Review Article

Photobiomodulation of human gingival fibroblasts with diode laser - A systematic review

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Abstract:

Low-level laser therapy (LLLT) is being extensively studied in the field of periodontics as a noninvasive technique to achieve better results after nonsurgical and surgical therapy. However, there is a lack of definitive guidelines for the use of LLLT to promote gingival and periodontal wound healing. The primary objective of this systematic review was to critically analyze the studies evaluating the effect of low-level diode laser on human gingival fibroblasts *in vitro* and to develop wavelength-specific guidelines for photobiomodulation of human gingival fibroblasts. A thorough electronic and manual search was conducted for relevant articles published until December 2019. Nine studies were included in the review after the initial screening of 1334 articles. Our data analysis revealed that LLLT with diode laser stimulates human gingival fibroblasts as there was the increase in cell viability, proliferation, migration, and protein synthesis in irradiated cells. The diode lasers in the 600–700 nm spectrum were effective in the 10 mW to 30 mW power range. Lasers in the 700–800 nm range were effective in the 25–50 mW power range and diode lasers in the 800–900 nm range were effective at a power setting of 10 mW. It was possible to ascertain a suitable power setting for a particular wavelength spectrum, but no other parameters could be defined due to a lack of reporting of details. Hence, the authors have developed guidelines for comprehensive reporting of in–vitro studies to facilitate future research and overcome existing lacunae in knowledge.

Key words:

Fibroblasts, low-level light therapy, systematic review, wound healing

INTRODUCTION

Low-level laser therapy (LLLT) or photobiomodulation (PBM) has attracted the attention of researchers worldwide. The thought of using laser energy to promote cellular regeneration and wound healing is exciting and has numerous plausible applications in health sciences. Hence, it is being extensively studied in the field of periodontics as a noninvasive technique to achieve better results after nonsurgical and surgical therapy. As the most abundant cells of the periodontium, the gingival and periodontal fibroblasts play a crucial role in wound healing and repair and various studies have been conducted to evaluate the effect of laser on these cells.[1] However, there is lack of consensus regarding the optimal irradiation parameters and a sound protocol to promote wound healing does not exist.[2]

LLLT remains controversial as the outcome is dependent on numerous parameters such as the wavelength, fluence, power density, time duration, frequency and even the slightest change in the parameters can adversely influence the result.[3] Low-level laser typically exhibits a biphasic response where an insufficient dose does not produce any effect but an adequate dose results in biostimulation and a dose higher than the optimal dose results in bioinhibition.[4] Thus biosimulation occurs only within a specific "therapeutic window" and such a "therapeutic window" specific to periodontal tissue response has not been established till date.[5] For any laser to exert its effect, the laser energy must be absorbed by the tissue. Therefore, an important aspect of laser dosimetry is the wavelength that is being used. The wavelength determines which photoacceptor molecule absorbs the light.[3] Dosage calculation in LLLT is thus complicated and cannot be generalized for a laser type or tissue healing in general. Generalization of results in the past may have resulted in contradictory outcomes in clinical studies. Since a multitude of factors plays a role, well-defined dosage

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guidelines can be made only for a specific laser.[6] In addition, there is a need for wavelength and therapy-specific guidelines for a particular cell type which can be easily extrapolated for clinical experiments.

The objectives of this study were to (1) conduct a systematic review of studies evaluating the effect of low-level diode laser on human gingival fibroblasts *in vitro* (2) To develop wavelength-specific guidelines for PBM of human gingival fibroblasts (3) To try and develop guidelines for comprehensive reporting of *in–vitro* studies evaluating effects of low-level laser on cell cultures.

MATERIALS AND METHODS

The systematic review was conducted with the following research question– "What are the recommended dosage parameters to achieve photobiostimulation of human gingival fibroblasts by diode laser?"

A thorough search was performed using three databases-PubMed, Google Scholar, and EBSCO, for relevant articles published until December 2019. No beginning date was provided for the search, to include as many relevant articles as available. The terms used for the electronic search were as follows– "low-level light therapy" (MeSH term), "LLLT," "low level laser irradiation," "LLLT," "photobiostimulation," "PBM," "PBM" and "gingival fibroblasts." A manual search was also performed to supplement the electronic search. The reference lists of important original articles and review articles were hand – searched and relevant citations were included for the selection process. All the search results were exported to Microsoft Word as well as to Zotero software (Center for History and New Media, George Mason University, Fairfax, Virginia, USA).

The duplicate articles were removed from the search results with the help of Zotero software. This was followed by the selection process which was independently performed by two reviewers and differences were solved with discussions. All the titles and abstracts were screened for relevant literature and full-text articles were shortlisted for the final selection process. Then articles were rejected if they did not suit the eligibility criteria and a final list of articles was approved for this review. The studies were included if (1) they evaluated the effect of LLLT using diode laser on human gingival fibroblasts specifically validity, cellular proliferation, cellular migration, and related protein synthesis and gene expression (2) The cell source was human gingival tissue and investigation was performed on cell cultures. The studies were excluded in the following situations (1) studies which evaluated the effect of light-emitting diode or other laser types, for example, He-Ne, Nd: YaG (2) studies which evaluated effect on cell types other than human gingival fibroblasts (3) studies in which a preestablished cell line was used for investigation (4) animal studies, clinical studies and literature reviews (5) studies for which full-text versions were not available (6) studies in which cells were co-cultured in the presence of other agents, for example, lipopolysaccharide, nuclear factor kappa B inhibitors, cytotoxic agents, etc.

Once the articles were selected the following data were extracted: Publication details, cell source, cell culture harvesting protocol, parameters involved in laser dosimetry (wavelength, power, energy, power density, energy density, time, mode of delivery), irradiation protocol (mode of application, distance of application, frequency), outcome measures and important observations, allocation concealment and use of power meter.

Finally, the quality and bias within selected studies were evaluated with the Office of Health Assessment and Translation (OHAT) Tool developed for studies related to environmental health sciences by Rooney *et al*.[7] The studies were assessed for the following parameters categorized as "All" in the OHAT risk of bias list: (1) confounding and modifying variables, (2) control for other exposures that might affect results, (3) study protocol, (4) outcome data analysis, (5) blinding, (6) exposure characterization, (7) outcome assessment, (8) reporting of outcomes, (9) internal validity via description of irradiation parameters, use of power meters and laser device calibration. Each question was verified for all the selected studies and an answer of "yes" or "no" was given depending on the perceived risk of bias.

RESULTS

The selection process has been depicted in detail through the PRISMA flowchart in Figure 1. The search revealed a total of 1368 articles of which 34 were in form of duplicates. Thousand three hundred and thirty-four titles and abstracts were screened for relevance and 31 articles were included for full-text screening. The eligibility criteria were applied and nine articles were selected for the systematic review.

The publication details, cell source, and sample size are summarized in Table 1 while cell culture methodology, study design characteristics, and conclusions are summarized in Table 2. The irradiation and dosimetry-related parameters along with outcome measures and observations have been grouped for similar wavelength and summarized in Tables 3-6.

Of the nine studies included in the review four had reported the demographics of donors and five had reported the number of biological samples taken for harvesting gingival fibroblasts [Table 1]. The gingival fibroblasts were grown from tissues obtained from healthy young donors (age ≤24 years) in all four studies. Only Pansani *et al*. in their study had harvested gingival fibroblasts from young as well as elderly patients.[8] Almost all studies had mentioned that the donors were systemically healthy. The studies by Almeida-Lopes *et al*. in the year 1998, 2001 and Azevedo *et al*. in 2006 were performed on the same primary cell line harvested from a 24 years old female.[9-11]

When cell culture and study design were analyzed it was observed that all studies evaluated the effect of LLLT using diode laser under ideal conditions, i.e. with 10% FBS except Almeida-Lopes *et al*.[9,10] and Azevedo *et al*.[11] These studies evaluated the effect under stressed conditions, i.e. with 5% FBS. Azevedo *et al*.[11] exposed the gingival fibroblasts to 5% serum concentration 2 days before irradiation whereas the duration of exposure was not mentioned by Almeida-Lopes *et al*.[9,10] The allocation of cells to experiment and control groups was not concealed to the observer in any study. The laser device was not calibrated before use in any study and four out of nine

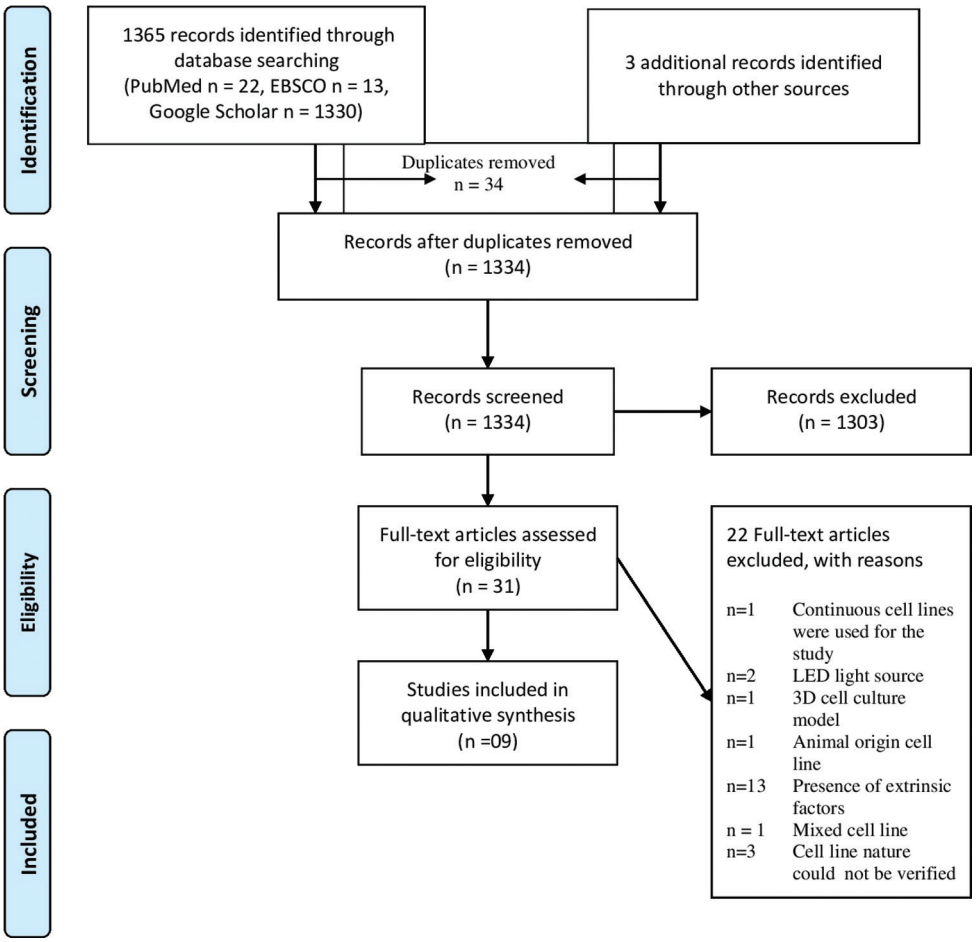

Figure 1: PRISMA flowchart depicting the study selection process. n – number

Table 1: Donor characteristics

Author	Donor details	Sample size
Almeida L (1998)	24 years, female	1
Kreisler M (2001)	NM	NM
Almeida L (2001)	24 years, female	1
Kreisler M (2002)	NM	NM
Azevedo LH (2006)	24 years, female	1
Saygun I (2008)	NM	NM
Damante (2009)	NM	1
Hakki SS (2011)	NM	NM
Pansani (2017)	18-5 years	3
	>65 years	3

NM – Not mentioned

studies reported the use of power meter to check laser output during the actual experiment [Table 2]. Almeida-Lopes *et al*.,[10] Azevedo *et al*.,[11] Kreisler *et al*.[12] and Damante *et al*.[13] described the protocol followed to prevent the influence of other variables such as ambient light and scattered laser energy but a majority of studies failed to describe the similarity of interventions in test and control groups.

The diode lasers used in the range of 600–700 nm had the following wavelength – 635 nm, 660 nm, 670 nm, 685 nm, and 692 nm. Almeida-Lopes *et al*.,[9,10] Azevedo *et al*.[11] and Saygun *et al*.[14] evaluated the effect on cell growth and all found that the viable cell counts were higher in the irradiated groups as compared to the control groups. Saygun *et al*. assessed for growth factor production in response to 685 nm laser stimulation and found that basic fibroblast growth factor (bFGF), insulin-like growth factor-1 (IGF-1) and IGF -binding protein 3 (IGFBP3) levels were higher in test groups.[14] However, Damante *et al*. found no significant difference in the control and test groups for bFGF production where cells were irradiated with 660 nm diode laser.[13] The irradiation parameters used by these authors in studies with favorable outcomes were as follows: 10–30 mW power, 2 J/cm^2 energy density, and successive irradiations.

The most common wavelength used in the range of 700–800 nm was 780 nm. Pansani *et al*.[8] and Almeida *et al*.[9,10] evaluated the effect on cell growth and found that the viable cell counts were higher in the irradiated groups as compared to the control groups.[8-10] Damante *et al*. found that bFGF levels were significantly greater (1.49 times) in cells irradiated with 780 nm as compared to controls.[13] Pansani *et al*. found that even though there was no effect on vascular endothelial growth factor (VEGF) synthesis, but VEGF gene expression was increased in irradiated groups and LLLT with 780 nm diode laser stimulated cell migration as well as collagen synthesis.[8] Favorable results were obtained with the following irradiation parameters: 25–50 mW power density, 2 J energy, 3–5 J/cm^2 energy density, 0.6–4 min duration, and repetitive irradiations.

Table 2: Characteristics in study design of selected studies

Author	Cell culture conditions	Groups	Plate	Replication	Allocation concealment	Use of power meter	laser device calibration	Important finding
Almeida-Lopes L (1998)	5% FBS 10% FBS	0%+635 nm 5%+635 nm 10%+635 nm 0%+780 nm 5%+780 nm 10%+780 nm	Petri dish (9 per treatment)	0	X	X	X	780 nm laser showed similar effect as 635 nm
Kreisler M (2001)	10% FBS	Control Laser - 20 protocols	24 well plate (6 wells per treatment)	0	X	Yes	X	Higher exposure time was associated with lower survival rate even for the lowest power setting in this study
Almeida-Lopes L (2001)	10% FBS 5%-10% FBS	Control 670 nm 780 nm 692 nm 786 nm	60 mm (1 per treatment)	0	X	X	X	Smaller laser exposure time results in higher proliferation
Kreisler M (2002)	10% FBS	Control 1.96 J/cm^2 once, twice, thrice 3.92 J/cm^2 once 7.84 J/cm^2 once	96 well plate (22 per treatment)	0	X	Yes	X	Repeated treatment may be necessary to achieve positive effect clinically
Azevedo LH (2006)	5% FBS	Control 10 mW 29 mW	35 mm diameter dishes (3 per treatment)	0	X	Yes	X	Power density influences cell growth in an inversely proportional manner
Saygun I (2008)	10% FBS	Control Single dose Double dose	NM (10 per treatmnent)	0	X	X	X	LLLT enhances the production of the growth factors
Damante (2009)	10% FBS	1% FBS 10% FBS 780 nm+3 J/cm^2 780 nm+5 J/cm^2 660 nm+3 J/cm^2 660 nm+5 J/cm^2	96 well plate (3 per treatment)	0	X	Yes	X	Increased production of bFGF could be one of the mechanisms by which infra-red laser stimulates wound healing
Hakki SS (2011)	10% FBS	Control Infected pocket group Periodontal pocket group Biostimulation group	96 well plate (6 per treatment)	Duplicate	X	X	X	LLLT induces growth factor mRNA expression
Pansani (2017)	10% FBS	Control Laser (Y) Laser (E) EGF (Y) EGF (E)	24 well plate (12 per treatment total)	0	X	X	X	LLLT can biostimulate gingival fibroblast functions involved in tissue repair in young and old patients

LLLT – Low-level laser therapy; FBS – Fetal-bovine serum; bFGF – Basic fibroblast growth factor; mRNA – Messenger RNA; EGF – Endothelial growth factor; NM – Not mentioned; Y – Young; E – Elderly

Only two studies evaluated the effect of diode LLLT on gingival fibroblasts in the 800–900 nm range and the wavelengths used were 809 nm and 810 nm namely. Kreisler *et al*. found that power setting of 10 mW stimulated cell growth, whereas ≥500 mW resulted in reduced cell viability.[12,15] Cellular proliferation was enhanced when the laser was used with the following parameters: 10 mW power, 1.96–7.84 J/cm^2 energy density achieved by irradiating for 75, 150, and 300 s, respectively. Kreisler *et al*. also found that the differences between the test and control groups were highly significant at 24 h postirradiation but decreased in an energy-dependent manner at 48 and 72 h postirradiation, and hence, they suggested that repeated irradiation may be necessary in the clinical environment.[12]

In the 900–1000 nm spectrum, only 940 nm diode laser has been evaluated for its efficacy to stimulate gingival fibroblasts. Hakki and Bozkurt evaluated the effect of 940 nm diode laser irradiation on cell growth and messenger ribonucleic acid (mRNA) expression of growth factors and Type I collagen.[16] The authors found that irradiation with this laser did not have any effect on cell growth, but there was a significant increase in IGF, VEGF, transforming growth factor-β and Type I collagen m– ribonucleic acid (RNA) expression at 48 h postirradiation. The parameters used in this study were –300 mW power, 6 J/cm^2 energy density, and single dose of irradiation using a 300 micron tip for 20 s/cm^2.

Direct comparisons between different wavelengths were made in three studies by Almeida-Lopes *et al*. and Damante *et al*. where they assessed the effect of visible (635–692 nm) versus infrared (780–786 nm) diode laser light.[9,10,13] Almeida-Lopes *et al*. in their study found no difference in the activity of 780 nm and 635 nm diode laser using different

Table 3: Irradiation parameters and results of low-level laser therapy with 600-700 nm diode laser

Author	Power (mW)	Energy	Power density	Energy density	Frequency with time interval	Duration	Spot size	Tip	Mode	Distance	Outcome measures and important observations
Almeida L (1998) 635 nm	4	2 J (8 J)	NM	NM	4 (time interval NM)	8.3 min	1 mm^2	NM	NM	Contact	Cell growth was evaluated at 4,896,144 h (manual count on Neubars chamber) Cell number in laser treated groups was higher than respective control groups for cells grown in 5% and 10% FBS
Almeida L (2001) 670 nm 692 nm	10 30	2 J	NM	NM	4 (12 h)	Automatic	NM	NM	NM	NM	Cell growth was evaluated at 2,4,6 days using Tryphan blue exclusion assay and hemocytometer Cell number was higher in control versus irradiated cell in 10% FBS; higher in irradiated cells than control in 5% FBS Cell number similar or higher than that of control cells grown on 10% serum concentration; cell number higher in irradiated cells than controls in 5% FBS group
Saygun I (2008) 685 nm	25	NM	NM	2 J/cm^2	Once twice (24 h interval)	NM	NM	Optical fibre	C	NM	Assessment at 24 h postirradiation Cell count by coulter counter: Proliferation was increased in single and double dose groups as compared to control Assay for bFGF, IGF-1, and IGFBP3 by ELISA: Increase in bFGF, IGF-1 was seen in single dose group; increase in bFGF, IGF-1, and IGFBP3 was seen in double dose group
Azevedo LH (2006) 660 nm	10 29	NM	142.85 W/cm^2 428.57 W/cm^2	2 J/cm^2	Twice (12 h interval)	14 s 4.8 s	0.07 cm^2	NM	NM	Contact through bottom	cell number at 2,4,9 days postirradiation measured using Tryphan blue exclusion assay and hemocytometer Cell numbers: Control <Group 2 <Group 1
Damante CA (2009) 660 nm	40±6.24	NM	1 W/cm^2	3 J/cm^2 5 J/cm^2	Twice (6 h interval)	3 s 5 s	NM	NM	NM	Contact through bottom	Growth factors measured at 24 h after irradiation KGF release was similar in all groups bFGF was significantly greater (1.49 times) in groups treated with infra-red laser than 660 nm laser groups and control groups

IGF-1 – Insulin-like growth factor-1; FBS – Fetal-bovine serum; bFGF – Basic fibroblast growth factor; IGFBP3 – Insulin-like growth factor-binding protein 3; NM – Not mentioned; KGF – Keratinocyte growth factor

settings except for the total energy which was kept constant at 8J.[9] In another study by Almeida-Lopes *et al*., similar results were observed with 786 nm and 692 nm diode laser when power setting (30 mW) and total energy (2J) was kept the same for both wavelengths.[10] However, in the same study, it was found that 780 nm laser showed better results as compared to 670 nm laser in terms of cell growth with different power settings but identical total energy.[10] Damante *et al*. found increased production of bFGF in gingival fibroblasts when irradiated with 780 nm diode laser as compared to 660 nm diode laser when all irradiation parameters were kept identical.[13] Thus in the two studies, no difference was observed between the visible and infrared diode laser, whereas two studies showed that diode laser with wavelength in the infrared spectrum resulted in better photobiostimulation of gingival fibroblasts.

When the studies were assessed for reporting of irradiation parameters it was found that a majority of studies failed to report energy per session, total energy, power density, spot size, and tip used for irradiation. All studies had reported the power settings and frequency of irradiation with time-lapse between subsequent irradiations. The duration of exposure was not mentioned by one study and three studies failed to report energy density, mode, and distance. Three important parameters that were not reported by a majority of studies were as follows: The quality assessment with OHAT Tool revealed that four studies had followed <4 criteria and five studies had followed ≥5 criteria. None of the selected studies had followed blinding and allocation concealment. None of the studies satisfied the criteria of internal validity as they failed to report at least one irradiation parameter or did not include methods to eliminate the effect of cross-irradiation and/or visible light.

Table 4: Irradiation parameters and results of low level laser therapy with 700-800 nm diode laser

Author	Power (mW)	Energy	Power density	Energy density	Frequency with time interval	Duration	Spot size	Tip	Mode	Distance	Outcome measures and important observations
Almeida L (1998) 780 nm	50	2 J (8 J)	NM	NM	4 (time interval NM)	0.6 min	1.3 mm^2	NM	NM	Contact	Cell growth at 4,896,144 h (manual count on Neubars chamber) Cell growth in laser treated groups was higher than respective control groups for cells grown in 5% and 10% FBS
Ameida L (2001) 780 nm 786 nm	50 30	2 J	NM	NM	4 (12 h interval)	Automatic	NM	NM	NM	NM	Cell growth at 2,4,6 days measured using Tryphan blue exclusion assay and hemocytometer Cell number higher in control versus irradiated cell in 10% FBS, 780 >670> control in 5% FBS Cell number similar or higher than that of control cells grown on 10% serum concentration Cell number higher in 786 versus controls in 5% FBS group 786 nm laser showed similar effect as 692 nm
Damante CA (2009) 780 nm	40±6.24	NM	1 W/ cm^2	3 J/cm^2 5 J/cm^2	Twice (6 h interval)	3 s 5 s	NM	NM	NM	Contact through bottom	Growth factors measured at 24 h after irradiation KGF release was similar in all groups bFGF was significantly greater (1.49 times) in groups treated with infra-red laser than 660 nm laser groups and control groups
Pansani TN (2017) 780 nm	25	NM	NM	3 J/cm^2	3 (24 h interval)	4 min	NM	NM	Continuous	NM	Evaluation done at 24 h, 4 h, 72 h postirradiation Cell viability: Higher in laser group as compared to controls and EGF group Cell migration: Laser and EGF stimulated cell migration in young cells, while only LLLT stimulated migration in elderly cells Collagen synthesis: Laser and EGF stimulated collagen production in both types of fibroblasts as compared to controls VEGF synthesis: Stimulated by EGF in both types of fibroblasts VEGF gene expression: Stimulated only by LLLT in both types of fibroblasts

LLLT – Low level laser therapy; bFGF – Basic fibroblast growth factor; VEGF – Vascular endothelial growth factor; EGF – Endothelial growth factor; FBS – Fetal bovine serum; NM – Not mentioned

The criteria used for quality assessment and the percentage of selected studies adhering to those are depicted in Figure 2.

DISCUSSION

Monolayer culture models facilitate testing of desired cells and allow researchers to test the effect of various substances or procedures on that particular cell type. They have reduced the need for animal studies and allow the analysis of tests which cannot be applied directly in the clinical environment.[17] This simple model has been used in periodontology to study the effect of laser irradiation on wound healing through cellular proliferation, viability, migration, protein synthesis, and gene expression. In our systematic review, we included studies conducted on human gingival fibroblasts using diode laser. The studies conducted on continuous cell lines were excluded as they are associated with many problems such as contamination and genotypic and phenotypic variation as compared to the primary cells.[18] Since the primary objective of the review was to evaluate the effect of LLLT on wound healing, studies in which the cells were grown in the presence of extrinsic factors and those which did not measure healing-related outcomes were also excluded from the review. This study reviewed articles published in the English language only. This may lead to the incorporation of a bias due to the exclusion of studies published in other languages.

As previously mentioned the wavelength is a major determinant of tissue absorption of laser energy. Cyctochrome c oxidase is an important photoacceptor in the monochromatic visible and infrared light spectrum and it mediates several biological responses to irradiation.[19] However, it is known that wavelengths above 900 nm are more absorbed by water than

Table 5: Irradiation parameters and results of low level laser therapy with 800-900 nm diode laser

Author	Power	Energy	Power density	Energy density	Frequency with time interval	Duration (s)	Spot size	Tip	Mode	Distance (mm)	Outcome measures and important observations
Kreisler M (2001) 810 nm	0.5 W 1 W 1.5 W 2 W 2.5 W	NM	NM	24.64-492.8 J/cm^2	1	60 120 180 240	NM	600 µ concentric circle movement	Continuous	0.5	Cell counting under light microscope at 24 h - tryphan blue staining None of the combinations resulted in cell stimulation
Kreisler M (2002) 809 nm	10 mW	NM	NM	1.96 J/cm^2 3.92 J/cm^2 7.84 J/cm^2	3 (24 h interval) 1 1	75 150 300	NM	600 µ 24° angle	Continuous	9	Cell proliferation with Alamar blue assay at 24,48, 72 h Cell count was higher in 1.96 J/cm^2, 3.92 J/cm^2 groups at 24 h Cell count was higher in 7.84 J/cm^2 group even at 48 h In groups with repeated laser treatment (1.96 J/cm^2) the cells showed increased proliferation activity at 24 and 48 h after irradiation

NM – Not mentioned

Table 6: Irradiation parameters and results of low level laser therapy with >900 nm diode laser

Author	Power	Energy	Power density	Energy density	Frequency with time interval	Duration	Spot size	Tip	Mode	Distance	Outcome measures and important observations
Hakki S (2011) 940 nm	0.3 W	NM	NM	6 J/cm^2	Once	20 s/cm^2	NM	300	C	0.5-1 mm	Proliferation: No difference Morphology: No difference mRNA expression: IGF, VEGF, TGF - β, Type I collagen (48 h), higher in test group

IGF – Insulin-like growth factor; VEGF – Vascular endothelial growth factor; TGF-β – Transforming growth factor-β; mRNA – Messenger RNA; NM – Not mentioned

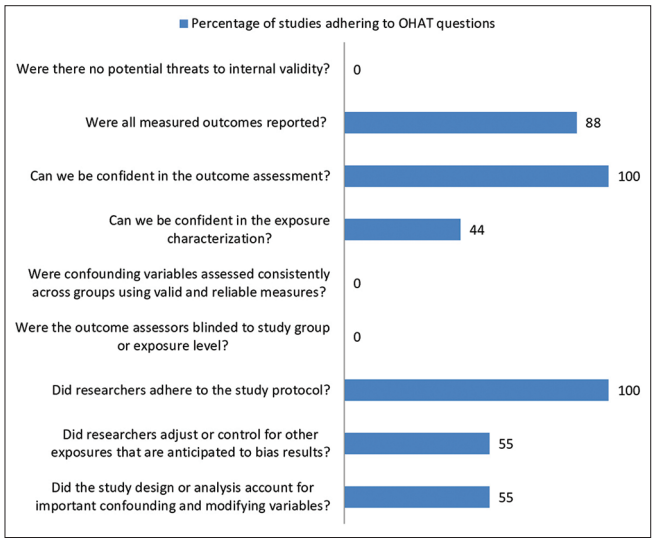

Figure 2: Quality assessment of included studies using Office of Health Assessment and Translation tool

cyctochrome c oxidase suggesting that other pathways may play a role in eliciting a biological response to irradiation in this spectrum.[3] This difference in the photoacceptor molecules was thought to influence laser dosimetry and have an effect on the tissue response and hence the authors have performed a wavelength-based analysis in this systematic review.

We found that irradiation with diode laser in the visible and infrared spectrum modulated gingival fibroblast behavior favoring wound healing. Studies by Almeida-Lopes *et al*. and Damante *et al*. have reported superior outcomes in the infrared spectrum with 780 nm laser as compared to visible spectrum in terms of cell proliferation and growth factor synthesis.[10,13] The diode lasers in the 600–700 nm spectrum were effective in the 10 mW to 30 mW power range whereas diode lasers in 700–800 nm range were effective in the 25–50 mW power range. The studies in the 800–900 nm range confirmed the biphasic response to cells to low-level laser energy as cell growth was promoted when the power setting was 10 mW whereas ≥0.5 W power setting resulted in adverse outcomes. So far, only one study has been conducted with diode wavelength >900 nm and it was found that even though LLLT did not have any effect on cell growth it stimulated secretion of growth factors and increased mRNA expression for Type I collagen. Overall, there is paucity of studies evaluating the effect of diode lasers with >800 nm wavelength. Due to lack of reporting of essential elements involved in laser dosimetry more definitive guidelines cannot be established for these ranges. Furthermore, while most studies evaluated the effect on cell viability and proliferation only a few have evaluated outcomes like growth factor synthesis, collagen production, and cellular migration.

The major limitation with these studies is the lack of comprehensive reporting of cell culture-related procedures, laser parameters, and procedures followed during the actual experiment. Hence, we were unable to summarize the exact protocol for stimulation of gingival fibroblasts in our review. For future research in this field, the authors need to incorporate the following: (1) quality control procedures and detailed reporting of all steps involved in cell culture – donor details,

method of establishing primary culture, growth media used, steps involved to prevent cross-contamination, characterization, cell doubling time, storage conditions, feeding cycles, passage used for research studies, (2) Description of each step in study protocol, for example, duration of serum starvation, change of culture medium before, during and after irradiation, methods employed to prevent the effect of ambient light, cross-irradiation, (3) Description of all parameters involved in laser dosimetry-tip used, distance from which irradiation is performed, spot size, power setting, energy setting, power density, energy density, mode of irradiation, frequency, and time gap between subsequent doses (4) Description of all outcome measures and the exact time of analysis.

Another aspect of cell culture related *in – vitro* studies we would like to highlight is the confusion regarding sample size calculation. Recently, Lazic *et al*. have highlighted the problem of pseudo-replication in *in-vitro* studies and its repercussions on the validity of study outcomes.[20] Lazic *et al*. have described criteria for true replication and have suggested that the experimental unit should be the number of independent repetitions of the experiment.[20] However, there are no guidelines for researchers regarding the minimum number of repetitions that one should conduct to achieve valid results, and hence, there is the greatest need of opinion from experts in this field.

CONCLUSION

Based on the findings of our systematic review we concluded that LLLT with diode laser stimulates human gingival fibroblasts as there as increase in cell viability, proliferation, migration, and protein synthesis in irradiated cells. While it was possible to define suitable power settings for a particular wavelength spectrum no other parameters could be defined due to lack of reporting of details. Further research in this field should focus on "good cell culture practice guidelines" as described by Hartung *et al*.[21] and reporting of all laser parameters involved in dosimetry to allow successful clinical translation of this therapy.

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Conflicts of interest
There are no conflicts of interest.

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