Near Infrared Laser Photobiomodulation of Periodontal Ligament Stem Cells

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Objective: *To determine the effect of different energy densities of near infrared diode lasers with wavelengths of 810 or 940 nm on the proliferation and survival of periodontal ligament derived stem cells (PDLSCs).*

Methods: *After isolation and characterisation, PDLSCs were cultured in clear 96-well plates. Each well was irradiated by either 810 nm (L1) or 940 nm (L2) lasers, with energy densities of 0.5, 1.5 and 2.5 J/cm^2 and an output power of 100 mW. A non-irradiated well was used as a control. Cellular viability was measured 24 hours after irradiation using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay and proliferation was measured 24, 48 and 72 hours after irradiation using trypan blue staining and counting. Propidium iodide (PI) staining was used to identify any pyknotic nuclei or nuclear fragmentation 72 hours after irradiation*

Results: *An increase in viability was observed only in the group with the 940 nm laser irradiation at energy density of 2.5 J/cm^2 ($P < 0.001$). The proliferation of cells was significantly increased in the group with 940 nm laser irradiation at energy density of 2.5 J/cm^2 at all the time points examined in comparison to other groups ($P < 0.001$). PI staining showed no change in cell nuclei in any of the groups.*

Conclusion: *Irradiation of PDLSCs with a 940 nm laser at an energy density of 2.5 J/cm^2 could promote efficient cell proliferation.*

Key words: *periodontal ligament, photobiomodulation, stem cells*

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Treatment and control of the inflammatory process and regeneration of lost periodontal supporting structures, especially in severe cases, are among the challenges facing periodontists. Different surgical and nonsurgical methods have been proposed for periodontal regeneration, with varying success rates[1,2]. New regenerative therapies and tissue engineering methods to improve the clinical outcome using stem cells in regenerative tissue engineering of the periodontium have attracted attention in recent years[3,4].

The presence of stem cells in dental and periodontal tissues has created a new approach to repairing dental and periodontal defects[3,5]. In this approach, the differentiation capacity of these cells in oral lesions can be exploited by isolating and replicating them and transferring them to the lesion site. Periodontal ligament (PDL) derived stem cells (PDLSCs) have been reported to have osteogenesis, angiogenesis and cementogenesis properties and have also been shown

to successfully regenerate PDLs[4,6,7]. They also appear to have a better regenerative capability of differentiating into periodontal tissues compared with dental pulp stem cells (DPSCs) and periapical follicle stem cells (PAFSCs) and are considered the best candidates for stem cell–based regenerative treatments in periodontology[4]. These cells have appropriate tissue acceptance and have recorded effective results in the treatment of periodontal defects in in vitro and in vivo studies[4,8-11].

Laser technology has drawn a great deal of attention and found many applications in various areas of dentistry in recent years[12]. Low level laser (light) therapy (LLLT) employs visible (generally red) or near-infrared (NIR) light generated from a laser or light emitting diode (LED) system with low power and energy densities. This kind of irradiation with its non-thermal and non-invasive nature has been shown to be effective in treating diverse injuries or pathological conditions in medicine[13]. The process is now better known as photobiomodulation (PBM) and is characterised by the ability to induce photobiological responses at cellular levels[14].

The mechanism of PBM at the cellular level has been attributed to the absorption of monochromatic visible and NIR radiation by photoreceptors, most importantly the components of the cellular respiratory chain and changes in cellular adenosine triphosphate (ATP) levels[15,16].

Laser has been previously used in periodontology for its antibacterial and anti-inflammatory effects in the treatment of periodontal and peri-implant diseases[17-20]. PBM has also been found to be able to modulate the immune response and reduce chronic inflammation[19,21-23], and can therefore potentially facilitate periodontal tissue repair.

At a cellular level, several studies have shown the effects of phototherapy on different cells, indicating that PBM improves the proliferation of cells without causing cytotoxic effects[24-26]. Irradiation conditions and parameters such as wavelength, power and energy densities and even the tissue being irradiated can influence the clinical outcomes of PBM[13,27,28].

According to recent systematic reviews, great heterogeneity can be observed in the methods and parameters of light irradiation, and only a few studies on infrared irradiation and PDLSCs are available[29-31]. Further research is still needed to identify the optimal characteristics of the PBM setting on these cells as a basis for future translation of use of this wavelength into clinical practice[24].

In a study on this cell line, Wu et al[32] showed that low-power 660 nm, 70 mW red laser can enhance the proliferation and osteogenic differentiation of human PDL cells via cyclic AMP (cAMP) regulation. Soares et al[33] also studied the effect of 660 nm, 30 mW laser irradiation on the proliferation rate of human PDLSCs (hPDLSCs) and the cells were irradiated at 0 and 48 hours using two different energy densities (0.5 J/cm², 1.0 J/cm²). They found that PBM using 660 nm light and an energy density of 1.0 J/cm² has a positive stimulatory effect on the proliferation of hPDLSCs[32,33]. Yamauchi et al[34] also evaluated the effects of a high-power, red LED light device with a wavelength of 650 nm at a power density of 1100 mW/cm^2 and a total irradiance of 200 mW/cm^2 on hPDLSCs. They tested energy densities ranging from 0 to 10 J/cm^2 to determine the optimal dose and observed a significant increase in PDLSC proliferation and osteogenic differentiation through the activation of ERK1/2 with 8 J/cm^2[34].

Since previous studies have mostly focused on the red wavelength and considering the good penetration depth of infrared light in periodontal tissues, in the present study, we sought to evaluate the effect of two NIR diode lasers with wavelengths of 810 and 940 nm which are more routinely used in dental practice. The effect of different energy densities (fluence) on the proliferation and survival of PDLSCs was evaluated. The present study seeks to add to the existing evidence and prove useful in future cell-based regenerative periodontal therapies.

Materials and methods

Cell isolation and culture

The remaining PDL tissue was scraped from the middle third of the root surface of two fully erupted third molars extracted for orthodontic reasons in one patient. The obtained periodontal tissue was cultured in a microplate containing Dulbecco's Modified Eagle Medium (DMEM, Gibco, Waltham, MA, USA) and 15% foetal bovine serum (FBS, Gibco), then enzymatically digested for 1 hour at 37°C in a solution of 3 mg⁄ml collagenase type I (Sigma-Aldrich, St Louis, MO, USA) and 4 mg/ml dispase (Gibco).

Characterisation of PDL derived mesenchymal stem cells

The mesenchymal nature of cells was confirmed by evaluating surface markers of CD90, CD105 and CD45 using the flow cytometric method[35]. Positive CD90 and CD105 mesenchymal markers and a negative result for CD45, which is a haematopoietic marker, indicated that

Table 1 Laser irradiation parameters.

Parameter (unit)	Value	
Centre wavelength (nm)	L1: 810	L2: 940
Operating mode	Continuous wave	Continuous wave
Radiant power (mW)	100	100
Beam spot size diameter (cm)	0.8	0.8
Beam spot size at target (cm^2)	0.5	0.5
Irradiance at target (mW/cm^2)	200	200
Energy density((J/cm^2)	0.5, 1.5, 2.5	0.5, 1.5, 2.5
Exposure duration (s)	3, 8, 13	3, 8, 13

the separated cells were mesenchymal cells. After the third cell passage, the cells were detached from the base of the plates by adding trypsin. The cell suspension was counted and 10^5-10^6 cells were added to each vial. The vials were then filled with 1 ml of phosphate buffer solution-bovine saline albumin (PBS-BSA) 3% solution (Sigma-Aldrich) and the suspension was centrifuged at 2000 rpm for 5 minutes. For each marker, a test and control isotype vial were used. Antibodies (Abcam, Cambridge, MA, USA) were added; accordingly, CD45 with a concentration of 1:200 was incubated (Binder, Tuttlingen, Germany) for 30 minutes at room temperature, and CD90 and CD105 markers with a concentration of 1:50 were incubated for 45 minutes at 37°C. The Rabbit isotype control was incubated for 30 minutes at a concentration of 1:200 at room temperature, then PBS was added to each sample to reach a volume of 1 ml. The suspensions were then centrifuged at 2000 rpm for 5 minutes (Hettich, Tuttlingen, Germany). The secondary antibody was added with a concentration of 1:4 and incubated for 45 minutes at 37°C. A volume of 1 ml was then reached by adding PBS and centrifuged once more at 2000 rpm for 5 minutes. The cells were washed again with PBS and centrifuged. At the last stage, the cell sedimentation was turned into a suspension with 4% paraformaldehyde and stored at 2°C to 8°C until it was read under the flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA).

The differentiation ability of the isolated cells was also evaluated. For this purpose, the cells were cultured in osteogenic medium of DMEM containing 10% FBS (Gibco) and 100 nM dexamethasone, 10 mM beta-glycerol-phosphate and 50 µg/ml ascorbic acid (all Sigma Aldrich) and adipogenetic differentiation medium containing DMEM with 10% FBS (Gibco, Paisley, UK), 66 nM insulin, 0.2 mM indomethacin, 100 nM dexamethasone (all Sigma Aldrich) and 0.5 mM IBMX (Pepro Tech, Rocky Hill, NJ, USA) for 21 days. After this time, staining with Alizarin red and Oil red (Sigma Aldrich) showed their successful osteogenic and adipogenetic differentiation.

Photobiomodulation therapy

The laser probe was adjusted perpendicular to each plate and the cells were irradiated from underneath each well to completely cover the area of a single well with a diameter of 8 mm. The third passage cells were cultured in clear 96-well micro titre plates at a density of 5×10^3 cells. There was one empty well between the seeded wells to prevent unintentional dispersion of light between wells during irradiation. Three energy densities, namely 0.5, 1.5 and 2.5 J/cm^2, were applied using two different laser devices of 810 nm (Picasso, AMD Lasers, West Jordan, UT, USA) as L1, and 940 nm (Epic 10, Biolase, CA, USA) as L2, with 400-micron tips and a continuous wave output power of 100 mW. The wells were irradiated from a distance of 15 mm for 2.5, 7.5 and 12.5 seconds, respectively (Table 1). The output power was 100 mW and checked with a power meter (Ophir Nova II, Ophir Optronics Solutions, Jerusalem, Israel) before irradiation. A continuous wave mode was used and a single session of laser irradiation was performed. The laser groups were compared with a control group without any laser irradiation. All tests were performed in triplicate and repeated twice.

Viability assessment

The 5×10^3 cells were placed into 96-well plates and grown for 24 hours, then the extracts were added to cell cultures. MTT colorimetric assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (Sigma Aldrich) was used to evaluate cell viability, then 24 hours after laser irradiation, 10 ml MTT, 90% αMEM and 15% FBS were added to cells and incubated for 3 hours. At this time, formazan crystals could be seen under an inverted microscope. Viable cells with a normal functional mitochondrial enzyme of succinate dehydrogenase changed water-soluble MTT dye to insoluble

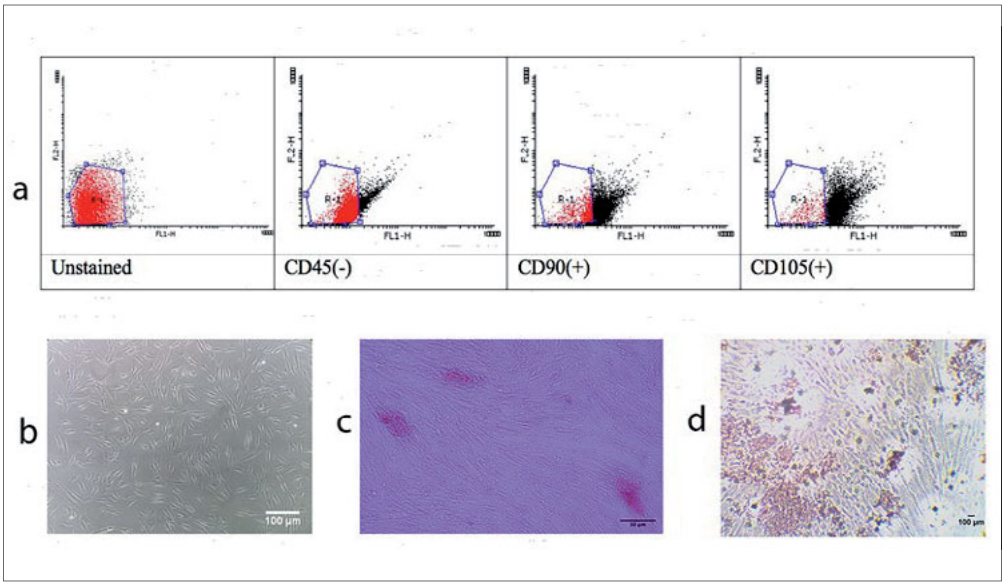

Fig 1 **(a)** Distribution of expression of CD45, CD90 and CD105 biomarkers. The black dots indicate the intensity of expression of the biomarkers and the red dots indicate the severity of the lack of biomarkers on the surface of the cells isolated from the periodontal ligament. **(b)** PDLSC with a spindle-shaped appearance. **(c)** Optical microscope photographs showing mineral deposition 21 days after culture in osteogenic medium and Alizarin red staining. **(d)** Oil red staining showing adipogenic differentiation of cells after 21 days.

purple formazan. After completion of incubation, the supernatant was removed and 100 ml dimethyl sulfide was pipetted to dissolve the crystals. An ELx808 microplate reader (BioTek, Bad Friedrichshall, Germany) was used to measure absorbance at 540 to 690 nm. The results were reported as percentages.

Proliferation rate assessment

To evaluate cell proliferation, cultured cells were stained with trypan blue 24, 48 and 72 hours after irradiation. They were counted using a neubauer chamber and their proportion to cell numbers at baseline were reported.

Effect of laser irradiation on cell nuclei

Cells were stained with propidium iodide (PI) to identify any pyknotic nuclei and nuclear fragmentation 72 hours after irradiation under a phase contrast microscope (BX51, Olympus, Tokyo, Japan).

Ethical considerations

The project was approved by the university's ethics committee (IR. ZUMS.94.5.11-7346). The teeth used for isolation of the cells were indicated to be removed for orthodontic reasons. The patient was informed about the study and signed an informed consent.

Statistical analysis

Data were analysed using a Student *t* test and three-way analysis of variance (ANOVA) with a Tukey post-hoc test and values were expressed as mean ± standard deviation (SD)/standard error (SE). All statistical analyses were performed at a significance level of 0.05 using R software, version 3.3.3 (R Core Team, Vienna, Austria) and SPSS version 16 (SPSS Statistics, IBM, Chicago, IL, USA).

Results

Characterisation of PDLSCs

The dot plot in Fig 1a displays the mesenchymal stem cell identifier markers. CD45 was at a minimum and negative and CD90, CD105 were positive, indicating the mesenchymal nature of isolated cells. PDLSCs were also differentiated into osteoblasts forming mineralised tissue after 21 days of culture in osteogenic medium and adipose tissue differentiation was also observed after 21 days by Oil red staining (Figs 1b and c).

PDLSC viability

A three-way ANOVA was used to compare the effects of laser, energy density and time on the MTT viability results based on percentages compared to controls.

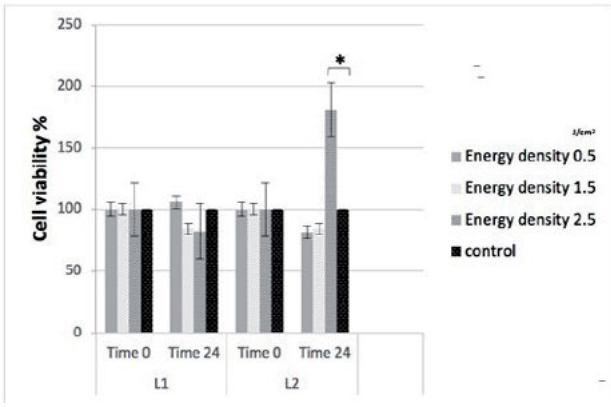

Fig 2 Effects of L1 810-nm and L2 940-nm laser irradiation on cell viability based on MTT assay after normalising to the control group.

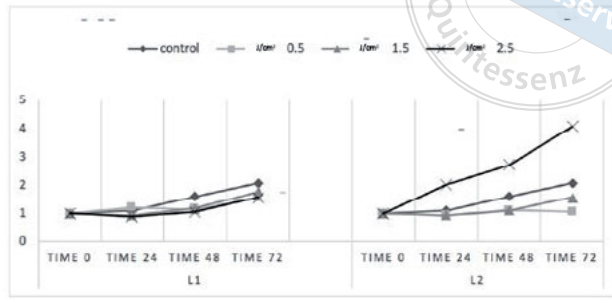

Fig 3 Effect of laser stimulation on PDLSC proliferation after 24, 48 and 72 hours based on trypan blue counting.

Table 2 Mean ± SD of proliferation in terms of time, energy density and type of laser.

		Control	L1			L2		
Energy density (J/cm^2)			0.5	2.5	1.5	0.5	1.5	2.5
Time point	T0	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
	T24	2.02 ± 0.18	0.93 ± 0.06	0.92 ± 0.14	0.90 ± 0.06	0.93 ± 0.06	1.20 ± 0.14	1.09 ± 0.08
	T48	2.71 ± 0.34	1.09 ± 0.38	1.12 ± 0.17	1.05 ± 0.09	1.19 ± 0.15	1.14 ± 0.17	1.59 ± 0.24
	T72	4.07 ± 0.27	1.57 ± 0.18	1.05 ± 0.08	1.57 ± 0.06	1.76 ± 0.10	1.55 ± 0.09	2.08 ± 0.04

The results showed that laser and energy density affect survival rate ($P < 0.05$), and that the effect of laser is dependent on time and energy density ($P < 0.05$). After 24 hours, an increase in cellular viability was observed only with the 940 nm laser with an energy density of 2.5 J/cm^2. This increase was statistically significant compared to the other settings of L2 and L1 and the control group ($P < 0.001$). None of the other comparisons between different energy densities of the L1 group were significant at the 0.05 level (Fig 2).

PLDSC proliferation

The mean ± SD of proliferation in terms of time, density and lasers are shown in Table 2. The highest mean proliferation was seen in the L2 group and the energy density of 2.5 J/cm^2 (Fig 3).

The comparison of proliferation between different energy densities in the L1 group did not show any statistically significant results. In the L2 group, the energy density of 2.5 J/cm^2 showed higher proliferation, with statistically significant differences at 24, 48 and 72 hours compared to the other energy densities in this group ($P < 0.001$).

L2-0.5 and L2-1.5 showed a statistically significant increase at 72 hours ($P < 0.001$) but were still lower compared to the control group. When comparing proliferation in the same energy densities between the L1 and L2 group, the L2-2.5 J/cm^2 group also had statistically significant superior proliferative results compared to the L1-2.5 J/cm^2 group at all time points ($P < 0.001$). The 1.5 J/cm^2 energy density groups showed no statistically significant difference at any of the time points. The results of the comparison of the 0.5 J/cm^2 L1 and L2 groups showed a statistically significant difference only after 72 hours, when the L1 group had higher proliferative results (1.05 versus 1.55 folds compared to baseline [T0]), which were both lower compared to the amounts of cell proliferation in the control group (Table 2).

Effect of laser irradiation on cell nuclei

PI-stained cells were carefully evaluated. No pyknotic nuclei or nuclear fragmentation was observed in any of the groups (Fig 4).

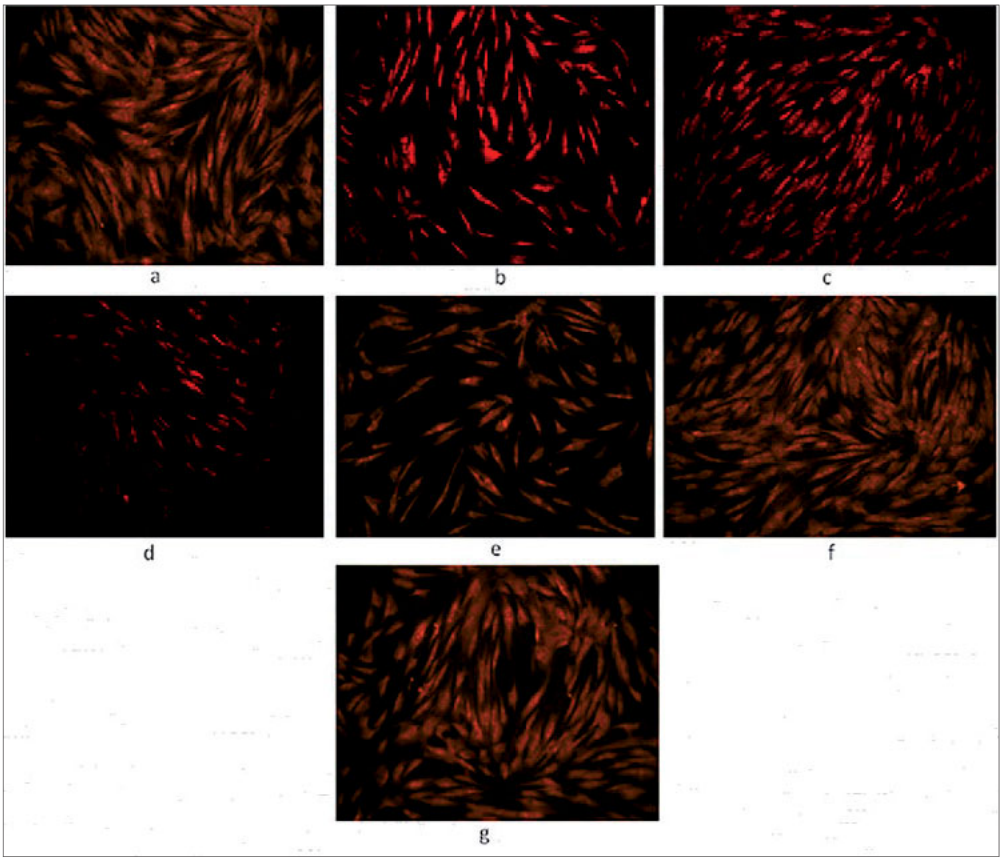

Fig 4 PI-stained cells from the different irradiation groups and nonirradiated control group observed under a phase contrast microscope. No pyknotic nuclei or nuclear fragmentation were observed. **(a)** 810 nm, 0.5 J/cm^2; **(b)** 810 nm, 1.5 J/cm^2; **(c)** 810 nm, 2.5 J/cm^2; **(d)** 940 nm, 0.5 J/cm^2; **(e)** 940 nm, 1.5 J/cm^2; **(f)** 940 nm, 2.5 J/cm^2; **(g)** Control (100x magnification).

Discussion

In the present study, the effect of different energy densities of two common NIR laser wavelengths in dentistry on the viability and proliferation of PDLSCs were comparatively evaluated. These wavelengths have a favourable penetration depth and the potential to be used as adjunctive tools in clinical practice. These wavelengths are much less studied and further investigation is needed to find an optimal setting for stimulating these cells using them.

According to our results, 940 nm laser with an energy density of 2.5 J/cm^2 (100 mW, CW) showed a better result compared to the control group, 810 nm laser with a 0.5 J/cm^2 setting also showed better results compared to its 1.5 and 2.5 J/cm^2 settings on PDLSC viability and proliferation. The results clearly demonstrate the sensitivity and importance of laser settings and wavelength. To our knowledge, this study is the first to compare diode lasers with these NIRs wavelengths on PDLSCs. Wavelengths in the 600 to 700 nm range are most often chosen to treat superficial tissue, and NIR is chosen for deeper seated tissues due to the longer optical penetration distances through tissue[14].

The most important law in photobiology is that photons of different wavelengths are absorbed by specific chromophores located inside cells or tissues. Lasers of 810 and 940 nm are well absorbed in tissue chromophores of haemoglobin and melanin. At a cellular level, Cytochrome C oxidase (CCO) has one absorption band in the red wavelength region around 660 nm and another in the NIR spectrum around 800 nm. The absorption bands of CCO are weaker for wavelengths greater than 900 nm. Other alternative chromophores such as water and ion channels have also been identified or proposed for these wavelengths and need to be elucidated further[14, 27].

In vitro studies on PBM have shown that the effect of lasers on cells is variable as it is dependent on many parameters[14,36]. The existence of a different 'window specificity' for every wavelength and energy dosage was postulated by Karu et al[37] in 1990. The results can be influenced by not only the wavelength but also the total dose of irradiation, application mode (pulsed or continuous), irradiation time and number and frequency of laser therapy sessions[13,27,28]. Since studies on PBM

do not have uniform methodological designs, it is complicated to compare the results with other wavelengths used in previous studies.

To our knowledge, only a few studies have been conducted into the 940 nm laser wavelength and its effect on proliferation and differentiation of mesenchymal stem cells. Jawad et al[38] studied the effect of different powers of 100, 200 and 300 mW with continuous wave mode of 940 nm diode LLLT on a human foetal osteoblast cell line cultured in 96-well plates and uniformly irradiated them for 3 and 6 minutes for a period of up to 7 days. The energy densities applied were much greater compared to the current study. The group exposed to 100 mW received 22.92 or 45.85 J/cm^2; the second group that was exposed to 200 mW received 45.85 or 91.79 J/cm^2; and the third group of 300 mW exposure received 68.78 or 137.57 J/cm^2 of energy. A significant increase in proliferation after 7 days was observed in all groups; however, at similar evaluation time points to those in the present study (1 and 3 days), no significant improvement in proliferation results was reported except for the group irradiated at 100 mW for 6 minutes which had the same output power as the one used in the present report. The researchers concluded that 300-mW irradiation significantly increased the amount of cell proliferation. By contrast, the 100 and 200 mW groups showed significantly better results only in cell differentiation[38].

The use of the lower laser powers and longer exposure time of LLLT was considered better than the higher power settings in improving osteogenic differentiation. Longer exposure times and repetition of irradiation might have also improved the effects observed with lower energy densities used in the present study.

A stimulatory effect of 940 nm laser was also reported in an animal study that investigated the radioprotective features of 940 nm laser on the lifespan and absolute counts of blood cells of gamma-irradiated mice[39]. Energy densities of 3, 12 or 18 J/cm^2 were tested at a fluence of 3 J/cm^2 and demonstrated interesting radioprotective features[39]. This setting significantly prolonged the lifespan of gamma-irradiated mice and the white blood cell, lymphocyte and neutrophil counts were higher in this group on day 12 after gamma irradiation[39]. The effective energy density recorded was quite similar to the results observed in the present study[39].

Only a few studies have been conducted into laser phototherapy of PDLSCs, and the previous studies were conducted using wavelengths different to those employed in the current study. As mentioned, Soares et al[33] studied the effect of laser therapy on PDLSCs with two sessions of 660 nm diode laser treatment in 48 hours at energy densities of 0.5 and 1.0 J/cm^2 for 16 and 33 seconds, respectively, and found that 1.0 J/cm^2 radiation recorded better results in terms of cell survival and proliferation compared to the 0.5 J/cm^2 laser and control groups at 48 and 72 hours[33]. In another study, Wu et al[32] provided PDLSCs with laser radiation from a 660-nm diode with energy densities of 1, 2 and 4 J/cm^2 for 66, 132 and 264 seconds, respectively, and evaluated them on days 1, 3 and 5, respectively. Irradiation of 2 J/cm^2 resulted in a significant increase in cell proliferation on days 3 and 5, and only two energy densities (2 and 4 J/cm^2) led to increased osteogenic differentiation of this group of cells[32]. These results demonstrate cell proliferation effects that are generally consistent with the findings of the present study, where a positive effect of a single session of laser irradiation was observed. Repetition of this single session of irradiation could result in an accumulative effect of laser energy and be useful in future in vivo studies.

In a recent study, Paschalidou et al[40] studied the effect of a similar single session of 940 nm laser irradiation with a 200-mW continuous mode on stem cells from the dental pulp of deciduous teeth (SHED) at energy fluences of 4, 8 and 16 J/cm^2. Their results indicated a statistically significant increase in proliferation based on MTT results after 48 and 72 hours in all energy densities of this wavelength; however, 8 J/cm^2 did not show a statistically significant increase at 24 hours and reported the lowest MTT results compared to the other two settings[40]. On the other hand, 8 J/cm^2 had the best effects on osteogenesis gene expression and biomineralisation[40]. This clearly shows how sensitive the results of PBM therapy can be and that even the same energy densities and laser parameters may influence proliferation and differentiation of cells in a different manner which needs to be investigated more thoroughly.

According to the results of the present study, the 810-nm laser with the energy densities and parameters used did not have a statistically significant positive effect on cell proliferation. Some studies have reported controversial findings regarding the effect of this wavelength on stem cells. Soleimani et al[41] evaluated 810-nm diode laser radiation on the proliferation of bone marrow mesenchymal stem cells and differentiated them into neuronal and bone cells. Radiation of 2, 3, 4 and 6 J/cm^2 was performed for 12, 18, 24 and 36 seconds respectively for three sessions at 1, 3 and 5 days after incubation, and MTT analysis for cell viability was performed on day 7[41]. The results showed that all of the above energies except 6 J/cm^2 resulted in a significant increase in cell viability[41]. Kreisler et al[42] also evalu-

ated the effect of low-level diode laser irradiation on human PDL fibroblasts with an 809-nm laser and three fluences of 1.96, 3.92 and 7.84 J/cm^2 were applied for 75, 150 and 300 seconds, respectively. The effect of one, two and three laser irradiation sessions at 24-hour intervals on cell proliferation was evaluated using Alamar Blue assay 24, 48 and 72 hours after irradiation, reported in relative fluorescence units (RFUs)[42]. Unlike in the present study, these researchers observed that at each energy level, these cells had a higher rate of proliferation than the non-irradiated cells for up to 72 hours[42]. When the laser treatments were repeated 24 and 48 hours after the first irradiation, the RFU values at days 1 and 2 were considerably higher, which the authors suggest may have been due to the repeated laser treatment or the longer incubation time prior to the first measurement[42]. We might have also recorded a better outcome with successive treatment sessions.

Wang et al[27] recently compared the laser irradiation of 810- and 980-nm diodes with adipose-derived stem cells. They found that the mechanisms of action of 810- and 980-nm lasers appear to have significant differences: 980 nm seems to rely on the activation of heat or light gated ion channels, whereas activation of CCO in mitochondria by 810 nm continues to be the most accepted mechanism[27]. The 980-nm laser with energy densities as low as 0.3 J/cm^2 resulted in ATP levels 40% higher than the control group but the 810-nm laser needed higher energy densities, with 3.0 J/cm^2 reporting the best results (20% higher than the control group)[27].

Based on this study and the present results, it seems that energy densities higher than those used in the present report are probably necessary to have biostimulatory effects with the 810-nm laser, and the effect of higher energy densities needs to be evaluated further in future studies. The differences in the results of the aforementioned studies and the present study and the difference observed between the two studied wavelengths are due to differences in irradiation conditions and characteristics of each wavelength that can influence the responses being measured. It must be noted that cell-based in vitro studies are highly technique sensitive. Further investigations are needed to elucidate the effect of different irradiation settings of these wavelengths on PDLSCs and the underlying molecular mechanisms. The differentiation of irradiated cells also needs to be investigated in future studies. Moreover, further clinical studies are needed for correct translation into clinical practice since it appears PBM can be considered an important future adjunctive tool in regenerative periodontal therapy and tissue engineering involving stem cells.

Conclusion

Based on the results of the present study, a 940-nm diode laser with an energy density of 2.5 J/cm^2 recorded better results compared to the same dose of 810-nm laser and controls and increased the viability and proliferation of PDLSCs.

Conflicts of interest

The authors declare no conflicts of interest related to this study.

Author contribution

Dr Mohammd Ayyoub RIGI-LADEZ contributed to the study design, data preparation and funding acquisition; Dr Seyedeh Sareh HENDI performed the data analysis and prepared the draft of the manuscript; Drs Alireza MIRZAEI and Reza FEKRAZAD contributed to the study design, supervision and editing of the final manuscript; Dr Leila GHOLAMI contributed to the study design and conducting of the study, data analysis and final revision.

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