et al. (Sella et al., 2015), 2015	808 nm, Ga−Al−As continuous laser, 0.2 W/cm2, 37 J/cm2, 5 s Irritated daily for 8 days.	*In vivo*	60 male Wistar rats	2-mm fragment of the femoral shaft	–	IHC	OCN OPN OSN	8, 13, and 18 days	1. Higher expression of OCN in the tissue samples from LLLT rats (subgroupA1) at day 8 as compared with that from the control ($p = 0.007$). 2. Laser treated rats had an anticipation on bone-remodeling response as compared with control rats. 3. OPN showed the expected behavior (increase on days 8 and 18 ($p = 0.033$) and decrease on day 13) in both groups 4. Similar expression changes for OSN.
Magri AMP et al. (Magri et al., 2015), 2015	808 nm, Ga−Al−As continuous laser, 3.57 W/cm2, 33 s 1) 30 J/cm2 2) 60 J/cm2 3) 120 J/cm2 Irritated three times per week.	*In vivo*	64 male Wistar rats	Tibia bone defect − 2 mm	–	IHC	RUNX-2, RANKL	15, 30 days	1. The irradiated laser groups presented a significant increase of RUNX-2 expression compared to control on 15th days ($p < 0.001$). 2. Thirty days post-surgery, no difference was observed for RUNX-2 immunoexpression for the experimental groups. 3. RANK-L immunostaining was observed in a similar pattern for all experimental groups15 days post-surgery. 4. After 30 days, a higher immune expression of RANK-L was observed in 30 J/cm2 and 120 J/cm2 compared to control ($p = 0.0131$ and $p = 0.0178$, respectively).
Park BJ et al. (Park et al., 2015), 2015	980-nm, Ga−Al−As diode laser, 13.95 J/cm2, 0, 1, 2, or 5 min Irritated instantly after surgery, and once daily.	*In vivo*	24 male Sprague −Dawley rats,	Maxillary first molars' socket	–	RT-PCR, Western blot	RT-PCR RUNX-2, COL1, OCN, PDGF, VEGF Western blot RUNX-2, COL1, OCN	1, 3,4, and 7 days	RT-PCR 1. LLLT increased the expressions of all tested genes in a time-dependent manner and that the highest levels of gene expression were in the 5-min group after 7 days ($p < 0.05$). 2. The increases in the percentages of expression in groups 1 to 4 after 3 days were similar to those after 7 days for each gene and were 8% ~30%. 3. Col1 showed the highest increase in percentage at about 30% ($p < 0.05$). 4. There were minor discrepancies of the expression levels of RUNX-2 and OCN between group 4 after 3 days and groups 2, 3, and 4 after 7 days ($p > 0.05$). 5. VEGF showed greater expression after 7 days than 3 days, with considerably increased expression in the 5-min group after 7 days ($p < 0.05$). 6. PDGF-B demonstrated progressive increases in gene expression as the irradiation time was prolonged and statistically significant increases in 5 min of irradiation after both 3 and 7 days ($p < 0.05$). Western blot 1. LLLT for 5min produces prominent increases in the

									expression levels of all tested proteins after both 3 and 7 days ($p < 0.05$). 2. RUNX-2 expression after 3 days of LLLT increased in a time dependent fashion, and the highest expression level after 3 days was similar to that of after 7 days ($p > 0.05$). 3. COL1, the increase in percentage after 3 days (99%) was more than that of after 7 days (86%). 4. The expression levels of VEGF showed a gradual increase proportionally with irradiation time, and group 4 showed a significantly greater increase after 7 days ($p < 0.05$). The expression of PDGF-B also increased in a time-dependent fashion, and a 5-min irradiation produced statistically significant increases in PDGF-B expression after 3 and 7 days ($p < 0.05$).
Tim CR et al. (Tim et al., 2014), 2014	830 nm, Ga−Al−As continuous laser, 57 W/cm2, 120 J/cm2, 34 s Irritated instantly after surgery, and repeated every two days.	*In vivo*	60 male Wistar rats	Tibia bone defect − 3 mm	–	IHC	RUNX-2, BMP-9, RANKL	15, 30, 45 days	1. The labeling for RUNX-2 occurred in both groups equally, without any significant differences among groups at days 15 and 30 postsurgery. 2. At the last set point evaluated in this study, the expression of RUNX-2 was significantly higher in laser treated group than in control ($p = 0.0001$). 3.15 days postsurgery, similar response to BMP-9 was noted for both groups, but a significantly higher BMP-9 expression was observed in laser treated rats when compared to control ones 30 days postsurgery ($p = 0.0171$) and no difference was found in the last experimental period. 4. No statistically significant differences of RANKL were detected among the groups.
Patrocínio-Silva TL et al. (Patrocínio-Silva et al., 2014), 2014	808 nm, continuous Laser, 120 J/cm2, 33 s Irritated three times per week.	*In vivo*	30 male Wistar rats	Tibia bone defect − 3 mm	–	IHC	RUNX-2, RANK-L	6 weeks	1. In the untreated diabetic rats, a weak RUNX-2 immunoexpression was detected. The diabetic laser-treated animals presented a strong positive RUNX-2 immunoexpression. 2. In the control group, RANK-L immunopositive cells were detected in bone tissue. In the laser treated rats, the same picture occurred, i.e., positive RANK-L immunoexpresssion was observed.
Saracino S et al. (Saracino et al., 2009), 2009	904−910 nm, Ga−Al−As Pulsed laser, 30 KHz, 200mW/cm2, 6.7 J/cm2, 5 min Irritated once daily for 5 days, then every two days till 20th days.	*In vitro*	–	–	MG-63	RT-PCR	BMP-4, BMP7, OCN, PPARa, PPARg, TGF-b2, COL1	4, 10, and 20 days	1. In laser-treated cells, the TGFb2 mRNA content was higher on days 10 and 20 (+30%, +40% vs. controls), whereas it was similar on day 4. 2. OCN, in laser-treated cells, was higher than in control cells only at day 10 (+60%) ($p > 0.05$). 3. COL1 showed significant changes on days 10 and 20 (+109%, +58% vs. controls) ($p < 0.05$). 4. As regards BMPs, a significant increase in laser-treated cells was evident inBMP-4 mRNA only on day 20 (+44%vs. control) ($p < 0.05$); whereas BMP-7 was induced on both day 10 and day 20, the larger increase being on day 10 (+50% vs. control) ($p < 0.05$). 5. PPARa and g mRNA contents increased in laser-treated cells at day 10 in comparison with control cells, although the increase in PPARg was larger (90% vs. 25% of PPARa) ($p < 0.05$).
Omasa S et al. (Omasa et al., 2012), 2012	830 nm, Ga−Al−As diode continuous laser, 200 mW, 195 J/cm2, 135sec Irritated instantly after surgery, and once daily.	In vivo	30 male Sprague-Dawley rats	*Implant placement*	–	RT-PCR	BMP-2	1,3,5, and 7 days	1. BMP-2 gene expression was 1.92-fold higher in the LLLT group than in the control group ($p < 0.05$) as early as 1 day after LLLT. 2. No significant difference in BMP-2 expression was observed between groups on days 3, 5, and 7.

(*continued on next page*)

Table 1 (*continued*)

Authors & year	Laser properties and treatment protocol	Type of study	Animal model	Defect type	Cell type	Method	Marker	Follow-up period	Results
Kesler G et al. (Kesler et al., 2011), 2011	2940 nm, Er:YAG Pulsed laser, 8 W/cm2, 32 J/cm2, 50 Hz, 500 −1000 mJ/pulse, 10−15 pulses Defect created by laser irradiation.	In vivo	56 male Wistar rats	2-mm crater depth in Tibia	–	IHC	PDGF	2, 4,8, and 14 days	1. After 2 days, the PDGF staining scores were very low for laser treated and control groups. 2. In the laser group, PDGF levels started to rise from the third day, reached a peak on day 4, and remained high for the rest of the study period (day 14). 3. The variability of the PDGF staining intensities (0−3°), as an ordinal dependent variable, was examined against the type of the rat (laser vs control group) and the day of sacrifice. Type of rat was found to be a significant predictor ($P = .000011$).

*__abbreviations:__ ANGPT-2: Angiopoietin 2, BGLAP: bone GLA protein (*also known as osteocalcin), BMSCs: bone marrow mesenchymal stem cells, cm: centimetre, COL1: Collagen type I, FGF: fibroblast growth factor, Ga−Al−As: gallium-aluminium-arsenide, h: hour, hADSCs: human adipose derived stem cell,IHC: immunohistochemistry, J: joule, LLLT: low level laser therapy, MAH: micro array hybridization, min: minute, mW: milliwatt, *Nd:YAG*: neodymium-doped yttrium aluminium garnet, nm: nanometer, OSX: osterix, OCN: osteocalcin, OPN: osteopontin, OSN: osteonectin, PPAR: peroxisome proliferators-activated receptor, RT-PCR: realtime polymerase chain reaction, sec: second, TGF: transforming growth factor, VEGF: vasculoendothelial growth factor, vWF: von Willebrand factor.

Table 2
Impact of photobiomodulation on biological markers in clinical investigations.

Authors & year	Laser properties and treatment protocol	Type of study	Sample size	Therapeutic type/ site	Method	Marker	Follow-up period	Results
Dominguez A et al. (Domínguez et al., 2015), 2015	670 nm, diode laser, 6.37 W/ cm2, 200 mW, 9 min Irritated one dosage instantly after surgery, and 1, 2, 3, 4, and 7 days postoperatively.	*Clinical trial*	10 - systematically healthy	Orthodontic tooth movement/maxilla	ELISA	RAKL, OPG	45 days	1. Up-regulation of RANKL level in CGF after two days in laser group compared to controls ($p < 0.05$). 2. Up-regulation of RANKL/OPG ratio in CGF after two days in laser group compared to controls ($p < 0.05$).
Mozzati M et al. (Mozzati et al., 2012), 2012	904−910 nm, Superpulsed gallium arsenide laser, 30 kHz, 200 W/cm2, 180 J/cm2, 200 mW, 15 min **Irritated one dosage instantly after surgery, and 3 and 5 days postoperatively.**	*Clinical trial − split mouth designed*	12 − hepatic failure	Extracted tooth socket − Alveolar bone (mandible/ maxilla)	RT-PCR	IL-1 beta, IL-6, IL-10, TGF-2, COX-2, BMP -4, BMP -7, PPAR, Col I, Col III	7 days	1. Down-regulation of IL-1 beta on day 7 between laser and control groups ($p < 0.05$). 2. Up-regulation of IL-6 and IL-10 on day 7 between laser and control groups ($p < 0.05$). 3. No significant difference of TGF-2, COX-2, BMP -4, BMP -7, PPAR, and Col I levels between laser and control groups ($p > 0.05$).
Mozzati M et al. (Mozzati et al., 2011), 2011	904−910 nm, Superpulsed gallium arsenide laser, 30 kHz, 200 W/cm2, 180 J/cm2, 200 mW, 15 min **Irritated one dosage instantly after surgery, and 3 and 5 days postoperatively.**	*Clinical trial − split mouth designed*	10 - systematically healthy	Extracted tooth socket − Alveolar bone (mandible/ maxilla)	RT-PCR	IL-1 beta, IL-6, IL-10, TGF-2, COX-2, BMP -4, BMP -7, PPAR, Col I, Col III	7 days	1. Down-regulation of IL-1 beta, IL-6, IL-10, and COX-2 on day 7 between laser and control groups ($p < 0.05$). 2. Up-regulation of Col I and III on day 7 between laser and control groups ($p < 0.05$). 3. No significant difference of TGF-2, BMP -4, BMP -7, and PPAR levels between laser and control groups ($p > 0.05$).

*__abbreviations__: BMP: bone morphogenetics protein, Col: Collagen, cm: centimetre, COX-2: cyclooxygenase-2, ELISA: enzyme-linked immunosorbent assay, GCF: gingival crevicular fluid, IHC: immunohistochemistry, IL: interleukin, J: joule, min: minute, mW: milliwatt, nm: nanometer, OPG: osteoprotegerin, PPAR: peroxisome proliferator activated receptor, sec: second, RANKL: receptor activator of nuclear factor KB ligand, RT-PCR: realtime polymerase chain reaction, TGF: transforming growth factor.

Table 3
Impact of photobiomodulation on inflammatory mediators' expression according to *in vitro*/*in vivo* studies.

Authors & year	Laser properties and treatment protocol	Type of study	Animal model	Defect type	Cell type	Method	Marker	Follow-up period	Results
Peat FJ et al. (Peat et al., 2018), 2017	1064 nm, Nd:YAG Pulsed laser, 9.77 J/cm2, 10 s Irritated one dosage instantly after surgery.	*In vitro*	–	–	Human BMSCs	RT-PCR	IL-1,-1b, −4, −6, −8, −10,-13, −17a, TNF, IFN-c, HSP90	24 h	1. No significant differences were found between irradiated and control BMSCs in the expression of the 10 analyzed cytokine genes at either 6 or 24 h post irradiation ($p > 0.05$). 2. No significant difference in HSP90 expression between irradiated and control BMSCs at either 6 or 24 h post irradiation ($p > 0.05$).
Tim CR et al. (Tim et al., 2016), 2016	830 nm, Ga−Al−As continuous laser, 2.8 J, 30 mW, 94 s Irritated one dosage instantly after surgery, and once daily for 7 days.	*In vivo*	60 male Wistar rats	Tibia bone defect − 3 mm	–	IHC, RT-PCR	COX-2, PTGIR, PTGS-2, MMD, IL-18, IL-1	36 h, 3, and 7 days	IHC 1. In the first and second experimental periods, COX-2 expression was predominantly detected in granulation tissue for both groups. Seven days post-surgery, for control group, the immunoreactivity of COX-2 was mainly observed in granulation tissue. 2. On three and seven days, significant difference were observed between laser treated and control groups ($p < 0.05$). RT-PCR 1. The inflammatory were significantly up- regulated at 36 h and 3 days after surgery, followed by a down regulation of the genes on day 7.
Tim CR et al. (Tim et al., 2014), 2014	830 nm, Ga−Al−As continuous laser, 57 W/cm2, 120 J/cm2, 34 s Irritated one dosage instantly after surgery, and every two days for 45 days.	*In vivo*	60 male Wistar rats	Tibia bone defect − 3 mm	–	IHC	COX-2	15, 30, 45 days	1. Similar findings for COX-2 immunoexpression were observed in both groups at the different experimental periods analyzed ($p > 0.05$).
Matsumoto MA et al. (Matsumoto et al., 2009), 2009	735 nm, Ga−Al−As continuous laser, 16 J/cm2, 1 min Irritated one dosage 24 h after surgery, and every two days for 21 days.	*In vivo*	48 male Wistar rats	Tibia bone defect − 5 mm	–	IHC	COX-2	2, 7, 14, and 21 days	1. After 2 days of surgery, COX-2 immunoreactivity could be seen in the central region of the lesion in rats of the control group. A similar pattern occurred in the group treated with low-level laser. 2. After 7 days, COX-2 immunoexpression could be seen in the granulation tissue in the control group, whereas COX-2 was positively detected in the surrounding bone tissue in the rats exposed to laser. 3. This was more evident on the 14th day, either in the control group or in the laser-exposed group. 4. Twenty-one days after the surgery, the control group and the group treated with laser showed COX-2 expression in some cells of the bone marrow.

*__abbreviations__: BMMSCs: bone-marrow mesenchymal stem cells, cm: centimetre, COX-2: cyclooxygenase-2, Ga−Al−As: gallium-aluminium-arsenide, h: hour, HSP90: Heat Shock Protein 90, IFN: interferons, IHC: immunohistochemistry, IL: interleukin, J: joule, MMD: macrophage differentiation-associated gene, min: minute, mW: milliwatt, *Nd:YAG*: neodymium-doped yttrium aluminium garnet,nm: nanometer, sec: second, RT-PCR: realtime polymerase chain reaction, TNF: tumor necrosis factor.

et al., 2004), and acceleration of regenerative processes (Posten et al., 2005) for the last decades. Previous studies demonstrated that PBM inducing mitochondrial respiration (Karu et al., 1999), promoting cell proliferation, and altering cellular regulatory proteins (Buravlev et al., 2015; Stein et al., 2005). In addition, in bone regeneration, PBM can promote secretion of various growth factors, expression of osteogenic genes, osteodifferentiation of stem cells, and inducing osteoblasts (Fávaro–Pípi et al., 2011; Wu et al., 2012). However, there are many parameters of PBM such as wavelength (Chung et al., 2012), energy density, and irradiance (Huang et al., 2009, 2011) influencing its therapeutic outcome. The future of laser therapies in bone regenerative medicine is dependent on having a better understanding of the points gathered from *in* vitro, in *vivo* laboratory studies, and primary clinical trials that can guide future clinical investigations and develop current treatment strategies. In the current study, we aimed to systematically reviewed application of PBM on bone regeneration among *in vitro* and *in vivo* studies which evaluated molecular alterations.

4.1. Osteogenic factors

Several signaling pathways participate in bone formation as a complicated process (Hoffmann and Gross, 2001; Yang et al., 2015). In osteoblastic differentiation pathway, transcription factors such as RUNX -2 and OSX initiate this process. RUNX -2 play a pivotal role on regulatory mechanism in osteogenic differentiation (McGee-Lawrence et al., 2013) and expression of OSX in RUNX -2 expressing precursors stimulates osteoprogenitor cells to differentiate into functional osteoblasts and osteocytes during bone formation (Zhou et al., 2010; Zhu et al., 2011). Among the reviewed studies, six of them assessed effect of laser irradiation on RUNX -2 expression (Magri et al., 2015; Park et al., 2015; Patrocínio-Silva et al., 2014; Tim et al., 2014, 2015; Wang et al., 2016). In all studies, laser treated group showed higher expression of RUNX -2 which indicated the potential impact of laser therapy on initiating the bone formative processes. All of these articles had control groups without laser irradiation. This finding has been confirmed by RT-PCR (Wang et al., 2016), IHC analysis (Magri et al., 2015), micro array hybridization (Tim et al., 2015) and Western blot (Park et al., 2015). One of the major limitations of these research articles seems to be the dearth of sufficient sample size. In fact, none of them reported the method for sample size calculation.

Wang et al. (2016) in 2016 represented that between various wavelength of laser irradiation (420 nm, 540 nm, 660 nm, and 810 nm) with the same physical and application properties, 540 nm LED array increased RUNX -2 expression more significantly after 7, 14, and 21 days application on human adipose-derived stem cells. Magri et al. (2015) in an *in vivo* experiment showed that there is no significant difference between laser treated groups via various energy densities (30, 60, 120 J/cm2) and the expression of RUNX -2. This finding did not observe in other evaluated marker (RANKL). In another *in vivo* investigation (Park et al., 2015), results indicated the impact of PBM' duration on RUNX -2 expression levels. It has been shown that 5 min application of PBM resulted in the highest level of RUNX -2 gene expression after seven days in comparison with lesser treatment duration. OSX has been assessed in one study (Wang et al., 2016). It seems that this transcription factor affect end stage of osteoblasts as a downstream of RUNX -2 (Sinha and Zhou, 2013). Wang et al. represented better OSX gene expression with 540 nm LED array at 21 days after bony defect creation in rat model (Wang et al., 2016).

RUNX -2 and OSX upregulate transcription of OCN, COL 1, OPN by binding promoters of their osteoblast specific genes (Ducy et al., 1997; Sinha and Zhou, 2013). OCN is a pro-osteoblastic regulatory factor in bone formation (Karsenty and Oury, 2014). This factor has been studied in six studies (Ateş et al., 2017; Park et al., 2015; Saracino et al., 2009; Sella et al., 2015; Tim et al., 2015; Wang et al., 2016). All studies except one (Ateş et al., 2017), reported upregulation of OCN gene expression after PBM application in comparison to untreated group (control). To the contrary, Ates et al. in 2017, represented that application of Ga–Al–As laser (809 nm wave length; 0.5, 1, and 2 (J/cm2) energy density) and 635 nm for 10, 20, 40 s on human osteoblasts significantly down regulated the expression of OCN according to energy density ($p < 0.05$). This discrepancy may occur since Ates's study is an *in vitro* investigation and three other studies (Park et al., 2015; Sella et al., 2015; Tim et al., 2015) are *in vivo.* In the other *in vitro* study (Wang et al., 2016), similar wave length (660 nm and 810 nm) were utilized; However, their energy density (3 J/cm2), treatment duration (188 s), and type of cells (Human adipose-derived stem cells) were different from aforementioned study. It seems that extended energy density and period of treatment can positively alter their findings from a down regulation in one (Ateş et al., 2017) to up regulation in another (Wang et al., 2016). Moreover, Saracino et al. (2009) used pulsed wave Gallium Arsenide laser (904–910 nm, pulse width 200 ns, 30 KHz, 200mW/cm2, 6.7 J/cm2, 5 min) on pre osteoblastic cells (MG-63). They showed that OCN expressed 60% higher in laser treated group compared to control. Hence, it can be concluded that PBM administration has an up regulatory impact on OCN expression.

Collagen type I is the most frequent type observed in extracellular matrix of bone tissue (Ferreira et al., 2012). Expression of COL 1 evaluated in three studies (Ateş et al., 2017; Park et al., 2015; Saracino et al., 2009). All studies showed significant higher expression of COL 1 after therapy with continuous (Ateş et al., 2017; Park et al., 2015) or pulsed wave (Saracino et al., 2009). Ates et al. (Ateş et al., 2017) reported better results for 635 nm, 50 mW, which has a positive relation with the time of application and energy densities. In addition, clinical trials in healing alveolar bone showed a significant increase in Col I and III expression after PBM application at 910 nm super pulsed gallium arsenide laser (Mozzati et al., 2011, 2012).

OPN plays a role in bone remodeling by helping osteoclasts to anchoring to the mineral matrix (Reinholt et al., 1990). OSN as a matrix glycoprotein acts in cellular interactions, collagen binding, and hard tissue mineralization (Young et al., 1992). Both of these osteogenic factors has been observed by Sella et al. in 2015 (Sella et al., 2015). They observed a significant increase for OPN and OSN at early stage of bone formation (before 13th day); However, the expression decreased at the next time point (18th day). They suggested that due to the early role of these factors in bone formation, laser therapy can ameliorate bone healing especially at initiative stages due to they perpetuated laser application during the follow up time.

BMP/Smad signaling pathway is another routes that seems to energy density by laser irradiation (Hirata et al., 2010). This pathway regulates mesenchymal stem cell differentiation during development, newly bone formation, healing, and homeostasis (Wu et al., 2016). In four studies, BMP -1, −2, −3, −4, −7, and −9 were evaluated (Omasa et al., 2012; Tim et al., 2015). Tim et al. in 2015 (Tim et al., 2015) and 2014 (Tim et al., 2014) reported higher qualitatively expression of BMP-1, -2, -3, -4, -7 and -9 by micro array hybridization method which was confirmed by RT-PCR after seven days. However, Omasa *at al* (Omasa et al., 2012). indicated significant higher expression of BMP 2 only one day after surgery and there was no statistical significant difference between laser treated and untreated groups. These studies applied 830 nm continuous Ga–Al–As diode laser; however, their energy densities and treatment protocol different. In fact, immediate exposure after surgery represented better results.

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RANKL is a transmembrane member of the tumor necrosis factor superfamily, involved in bone tissue remodeling via acting on activation and differentiation of osteoclasts (Baharuddin et al., 2015). Magri et al. found significant higher expression of RANKL after 808 nm, Ga–Al–As laser therapy for 33 s in rats. However, in another similar investigation (Tim et al., 2014), the comparison between laser treated and untreated groups were not significantly different. The only varied parameter between these studies was energy density rate (3.57 W/cm2 vs. 57 W/cm2) which can explain the controversy. Moreover, one clinical trials demonstrated that RANKL up regulated after diode laser therapy in gingival crevicular fluid (Domínguez et al., 2015). In this study, PBM was applied in order to facilitate orthodontic tooth movement in maxilla. According to the biological effect of RANKL in bone remodeling and the importance of bone remodeling in tooth movements, beneficial impacts of PBM can be explained.

4.2. Angiogenic factors

Vascularization is a key component of bone regeneration. In fact, it can restrict bone repair approaches (Hankenson et al., 2011). Thus, finding alternative therapies that can ameliorate angiogenesis is an attractive subject for researchers. In this regard, PBM can non-invasively increase neovascularization in various tissues (Cury et al., 2013; Tim et al., 2016). Three studies investigated the effect of PBM on VEGF (Park et al., 2015; Peat et al., 2018; Tim et al., 2015) and two of them (Tim et al., 2015, 2016) assessed ANGPT-2 as signaling molecules for angiogenesis. Peat et al. applied 1064 nm Nd:YAG pulsed laser on human mesenchymal stem cells and showed a significant increase in VEGF production in comparison to control cells after 24 h. In 2016, 2015, Tim et al., 2015, 2016 indicated an enhancement of VEGF and ANGPT-2 at early stages of bone healing (after 36 h). Park et al. (2015) reported a time-dependent manner for expression of VEGF after 980-nm Ga–Al–As diode laser for 1, 2, and 5 min applications. In addition, Wang et al. (2018) and Chen et al. (2018) in 2018 showed that 660 nm Gaa-Al–As laser can increase vWF expression in hADSCs after 16 weeks compared with controls.

4.3. Other biological factors

Osteoinductive exogenous growth factors have been utilized for bone tissue engineering with promising results (Kitaori et al., 2009; Komaki et al., 2006; Lieberman et al., 2002; Stevenson et al., 1994; Wulsten et al., 2011). Nonetheless, finding a way to promote endogenous growth factors can be more efficient for bone healing. Previous studies showed promoting effect of PBM on various growth factors (de Freitas and Hamblin, 2016). TGF-β is a strong stimulator of collagen production, inducing the expression of extracellular matrix components and inhibiting its degradation by inhibiting matrix metalloproteinases (MMPs). Expression of TGF-β was assessed in three studies (Peat et al., 2018; Saracino et al., 2009; Tim et al., 2015). Although one of them reported no significant effect for laser irradiation (Peat et al., 2018), the others indicated significant upregulation of its expression (Saracino et al., 2009; Tim et al., 2015). This controversy can explain by the different applied laser (Nd:YAG vs. Ga–Al–As laser). In fact, Ga–Al–As laser represented statistically significant up regulation of TGF-β in comparison to untreated group.

PDGF increases the expression level of VEGF in mural cells and stimulates fibroblasts to produce and secrete collagenases, which are essential for cell migration in angiogenesis (Heldin and Westermark, 1999). PDGF was reported to positively affect vessel formation and ultimately bone formation (Chang et al., 2010). Despite of many other factors, studies showed a delayed upregulation of PDGF after laser therapy (Kesler et al., 2011; Park et al., 2015; Tim et al., 2015). It seems this effect mimic this molecules function in bone healing process.

FGFs is known to be a potent mitogen and chemoattractant for endothelial cells and fibroblasts, as well as accelerating the formation of granulation tissue and to induce re-epithelization. This factor is not related to bone regeneration as much as other factors. However, Tim et al. investigated it as related factor in bone healing process by two different studies (Tim et al., 2015, 2016). Their findings represented the positive effect of PBM on expression of these factors.

4.4. Inflammation mediators

COX is a terminal molecule in an electron transferring chain from cytochrome *c* to O_2. PBM intensifies ATP, cyclic adenosine monophosphate, and reactive oxygen species (Bayat et al., 2018). It has been shown that PBM increases the electrons' availability in catalytic center of COX. Among reviewed articles, three studies measured expression of COX -2 by IHC analysis (Matsumoto et al., 2009; Tim et al., 2014, 2016). Although in one study similar expressions were observed in irradiated and control groups at 15, 30, 45 days after surgery (Tim et al., 2014), two other articles represented higher expression of COX -2 especially first days after surgery (Matsumoto et al., 2009; Tim et al., 2016). This controversy may occurred since the long period of follow up in Tim et al. study, 2014 (Tim et al., 2014). It has been suggested that expression of COX -2 can be beneficial for bone healing. In early stage of bone formation, COX -2 is associated with the maturation of osteoblasts and modulating differentiation genes (Forwood, 1996; Sato et al., 1997; Zhang et al., 2002). In addition the other hand, inflammatory mediators such as IL-1ra, IL-1b, IL-4, IL-6, IL8, IL-10, IL-13, IL-17a, TNF, IFN-c, HSP90, PTGIR, PTGS-2, MMD, and IL-18 were studied in two articles (Peat et al., 2018; Tim et al., 2016). Nevertheless, none of them found any significant relation between PBM and their expression. In clinical trials, IL-1 beta secretion was prevented by PBM application that may explain PBM's impact on reducing the inflammation (Mozzati et al., 2011, 2012). However, included clinical trials showed controversial findings regarding IL-6 and IL-10. These ILs act as anti-inflammatory cytokines and are beneficial for bone healing (Chang and Bistrian, 1998; Opal and Depalo, 2000).

5. Conclusions

Based on the studies evaluated here, PBM administration demonstrated promising results for bone tissue regenerating processes. PBM can up regulate numerous osteogenic factors, angiogenic factors, growth factors, and inflammation mediators that facilitate bone formation. PBM significantly increased the expression of OCN, RUNX-2, BMP-2, -9, COX-2 via 830 nm, Ga–Al–As laser. In addition, 635 nm, Ga–Al–As laser and 809 nm, Ga–Al–As laser induced significantly higher expression of OCN, Collagen type I, and RUNX-2. PBM by 1064 nm and Nd:YAG laser significantly increased VEGF expression. These effects depend on many parameters which energy density is more important than the others. Although since the heterogeneity of the studies and their limitations, an evidence-based decision for definite therapeutic application of PBM is still unattainable, the findings of our review can help other researchers to ameliorate their study design and elect more efficient approach for their investigation.

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References

Fávaro–Pípi, E., Ribeiro, D.A., Ribeiro, J.U., Bossini, P., Oliveira, P., Parizotto, N.A., Tim, C., de Araújo, H.S.S., Renno, A.C.M., 2011. Low-level laser therapy induces differential expression of osteogenic genes during bone repair in rats. Photomedicine and laser surgery 29, 311–317.

Aihara, N., Yamaguchi, M., Kasai, K., 2006. Low-energy irradiation stimulates formation of osteoclast-like cells via RANK expression in vitro. Laser Med. Sci. 21, 24–33.

Al-Watban, F.A., Zhang, X., 2004. The comparison of effects between pulsed and CW lasers on wound healing. J. Clin. Laser Med. Surg. 22, 15–18.

AlGhamdi, K.M., Kumar, A., Moussa, N.A., 2012. Low-level laser therapy: a useful technique for enhancing the proliferation of various cultured cells. Laser Med. Sci. 27, 237–249.

Ateş, G.B., Can, A.A., Gülsoy, M., 2017. Investigation of photobiomodulation potentiality by 635 and 809 nm lasers on human osteoblasts. Laser Med. Sci. 32, 591–599.

Baharuddin, N., Coates, D., Cullinan, M., Seymour, G., Duncan, W., 2015. Localization of RANK, RANKL and osteoprotegerin during healing of surgically created periodontal defects in sheep. J. Periodontal. Res. 50, 211–219.

Basford, J.R., 1995. Low intensity laser therapy: still not an established clinical tool. Laser Surg. Med. 16, 331–342.

Bayat, M., Virdi, A., Rezaei, F., Chien, S., 2018 Mar 1. Comparison of the in vitro effects of low-level laser therapy and low-intensity pulsed ultrasound therapy on bony cells and stem cells. Prog. Biophys. Mol. Biol. 133, 36–48.

Bossini, P.S., Muniz Rennó, A.C., Ribeiro, D.A., Fangel, R., Peitl, O., Zanotto, E.D., Parizotto, N.A., 2011. Biosilicate® and low-level laser therapy improve bone repair in osteoporotic rats. J. Tissue Eng. Regenerat. Med. 5, 229–237.

Buravlev, E.A., Zhidkova, T.V., Osipov, A.N., Vladimirov, Y.A., 2015. Are the mitochondrial respiratory complexes blocked by NO the targets for the laser and LED therapy? Laser Med. Sci. 30, 173–180.

Chang, H.R., Bistrian, B., 1998. The role of cytokines in the catabolic consequences of infection and injury. J. Parenter. Enteral Nutr. 22, 156–166.

Chang, P.-C., Seol, Y.-J., Cirelli, J.A., Pellegrini, G., Jin, Q., Franco, L.M., Goldstein, S.A., Chandler, L.A., Sosnowski, B., Giannobile, W.V., 2010. PDGF-B gene therapy accelerates bone engineering and oral implant osseointegration. Gene Ther. 17, 95–104.

Chen, C.H., Wang, Y.H., Ho, M.L., 2018. Low power laser irradiation and human adipose-derived stem cell treatments promote bone regeneration of critical-sized calvarial defects in rats. Annals of Physical and Rehabilitation Medicine 61, e418–e419.

Chung, H., Dai, T., Sharma, S.K., Huang, Y.-Y., Carroll, J.D., Hamblin, M.R., 2012. The nuts and bolts of low-level laser (light) therapy. Ann. Biomed. Eng. 40, 516–533.

Coombe, A.R., Ho, C.T., Darendeliler, M.A., Hunter, N., Philips, J.R., Chapple, C.C., Yum, L.W., 2001. The effects of low level laser irradiation on osteoblastic cells. Clin. Orthod. Res. 4, 3–14.

Cury, V., Moretti, A.I., Assis, L., Bossini, P., Crusca Jde, S., Neto, C.B., Fangel, R., de Souza, H.P., Hamblin, M.R., Parizotto, N.A., 2013. Low level laser therapy increases angiogenesis in a model of ischemic skin flap in rats mediated by VEGF, HIF-1alpha and MMP-2. J. Photochem. Photobiol., B 125, 164–170.

da Silva, J.P., da Silva, M.A., Almeida, A.P.F., Junior, I.L., Matos, A.P., 2010. Laser therapy in the tissue repair process: a literature review. Photomedicine and laser surgery 28, 17–21.

de Freitas, L.F., Hamblin, M.R., 2016. Proposed mechanisms of photobiomodulation or low-level light therapy. IEEE J. Sel. Top. Quantum Electron. 22, 348–364.

de Vasconcellos, L.M.R., Barbara, M.A.M., da Silva Rovai, E., de Oliveira França, M., Ebrahim, Z.F., de Vasconcellos, L.G.O., Porto, C.D., Cairo, C.A.A., 2016. Titanium scaffold osteogenesis in healthy and osteoporotic rats is improved by the use of low-level laser therapy (GaAlAs). Laser Med. Sci. 31, 899–905.

Domínguez, A., Gómez, C., Palma, J.C., 2015. Effects of low-level laser therapy on orthodontics: rate of tooth movement, pain, and release of RANKL and OPG in GCF. Laser Med. Sci. 30, 915–923.

Dostalova, T., Kroulikova, V., Podzimek, S., Jelinková, H., 2017. Low-level laser therapy after wisdom teeth surgery: evaluation of immunologic markers (secretory immunoglobulin a and lysozyme levels) and thermographic examination: placebo controlled study. Photomedicine and laser surgery 35, 616–621.

Ducy, P., Zhang, R., Geoffroy, V., Ridall, A.L., Karsenty, G., 1997. Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation. Cell 89, 747–754.

El-Maghraby, E.M., El-Rouby, D.H., Saafan, A.M., 2013. Assessment of the effect of low-energy diode laser irradiation on gamma irradiated rats' mandibles. Arch. Oral Biol. 58, 796–805.

Enwemeka, C.S., Parker, J.C., Dowdy, D.S., Harkness, E.E., Harkness, L.E., Woodruff, L.D., 2004. The efficacy of low-power lasers in tissue repair and pain control: a meta-analysis study. Photomed. Laser Ther. 22, 323–329.

Fallahnezhad, S., Piryaei, A., Darbandi, H., Amini, A., Ghoreishi, S.K., Jalalifirouzkouhi, R., Bayat, M., 2018. Effect of low-level laser therapy and oxytocin on osteoporotic bone marrow-derived mesenchymal stem cells. J. Cell. Biochem. 119, 983–997.

Farivar, S., Malekshahabi, T., Shiari, R., 2014. Biological effects of low level laser therapy. J. Laser Med. Sci. 5, 58.

Fekrazad, R., Ghuchani, M.S., Eslaminejad, M., Taghiyar, L., Kalhori, K., Pedram, M., Shayan, A., Aghdami, N., Abrahamse, H., 2015. The effects of combined low level laser therapy and mesenchymal stem cells on bone regeneration in rabbit calvarial defects. J. Photochem. Photobiol. B Biol. 151, 180–185.

Ferreira, A.M., Gentile, P., Chiono, V., Ciardelli, G., 2012. Collagen for bone tissue regeneration. Acta Biomater. 8, 3191–3200.

Forwood, M., 1996. Inducible cyclo-oxygenase (COX-2) mediates the induction of bone formation by mechanical loading in vivo. J. Bone Miner. Res. 11, 1688–1693.

Fredoni, M., Ghatrehsamani, M., Abdollahifar, M.A., Bayat, S., Bayat, M., 2017. Evaluation of the effects of photobiomodulation on vertebras in two rat models of experimental osteoporosis. Laser Med. Sci. 32, 1545–1560.

Funk, J.O., Kruse, A., Kirchner, H., 1992. Cytokine production after helium neon laser irradiation in cultures of human peripheral blood mononuclear cells. J. Photochem. Photobiol. B Biol. 16, 347–355.

Garcia, V.G., da Conceicao, J.M., Fernandes, L.A., de Almeida, J.M., Nagata, M.J., Bosco, A.F., Theodoro, L.H., 2013. Effects of LLLT in combination with bisphosphonate on bone healing in critical size defects: a histological and histometric study in rat calvaria. Laser Med. Sci. 28, 407–414.

Gerbi, M.E.M., Pinheiro, A.L.B., Marzola, C., Júnior, F.D.A.L., Ramalho, L., Ponzi, E.A., Soares, A.O., Carvalho, L.C.B., Lima, H.V., Gonçalves, T.O., 2005. Assessment of bone repair associated with the use of organic bovine bone and membrane irradiated at 830 nm. Photomed. Laser Ther. 23, 382–388.

Gomes, F., Mayer, L., Massotti, F., Baraldi, C., Ponzoni, D., Webber, J., De Oliveira, M., 2015. Low-level laser therapy improves peri-implant bone formation: resonance frequency, electron microscopy, and stereology findings in a rabbit model. Int. J. Oral Maxillofac. Surg. 44, 245–251.

Hankenson, K.D., Dishowitz, M., Gray, C., Schenker, M., 2011. Angiogenesis in bone regeneration. Injury 42, 556–561.

Heldin, C.-H., Westermark, B., 1999. Mechanism of action and in vivo role of platelet-derived growth factor. Physiol. Rev. 79, 1283–1316.

Hirata, S., Kitamura, C., Fukushima, H., Nakamichi, I., Abiko, Y., Terashita, M., Jimi, E., 2010. Low-level laser irradiation enhances BMP-induced osteoblast differentiation by stimulating the BMP/Smad signaling pathway. J. Cell. Biochem. 111, 1445–1452.

Hoffmann, A., Gross, G., 2001. BMP Signaling Pathways in Cartilage and Bone Formation, Critical Reviews™ in Eukaryotic Gene Expression, vol. 11.

Huang, Y.-Y., Chen, A.C.-H., Carroll, J.D., Hamblin, M.R., 2009. Biphasic dose response in low level light therapy. Dose-Response 7 dose-response. 09-027. Hamblin.

Huang, Y.-Y., Sharma, S.K., Carroll, J., Hamblin, M.R., 2011. Biphasic dose response in low level light therapy–an update. Dose-Response 9 dose-response. 11-009. Hamblin.

Huang, Z., Chen, J., Ma, J., Shen, B., Pei, F., Kraus, V.B., 2015. Effectiveness of low-level laser therapy in patients with knee osteoarthritis: a systematic review and meta-analysis. Osteoarthritis Cartilage 23, 1437–1444.

Huertas, R.M., Luna-Bertos, E.D., Ramos-Torrecillas, J., Leyva, F.M., Ruiz, C., Garcia-Martinez, O., 2014. Effect and clinical implications of the low-energy diode laser on bone cell proliferation. Biol. Res. Nurs. 16, 191–196.

Jawad, M.M., Husein, A., Azlina, A., Alam, M.K., Hassan, R., Shaari, R., 2013. Effect of 940 nm low-level laser therapy on osteogenesis in vitro. J. Biomed. Opt. 18, 128001.

Karsenty, G., Oury, F., 2014. Regulation of male fertility by the bone-derived hormone osteocalcin. Mol. Cell. Endocrinol. 382, 521–526.

Karu, T., Piatibrat, L., Kalendo, G., 1999. Suppression of the intracellular concentration of ATP by irradiating with a laser pulse of wavelength lambda= 820 nm. In: Book Suppression of the Intracellular Concentration of ATP by Irradiating with a Laser Pulse of Wavelength Lambda= 820 Nm. City, p. 399.

Kesler, G., Shvero, D.K., Tov, Y.S., Romanos, G., 2011. Platelet derived growth factor secretion and bone healing after Er: YAG laser bone irradiation. J. Oral Implantol. 37, 195–204.

Kitaori, T., Ito, H., Schwarz, E.M., Tsutsumi, R., Yoshitomi, H., Oishi, S., Nakano, M., Fujii, N., Nagasawa, T., Nakamura, T., 2009. Stromal cell–derived factor 1/CXCR4 signaling is critical for the recruitment of mesenchymal stem cells to the fracture site during skeletal repair in a mouse model. Arthritis Rheum. 60, 813–823.

Komaki, H., Tanaka, T., Chazono, M., Kikuchi, T., 2006. Repair of segmental bone defects in rabbit tibiae using a complex of β-tricalcium phosphate, type I collagen, and fibroblast growth factor-2. Biomaterials 27, 5118–5126.

Korany, N.S., Mehanni, S.S., Hakam, H.M., El-Maghraby, E.M., 2012. Evaluation of socket healing in irradiated rats after diode laser exposure (histological and morphometric studies). Arch. Oral Biol. 57, 884–891.

Lieberman, J.R., Daluiski, A., Einhorn, T.A., 2002. The role of growth factors in the repair of bone. J Bone Joint Surg Am 84, 1032–1044.

Magri, A.M.P., Fernandes, K.R., Assis, L., Mendes, N.A., da Silva Santos, A.L.Y., de Oliveira Dantas, E., Rennó, A.C., 2015. Photobiomodulation and bone healing in diabetic rats: evaluation of bone response using a tibial defect experimental model. Laser Med. Sci. 30, 1949–1957.

Mandić, B., Lazić, Z., Marković, A., Mandić, B., Mandić, M., Đinić, A., Miličić, B., 2015. Influence of postoperative low-level laser therapy on the osseointegration of self-tapping implants in the posterior maxilla: a 6-week split-mouth clinical study. Vojnosanit. Pregl. 72, 233–240.

Matsumoto, M.A., Ferino, R.V., Monteleone, G.F., Ribeiro, D.A., 2009. Low-level laser therapy modulates cyclo-oxygenase-2 expression during bone repair in rats.

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Laser Med. Sci. 24, 195–201.
McGee-Lawrence, M.E., Li, X., Bledsoe, K.L., Wu, H., Hawse, J.R., Subramaniam, M., Razidlo, D.F., Stensgard, B.A., Stein, G.S., Van Wijnen, A.J., 2013. Runx2 protein represses Axin2 expression in osteoblasts and is required for craniosynostosis in Axin2-deficient mice. J. Biol. Chem. 288, 5291–5302.
Moher, D., Shamseer, L., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., Shekelle, P., Stewart, L.A., 2015. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst. Rev. 4, 1.
Mohsenifar, Z., Fridoni, M., Ghatrehsamani, M., Abdollahifar, M.A., Abbaszadeh, H., Mostafavinia, A., Fallahnezhad, S., Asghari, M., Bayat, S., Bayat, M., 2016. Evaluation of the effects of pulsed wave LLLT on tibial diaphysis in two rat models of experimental osteoporosis, as examined by stereological and real-time PCR gene expression analyses. Laser Med. Sci. 31, 721–732.
Mostafavinia, A., Ahadi, R., Abdollahifar, M., Ghorishi, S.K., Jalalifirouzkouhi, A., Bayat, M., 2017a. Evaluation of the effects of photobiomodulation on biomechanical properties and hounsfield unit of partial osteotomy healing in an experimental rat model of type I diabetes and osteoporosis. Photomed Laser Surg 35, 520–529.
Mostafavinia, A., Dehdehi, L., Ghoreishi, S.K., Hajihossainlou, B., Bayat, M., 2017b. Effect of in vivo low-level laser therapy on bone marrow-derived mesenchymal stem cells in ovariectomy-induced osteoporosis of rats. J. Photochem. Photobiol., B 175, 29–36.
Mozzati, M., Martinasso, G., Cocero, N., Pol, R., Maggiora, M., Muzio, G., Canuto, R.A., 2011. Influence of superpulsed laser therapy on healing processes following tooth extraction. Photomedicine and laser surgery 29, 565–571.
Mozzati, M., Martinasso, G., Cocero, N., Pol, R., Maggiora, M., Muzio, G., Canuto, R.A., 2012. Superpulsed laser therapy on healing process after tooth extraction in patients waiting for liver transplantation. Laser Med. Sci. 27, 353–359.
Neiburger, E., 1999. Rapid healing of gingival incisions by the helium-neon diode laser. J. Mass. Dent. Soc. 48, 8-13, 40.
Ninomiya, T., Hosoya, A., Nakamura, H., Sano, K., Nishisaka, T., Ozawa, H., 2007. Increase of bone volume by a nanosecond pulsed laser irradiation is caused by a decreased osteoclast number and an activated osteoblasts. Bone 40, 140–148.
Omasa, S., Motoyoshi, M., Arai, Y., Ejima, K.-I., Shimizu, N., 2012. Low-level laser therapy enhances the stability of orthodontic mini-implants via bone formation related to BMP-2 expression in a rat model. Photomedicine and laser surgery 30, 255–261.
Opal, S.M., Depalo, V.A., 2000. Anti-inflammatory cytokines. Chest 117, 1162–1172.
Park, J.B., Ahn, S.-J., Kang, Y.-G., Kim, E.-C., Heo, J.S., Kang, K.L., 2015. Effects of increased low-level diode laser irradiation time on extraction socket healing in rats. Laser Med. Sci. 30, 719–726.
Patrocínio-Silva, T.L., de Souza, A.M.F., Goulart, R.L., Pegorari, C.F., Oliveira, J.R., Fernandes, K., Magri, A., Pereira, R.M.R., Araki, D.R., Nagaoka, M.R., 2014. The effects of low-level laser irradiation on bone tissue in diabetic rats. Laser Med. Sci. 29, 1357–1364.
Peat, F.J., Colbath, A.C., Bentsen, L.M., Goodrich, L.R., King, M.R., 2018 Feb 1. Vitro effects of high-intensity laser photobiomodulation on equine bone marrow-derived mesenchymal stem cell viability and cytokine expression. Photomedicine and Laser Surgery 36 (2), 83–91.
Posten, W., Wrone, D.A., Dover, J.S., Arndt, K.A., Silapunt, S., Alam, M., 2005. Low-level laser therapy for wound healing: mechanism and efficacy. Dermatol. Surg. 31, 334–340.
Reinholt, F.P., Hultenby, K., Oldberg, A., Heinegård, D., 1990. Osteopontin–a possible anchor of osteoclasts to bone. Proc. Natl. Acad. Sci. Unit. States Am. 87, 4473–4475.
Safavi, S.M., Kazemi, B., Esmaeili, M., Fallah, A., Modarresi, A., Mir, M., 2008. Effects of low-level He–Ne laser irradiation on the gene expression of IL-1β, TNF-α, IFN-γ, TGF-β, bFGF, and PDGF in rat's gingiva. Laser Med. Sci. 23, 331–335.
Santulli, G., R Marks, A., 2015. Essential roles of intracellular calcium release channels in muscle, brain, metabolism, and aging. Curr. Mol. Pharmacol. 8, 206–222.
Saracino, S., Mozzati, M., Martinasso, G., Pol, R., Canuto, R.A., Muzio, G., 2009. Superpulsed laser irradiation increases osteoblast activity via modulation of bone morphogenetic factors. Laser Surg. Med. 41, 298–304.
Sato, Y., Arai, N., Negishi, A., Ohya, K., 1997. Expression of cyclooxygenase genes and involvement of endogenous prostaglandin during osteogenesis in the rat tibial bone marrow cavity. J. Med. Dent. Sci. 44, 81–92.
Scalize, P.H., de Sousa, L.G., Regalo, S.C., Semprini, M., Pitol, D.L., da Silva, G.A., de Almeida Coelho, J., Coppi, A.A., Laad, A.A., Prado, K.F., Siessere, S., 2015. Low-level laser therapy improves bone formation: stereology findings for osteoporosis in rat model. Laser Med. Sci. 30, 1599–1607.
Schneede, P., Jelkmann, W., Schramm, U., Fricke, H., Steinmetz, M., Hofstetter, A., 1988. Effects of the helium-neon laser on rat kidney epithelial cells in culture. Laser Med. Sci. 3, 249–257.
Sella, V.R.G., do Bomfim, F.R.C., Machado, P.C.D., da Silva Morsoleto, M.J.M., Chohfi, M., Plapler, H., 2015. Effect of low-level laser therapy on bone repair: a randomized controlled experimental study. Laser Med. Sci. 30, 1061–1068.
Sene, G.A.L., Sousa, F.F.d.A., Fazan, V.S., Barbieri, C.H., 2013. Effects of laser therapy in peripheral nerve regeneration. Acta Ortopédica Bras. 21, 266–270.
Sharma, S.K., Kharkwal, G.B., Sajo, M., Huang, Y.Y., De Taboada, L., McCarthy, T., Hamblin, M.R., 2011. Dose response effects of 810 nm laser light on mouse primary cortical neurons. Laser Surg. Med. 43, 851–859.
Sinha, K.M., Zhou, X., 2013. Genetic and molecular control of Osterix in skeletal formation. J. Cell. Biochem. 114, 975–984.
Stein, A., Benayahu, D., Maltz, L., Oron, U., 2005. Low-level laser irradiation promotes proliferation and differentiation of human osteoblasts in vitro. Photomed. Laser Ther. 23, 161–166.
Stevenson, S., Cunningham, N., Toth, J., Davy, D., Reddi, A.H., 1994. The effect of osteogenin (a bone morphogenetic protein) on the formation of bone in orthotopic segmental defects in rats. J Bone Joint Surg Am 76, 1676–1687.
Tim, C.R., Pinto, K.N.Z., Rossi, B.R.O., Fernandes, K., Matsumoto, M.A., Parizotto, N.A., Rennó, A.C.M., 2014. Low-level laser therapy enhances the expression of osteogenic factors during bone repair in rats. Laser Med. Sci. 29, 147–156.
Tim, C.R., Bossini, P.S., Kido, H.W., Malavazi, I., von Zeska Kress, M.R., Carazzolle, M.F., Parizotto, N.A., Rennó, A.C., 2015. Effects of low-level laser therapy on the expression of osteogenic genes during the initial stages of bone healing in rats: a microarray analysis. Laser Med. Sci. 30, 2325–2333.
Tim, C.R., Bossini, P.S., Kido, H.W., Malavazi, I., von Zeska Kress, M.R., Carazzolle, M.F., Parizotto, N.A., Rennó, A.C., 2016. Effects of low level laser therapy on inflammatory and angiogenic gene expression during the process of bone healing: a microarray analysis. J. Photochem. Photobiol. B Biol. 154, 8–15.
Ueda, Y., Shimizu, N., 2003. Effects of pulse frequency of low-level laser therapy (LLLT) on bone nodule formation in rat calvarial cells. J. Clin. Laser Med. Surg. 21, 271–277.
Ustaoglu, G., Ercan, E., Tunali, M., 2017. Low-level laser therapy in enhancing wound healing and preserving tissue thickness at free gingival graft donor sites: a randomized, controlled clinical study. Photomedicine and laser surgery 35, 223–230.
Wang, Y., Huang, Y.-Y., Wang, Y., Lyu, P., Hamblin, M.R., 2016. Photobiomodulation (blue and green light) encourages osteoblastic-differentiation of human adipose-derived stem cells: role of intracellular calcium and light-gated ion channels. Sci. Rep. 6, 33719.
Wang, Y.-H., Wu, J.-Y., Kong, S.C., Chiang, M.-H., Ho, M.-L., Yeh, M.-L., Chen, C.-H., 2018. Low power laser irradiation and human adipose-derived stem cell treatments promote bone regeneration in critical-sized calvarial defects in rats. PLoS One 13 e0195337.
Woodruff, L.D., Bounkeo, J.M., Brannon, W.M., Dawes, K.S., Barham, C.D., Waddell, D.L., Enwemeka, C.S., 2004. The efficacy of laser therapy in wound repair: a meta-analysis of the literature. Photomedicine and laser surgery 22, 241–247.
Wu, Z.H., Zhou, Y., Chen, J.Y., Zhou, L.W., 2010. Mitochondrial signaling for histamine releases in laser-irradiated RBL-2H3 mast cells. Laser Surg. Med. 42, 503–509.
Wu, Y.-h., Wang, J., Gong, D.-x., Gu, H.-y., Hu, S.-s., Zhang, H., 2012. Effects of low-level laser irradiation on mesenchymal stem cell proliferation: a microarray analysis. Laser Med. Sci. 27, 509–519.
Wu, M., Chen, G., Li, Y.-P., 2016. TGF-β and BMP signaling in osteoblast, skeletal development, and bone formation, homeostasis and disease. Bone research 4, 16009.
Wulsten, D., Glatt, V., Ellinghaus, A., Schmidt-Bleek, K., Petersen, A., Schell, H., Lienau, J., Sebald, W., Ploger, F., Seemann, P., 2011. Time kinetics of bone defect healing in response to BMP-2 and GDF-5 characterised by in vivo biomechanics. Eur. Cells Mater. 21, 177–192.
Yang, J., Andre, P., Ye, L., Yang, Y.-Z., 2015. The Hedgehog signalling pathway in bone formation. Int. J. Oral Sci. 7, 73–79.
Young, M.F., Kerr, J.M., Ibaraki, K., Heegaard, A.-M., Robey, P.G., 1992. Structure, expression, and regulation of the major noncollagenous matrix proteins of bone. Clin. Orthop. Relat. Res. 281, 275–294.
Zhang, X., Schwarz, E.M., Young, D.A., Puzas, J.E., Rosier, R.N., O'Keefe, R.J., 2002. Cyclooxygenase-2 regulates mesenchymal cell differentiation into the osteoblast lineage and is critically involved in bone repair. J. Clin. Investig. 109, 1405.
Zhou, X., Zhang, Z., Feng, J.Q., Dusevich, V.M., Sinha, K., Zhang, H., Darnay, B.G., de Crombrugghe, B., 2010. Multiple functions of Osterix are required for bone growth and homeostasis in postnatal mice. Proc. Natl. Acad. Sci. Unit. States Am. 107, 12919–12924.
Zhu, J., Shimizu, E., Zhang, X., Partridge, N.C., Qin, L., 2011. EGFR signaling suppresses osteoblast differentiation and inhibits expression of master osteoblastic transcription factors Runx2 and Osterix. J. Cell. Biochem. 112, 1749–1760.

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