Review Article

Effect of Low Level Laser Therapy on Proliferation and Differentiation of the Cells Contributing in Bone Regeneration

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Abstract:

Introduction: Low level laser therapy (LLLT) also known as photobiomodulation, is a treatment that uses low-level lasers or light-emitting diodes (LEDs) to change cellular function and is a clinically well accepted tool in regenerative medicine and dentistry. Considering the variety of laser, exposure, cells and study types, the exact effects of low level laser therapy seems to be unclear. The aim of this study was to review the data published in the field of the effects of low level laser therapy on proliferation and differentiation of the cells contributing in bone regeneration.

Methods: To access relevant articles, an electronic search in PubMed was conducted from 2001 to April 2014. English language published papers on low level laser therapy were found using the selected keywords .The full texts of potentially suitable articles were obtained for final assessment according to the exclusion and inclusion criteria.

Results: 240 articles were found from 2001 to April 2014. Following the initial screening of titles and abstracts as well as the final screening of full texts, 22 articles completely fulfilled the inclusion criteria of this study. Wavelength used in LLLT irradiation varied between 600 to 1000 nm with an energy density of 0.04–60J/cm^2. Although almost all studies agreed on getting positive effects from LLLT, some had opposing results.

Conclusion: Low level laser with low-energy density range appears to exert a biostimulatory effect on bone tissue, enhance osteoblastic proliferation and differentiation on cell lines used in in vitro studies. Despite the fact that many researches have been recently done on the effects of LLLT on different cell lines, without knowing the precise mechanism and effects, we are not able to offer a clinical treatment protocol. This paper is a beginning to help further progress and extend practical use of LLLT in future.

Keywords: low-level laser therapies; cell line; bone regenerations

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Introduction

Laser dentistry has developed in two different fields: laser surgery and therapy. Low level laser therapy (LLLT) for improving tissue healing and regeneration is also known as photobiomodulation. LLLT is a treatment that uses low-level lasers or light-emitting diodes (LEDs) to change cellular function and is a clinically well

accepted tool in regenerative medicine and dentistry to improve healing processes and management of functional disorders[1]. Laser therapy gives us a better inflammatory response by reducing edema and pain[2]. It has been reported that Low-level lasers exert biostimulatory effects on various cell types, including osteogenic cells and bone tissue[3,4]. Enhanced alkaline phosphatase activity and improved osteocalcin gene expression are other effects of Low Level laser therapy[5]. While injected chemicals and some other medicaments have the problem of systemic side effects, lasers carry the advantage of not having much unwanted impacts on the patients' health status[6]. However, some researchers suggest further studies to clarify the exact action mechanisms of laser[1,5].

Although diode lasers are being frequently used for dental treatments, different types of laser, such as He-Ne, argon have been successfully used for various cell activation. Also each kind of Laser therapy has its own wavelength, power density, energy density, type of exposure and treatment duration[7].

Many in vivo and in vitro studies have demonstrated the positive effects of LLLT on tissues. LLLT enhances the survival of Adipose-derived mesenchymal stem cells (ASCs) and stimulates the secretion of growth factors in the wound bed[8]. Fernandes et al. in a clinical research used LLLT during the initial phase of bone healing in a model of bone defect in rats. The results indicated a higher inflammatory cells recruitment and a better tissue organization at the site of the injury, with the presence of granulation tissue and new bone formation[9]. Improved opening of the mid-palatal suture and accelerated bone regeneration are other clinical effects of low-level lasers in rapid maxillary expansion process[10]. LLLT also stimulates repair process of the radiation-related damages in alveolar bones[11], and can enhance mineralization in sockets[12]

The precise mechanism of LLLT has not been completely explained, while several in vitro studies have shown that LLLT has stimulating effects on osteoblast-like cells and accelerates the repair process of the bone[3,13]. Other study reported delayed fracture healing or no effects after low level laser irradiation[5]. Bloise et al. used diode laser (659 nm) on human osteoblast-like cell line (Saos-2),which resulted in enhanced proliferation and differentiation[14]. Same results were shown in the study carried out by Stein et al. using a helium-neon (He-Ne) laser (632 nm) on human osteoblast cell line which also promoted cell proliferation and maturation of human osteoblasts[4]. However, Bouvet-Gerbettaz et al. used a diode Laser (808 nm) to assess bone cell proliferation as well as osteoblastic and osteoclastic differentiation on murine bone marrow cells and found no significant change between the control (non-radiated) and LLLT groups[15]. On the other hand, in another study, a single exposure of 830 nm Diode on osteoblastic (MC3T3) cell line resulted in a reduction in cell growth compared to non-irradiated controls[5].

Despite the fact that many researches have been recently done on the effects of LLLT on different cell lines, without knowing the precise mechanism and effects, we are not able to offer a clinical treatment protocol. This study was designed to review articles, trying to develop primary understanding about functions and effects of LLLT on different tissues. This paper is a beginning to help further progress and extend practical use of LLLT in future.

Methods

Search strategy

To access relevant articles, an electronic search in PubMed was conducted from 2001 to April 2014. English language published papers on low level laser therapy were found using the following keywords alone or ensemble: low-level laser therapy, cell line, stem cell differentiation and bone regeneration. Initial paper selection was performed by examining titles and abstracts of all selected papers. The full texts of potentially suitable articles were obtained for final assessment according to the exclusion and inclusion criteria.

Study selection

All in vivo and in vitro studies applying LLLT on defined cell lines by using outlined assessment tests and reaching clear results were included. Different types of low-level laser with different types of irradiation were also included in this study.

Results

240 articles were found from 2001 to April 2014. Following the initial screening of titles and abstracts and final screening of full texts, 22 articles completely fulfilled the inclusion criteria of this study. All data were calculated in a table for better analyzing and comparing the studies which have used a specific type of cell line. (Table 1)

Among both in vitro and in vivo studies, diode laser

Table 1. Review of published data in using the effect of Low Level Laser Therapy on proliferation and differentiation of the cells contributing in bone regeneration

No	Author & year	Type of LLLT	Type of irradiation	Type of cells	Criteria	Assessment Tests/Assay	Conclusion
1	Huertas et al.2014[3]	Diode (940 nm)	Pulsed radiation Energy outputs: 1-5 J Intensities: 0.5, 1, 1.5 and 2 W/cm^2	MG-63 cell	Cell proliferation	MTT assay	Pulsed low-level laser with low-energy density range appears to exert a biostimulatory effect on bone tissue.
2	Migliario et al.2014[13]	Diode (980 nm)	Continuous mode Energy outputs: 1-50 J Intensities: 1.57, 7.87, 15.74, 39.37 and 78.75 J/cm^2	Murine pre-osteoblasts MC3T3 cells	Cell proliferation	Manual cell count	LLLT may be a useful tool for bone regeneration therapy.
3	Choi et al.2013[17]	He-Ne (632.8 nm)	Continuous mode Energy out puts: 17.0 mW Intensities: 0, 1 and 3 J/cm^2	Adipose-derived mesenchymal stem cell (ASC)	Osteogenic potential	-Histological and immunofluorescence assessment -Three-dimensional micro-computed tomography -Western blot analysis	LLLT enhanced the proliferation and the survival of ASCs at 14 days ASC-seeded grafts promote bone regeneration, and the application of LLLT on ASC-seeded ADM results in rapid bone formation.
4	Bloise et al.2013[14]	Diode (659 nm)	Single transverse- mode Energy out puts: 10 mW Intensities: 1, 3 J/cm^2	Human osteoblast-like cell line (Saos-2 cell line)	1.Proliferation 2.Differantiation	-Calcium deposition -Alkaline phosphatase activity	LLLT is a helpful application for bone tissue regeneration.
5	Jawad et al.2013[20]	Diode (940 nm)	Continuous mode Energy out puts: 100, 200, 300 mw	Human fetal osteoblast cell line	1.Proliferation 2.Differantiation	-MTT assay. -Alkaline phosphatase -Osteocalcin activity assays.	LLLT may play an important role in stimulating osteoblast cells for improved bone formation.
6	Wu et al.2013[23]	Diode (660 nm)	Energy out puts: 15 -17 mW.cm^{-2}, Intensities: 1, 2 and 4 J/cm^2	Human PDL (hPDL) cells	1.Proliferation 2.Differantiation 3. Osteogenic marker gene expression 4.Cytotoxicity	-MTT assay -Alizarin Red S staining -Alkaline phosphatase (ALP) activity. -Osteogenic marker gene expression: 5RT-PCR -Lactate dehydrogenase (LDH) leakage measurement	Potential use of LPLI in clinical applications for periodontal tissue regeneration.
7	Pyo et al.2013[21]	Diode (808 nm)	Continuous mode Energy out puts: 1000 mW Intensities: 1.2, 2.4 and 3.6 J/cm^2	Hypoxic-cultured Human fetal osteoblast cells (cell line 1.19)	1.Cell viability 2.Expression of hypoxia-inducible factor-1s (HIF-1s), bone morphogenic protein-2 (BMP-2), osteocalcin, type I collagen, transforming growth factor-β1 (TGF-β1), and Akt	-MTT assay -Western blot assay -Quantitative reverse transcriptional assay	LLLT induces the expression of BMP-2, osteocalcin, and TGF-β1 in 1 % hypoxic-cultured human osteoblasts.

Table 1. Continued

No	Author & year	Type of LLLT	Type of irradiation	Type of cells	Criteria	Assessment Tests/Assay	Conclusion
8	Aleksic et al. 2010[25]	Er: YAG laser (2.94 nm)	Pulsed radiation Energy out puts: 30–350 mJ Intensities: 0.7– 17.2J/cm^2	Mouse-derived osteoblastic cell line MC3T3-E1	1. Cell proliferation 2.Cell death 3.Mitogen-activated protein kinase (MAPK) pathways	- Lactate dehydrogenase measurement -Western blot	Er: YAG laser may be able to promote bone healing following periodontal and peri implant therapy.
9	Renno et al. 2010[5]	Diode (830 nm)	Single exposure Energy out puts: 30mW Intensities: 10 J/cm^2	Osteoblastic (MC3T3) cell line	1.Proliferation 2.Cell growth	- CellTiter 96Aqueous One Solution Cell Proliferation Assay	Reduction in cell proliferation compared to non-irradiated controls.
10	Stein et al. 2008[22]	9Diode (670 nm)	Continuous mode Energy outputs: 400 mW Intensities: 1 or 2 J/cm^2	Human osteosarcoma cell line SaOS-2	1.Total cellular protein synthesis 2.Alkaline phosphatase (ALP) -specific activity 3. attached cell viability	-Micro BCA™ protein assay -Alkaline phosphatase (ALP) -specific activity: colorimetric end-point assay -Metabolic XTT-Assay	Combined treatment with phenothiazine chloride and LLLT does not result in a synergistic enhancement of the biostimulatory effect of LLLT. No evidence for antagonizing effects on growth and, differentiation of human osteoblasts.
11	Bouvet-Gerbettaz et al. 2009[15]	Diode (808 nm)	Continuous mode Intensities: 4 J/cm^2	Murine bone marrow cells	1. Bone cell proliferation, 2.Osteoblastic and osteoclastic differentiation	-Specific staining and microscopic analysis of the culture -Quantitative RT-PCR	LLLT does not alter murine bone progenitor cell proliferation and differentiation.
12	Renno et al. 2007[26]	(670-nm, 780-nm, and 830-nm)	Single exposure Energy outputs: 10 mW Intensities: 0.5, 1, 5, and 10 J/cm^2.	Neonatal, murine, calvarial, osteoblastic (MC3T3) and Human osteosarcoma (MG63) cell lines	1.Cell proliferation 2.Alkaline phosphatase activity	-Cell proliferation assay -Alkaline phosphatase assays (EnzoLyteTM pNPP Alkaline Phosphatase Assay (Colorimetric) Kit)	Each cell line responds differently to specific wavelength and dose combinations.
13	Martinasso et al. 2007[27]	Superpulsed low-level laser therapy (SLLLT)	Superpulsed radiation Energy outputs: 60 J	Human osteoblast-like cells MG-63.	1.Cell proliferation 2. Markers of osteoblast activity	-Osteocalcin measurement -Alkaline phosphatase measurement -Real time PCR.	Repeated SLLLT irradiation stimulates cell proliferation in human osteoblast-like cells and, importantly, increases the expression of proteins essential for bone formation.
14	Aihara et al. 2006[16]	Diode laser (810 nm)	Continuous mode Energy outputs: 50 mW Intensities: 9.33, 27.99, 55.98, or 93.30 J/cm^2,	Rat osteoclast precursor cells	Formation of osteoclast-like cell	-Immunohistochemical staining Reverse transcription-polymerase chain reaction -Pit formation assay	Low-energy laser irradiation facilitates differentiation and activation of osteoclasts via RANK expression.

Table 1. Continued

No	Author & year	Type of LLLT	Type of irradiation	Type of cells	Criteria	Assessment Tests/Assay	Conclusion
15	Stein et al. 2005[4]	He-Ne laser (632 nm)	energy output: 10mW Intensities: 0.43 J/cm^2	Human osteoblast cell line	1.Cell proliferation 2.Differantiation	-MTT assay - Histochemical staining of ALP - Immunohistochemistry	LLLT promotes proliferation and maturation of human osteoblasts in vitro.
16	Hamajima et al. 2003[19]	Diode (830 nm)	Continuous mode Energy output: 500 mW Intensities: 7.64 J/cm^2	Mouse calvaria-derived osteoblastic cell line, MC3T3-E1	Bone formation	-Reverse transcription polymerase chain reaction (RT-PCR) method -Real-time PCR method	Increased expression of the osteoglycin gene by LLLI in the early proliferation stage of cultured osteoblastic cells may play an important role in the stimulation of bone formation in concert with matrix proteins and growth factors.
17	Coombe et al. 2001[18]	Diode (830 nm)	Single radiation Energy output 90 mW Intensities: 1.7 to 25.1 J/cm^2	Human osteosarcoma cell line, SAOS-2	1.Cell viability 2.Proliferation	-Alkaline phosphatase activity -Intracellular calcium concentration	The heat shock response and increased intracellular calcium indicate that the cells do respond to low level laser irradiation.

was the most frequently used laser. 18 studies used diode laser[3-5,9,12-24]. 2 studies used He-Ne laser[4,17] and 1 study used Er:YAG (Erbium-Doped Yttrium Aluminum Garnet) laser in their experiments[25]. One study didn't clarify its type of laser[26]. Only 1 study used Superpulsed low-level laser therapy (SLLLT)[27]. Laser wavelength was different between the selected studies and varied from 632 to 980 nm. In one study, laser wavelength was not clarified.[12]

Different types of irradiation were applied in the studies selected, such as: single, pulsed, superpulsed exposure and continuous mode. Also each type of study had its specific energy density and power output. Different types of cell lines were used in studies such as: Human osteoblastic-like and osteosarcoma cell line (Saos-2), Human periodontal ligament (PDL)cells, Human osteoblast-like cells MG-63,Osteoblastic MC3T3 cell line, Adipose derived mesenchymal stem cell, Mouse calvaria derived osteoblastic cell line MC3T3-E1,Murine bone marrow cells and Murine preosteoblastic MC3T3 cells. In vivo studies were mainly done on animals.

The most common criteria assessed in almost all the studies were cell proliferation and cell differentiation. Osteogenic genes expression, cytotoxicity, expression of hypoxia-inducible factor-1s (HIF-1s), bone morphogenic protein-2 (BMP-2), osteocalcin, type I collagen and transforming growth factor-β1 (TGF-β1) were other important criteria among the studies. The most common assessment tests among the studies were: the 3-(4,5-dimethylthiazol-2yl)-2,5 diphenyl tetrazolium bromide (MTT) assay, alkaline phosphatase assays, Western blot analysis, reverse transcription polymerase chain reaction (RT-PCR) and real-time PCR method.

In general, almost all the studies resulted in having positive effects while using low-level lasers in comparison to the control group (non-irradiated). In 2007, a study was conducted using super pulsed low level laser therapy on human osteoblast-like MG-63 cells to assess cell proliferation and markers of osteoblast activity[27]. The results showed increased number of cells and greater osteocalcin and alkaline phosphatase especially after 3 days of treatment. Seven years later, Huertas et al. did an in vitro study using diode laser on cultured MG-63 cell to assess their proliferation by MMT test which showed increased cell proliferation at intensities of 0.5, 1 and 1.5 W/cm^2 versus controls. They also concluded that low energy density variation positively correlated with cell growth[3]. In two of the selected articles, diode laser was used to see its effect on human osteosarcoma cell line SAOA-2 to see its effects on cell viability through alkaline phosphatase activity[18,22]. In the experience

performed by Stein et al. a 670 nm nonthermal diode laser unit was used with an output of 400mW and energy density of 1 or 2 J/cm^2 in continuous mode[4]. Their outcomes demonstrated an increase in cell viability and ALP-specific activity with a laser dose of 1J/cm^2 and lower levels of ALP-specific with 2J/cm^2 dose[22]. On the contrary, different results were obtained in the study of Coombe et al[18]. In their study, a diode laser with 830 nm was used with an output of almost 4.5 times less than the previous study and energy densities of 0.3, 0.5,1 and 2 joules. The results came out as: no changes in cell viability, proliferation or activation and also no significant early or late effects on protein expression and alkaline phosphatase activity[18]. Same results were observed in the study carried out by Bouvet-Gerbettaz et al in 2009 to see the effects of continuous irradiation of diode laser (808nm) on Murine bone marrow cells using a specific staining and microscopic analysis of the cultures after various times and quantitative RT-PCR[15] .The results were similar proliferation response of bone marrow mesenchymal stem cells as well as osteoclast or osteoblast differentiation of the corresponding progenitors between control and LLLT conditions.

In 2006, Aihara et al. used a diode laser on rat osteoclast precursor cells to see the formation of osteoclast-like cells, which resulted in an increased number of tartrate-resistant acid phosphatase positive multinucleate cells by approximately 1.3-fold in the 3- and 6-min irradiation groups and a faster appearance of osteoclasts in the laser irradiation groups[16].

To analyze the effects of He-Ne laser on human osteoblast like cell, a study was done in 2005 which showed higher ALP activity and expression of osteopontin[4]. In 2013, same type of laser and wavelength with a different power output was irradiated on an adipose-derived mesenchymal stem cell (ASC)-seeded acellular dermal matrix (ADM) to analyze its osteogenic potency, which resulted in a faster bone regeneration in comparison to the control group[17].

One of the most frequent used cell line within the studies were MC3T3 cells, in which diode laser was irradiated, but apparently studies differed in their results[5,13,19,25,26]. In 2007, Renno et al. used a Diode laser at three different wavelengths: 670-nm, 780-nm, and 830-nm with a single irradiation .The results were significant increase in osteoblast proliferation after 830-nm laser irradiation (at 10 J/cm^2) but decrease after 780-nm laser irradiation (at 1, 5, and 10 J/cm^2)[26]. In 2010, they did another research using a diode (830 nm) with a single exposure at 10 J/cm^2 on osteoblastic (MC3T3) cell line (on bioscaffolds), which showed a 13% decrease in MC3T3 cell proliferation on glass-ceramic discs compared to control (non-irradiated) discs. It also resulted in a reduction in cell growth compared to non-irradiated controls[5]. In another study in 2010, an Er:YAG laser was applied on the same cell line and showed that under a number of parameter combinations, high osteoblast proliferation was observed[25]. In the study carried out by Wu et al. human PDL (hPDL) cells were exposed to GaAlAs laser with a wavelength of 660-nm to see its effect on proliferation, differentiation and osteogenic marker gene expression[23]. The low level laser irradiation significantly promoted hPDL cell proliferation at days 3 and 5 and at energy doses of 2 and 4 J/cm^2 showed potential osteogenic capacity, as it stimulated ALP activity, calcium deposition, and osteogenic gene expression[23].

Another effect of low-level laser irradiation is that it promotes the expression of BMP-2, osteocalcin, and TGF-β1 but has no effect on type I collagen expression in hypoxic-cultured osteoblast based on the research done by Pyo et al. in 2013[21].

Discussion

Low level laser therapy as a clinically well accepted tool in regenerative medicine and dentistry improves healing processes and management of functional disorders[1]. Providing direct biostimulative light energy to cells is the main target of low level laser therapy. Laser energy results in stimulation of molecules of cells, while having no significant increase in tissue temperature[28].

Low-level laser therapy (LLLT) as a simple and noninvasive technique, highly effective in different branches of regenerative medicine, has favorable results on a variety of pathological conditions, such as pain and inflammation reduction[13], Chondral[29] and fibroblast proliferation[30], collagen synthesis and nerve regeneration are other effects of LLLT[31,32].

In order to see the effects of LLLT on osteoblast's proliferation, differentiation and maturation, specific cell lines should be used such as: MC3T3, MG-63 and SAOS-2. To assess cell's proliferation, (3-(4,5-dimethylthiazol-2yl)-2,5 diphenyl tetrazolium bromide) (MTT) assay is essential; also to see the effects of LLLT on cell differantiation alkaline phosphatase and osteocalcin activity assays are two helpful tests.

Wave length used in LLLT irradiation varies between 600 to 1000 nm with an energy density of 0.04–60J/cm^2. Different laser light sources, like helium-neon

and gallium-aluminium-arsenide (GaAlAs), are being frequently used in clinical studies such as: surgical treatments of oral lesions, uncovery of implants, bacteria reduction in root canals or periodontal pockets and dentine hypersensitivity reduction. Diode lasers are known to have a high penetration depth compared to other laser types[13,3].

Although irradiation in laser therapy is local, it may have some systemic effects on other parts of the body, and since the exact mechanism of LLLT action is still unclear, we may not be aware of these effects. In 2011 Silva et al. indicated that LLLT is a powerful instrument to prevent oral mucositis in the patients who have undergone hematopoitic stem cell transplantation (HSCT)[33]. Another study showed that LLLT had systemic and skeletal muscle anti-inflamatory effects in rats with heart failure, due to reduced plasma IL-6, TNF-α levels[34]. LLLT can also reduce postoperative pain by activation of peripheral opioid receptors[35].

Conclusion

Based on all the results and conclusions of reviewed articles, considering the variety of laser, exposure, cells and study types, exact effects of low level laser therapy seems to be unclear. Although almost all the studies agreed on getting positive effects from LLLT, some have opposing results. Low level laser with low-energy density range appears to exert a biostimulatory effect on bone tissue, enhance osteoblastic proliferation and differentiation on cell lines used in in vitro studies and therefore may be a useful tool for bone regeneration therapy. Opposing results such as no effect on osteoblastic proliferation and differantiation, or even a reduction in these two criteria, are also mentioned within the studies.

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