International Journal of Dentistry and Oral Science (IJDOS)
ISSN: 2377-8075

Evaluation of the Effect of Near Infra-red Photobiomodulation on Buccal Fat Pad-Derived Stem Cells

Research Article

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Abstract

Background: Laser photobiomodulation can be a useful adjunctive method in tissue engineering in enhancement of proliferation and differentiation of mesenchymal stem cells. Buccal fat pad-derived stem cells (BFPSCs)has been introduced as a promising source for craniofacial bone tissue engineering. Current study aimed to evaluate the effects of near infra-red photobiomodulation on (BFPSCs)behavior.

Methods: After cell isolation from a surgically excised sample of human buccal fat pad, third passage cells were irradiated twice daily for three consecutive days. Irradiation was performed with 6 different laser settings by two modes of continuous and pulsed (50% duty cycle) and energy densities of 3 and 6 J/cm2 and two different output powers (0.1W and 0.3W) using a 940nm laser. Anon-irradiated group served as control. The test was repeated in three different daysand every time cell viability was evaluated by MTT assay at intervals of 24, 48 and 72h. Based on viability results a setting was chosen for evaluation of osteogenic differentiation by Alizarin red staining.

Results: The highest proliferation was observed at irradiation of 3J, 0.3W, pulsed at 24h and 48h, however, after 72h the highest proliferation rate was related to 6J ,0.1W, pulsed. Considering the effect of 3J 0.3W pulsed modeon cell proliferation at an earlier time, this setting was used for osteogenic differentiation assay. Both microscopic and quantitative analysis of Alizarin Red staining showed that cells subjected to the 3J 0.3W Pulsed irradiation also resulted in an increase in mineralization of BFPSCs cultured in osteogenic induction medium compared to the negative control ($p<0.05$).

Conclusion: According to the results of this study a pulsed mode of irradiations showed better viability results. Although the 3J/cm2 0.3W, Pulsed irradiation showed significantly better results for viability and proliferation, however no statistically significant effect was observed in osteogenic differentiation.

Keywords: Buccal Fat Pad-Derived Stem Cells; Photobiomodulation: Cell Proliferation: Cell Differentiation.

Introduction

Evidence of the use of Photobiomodulation (PBM) by humans goes back to thousands of years ago in ancient civilizations where they used sunlight sometimes combined with plants for treatment of skin diseases [1]. Years later the Nobel Prize of Physiology and Medicine was awarded to Nils Finsen for his invention in using arc lamps to treat cutaneous tuberculosis and prevention of scarring from smallpox [2, 3]. Low level laser (light) therapy (LLLT) or more recently regarded as photobiomodulation is a nonthermal process that its biostimulatory effect was accidently discovered by Endre Mester in 1960 [4, 5]. Ever since various wavelengths of

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Received: October 24, 2020
Accepted: December 01, 2020
Published: December 07, 2020

Citation: Leila Gholami, Saeid Afshar, Roghayeh Mahmoudi, Ali Asghar Arkian, Gilda Parsamanesh, Maryam Rezai Rad, Kaveh Baghaei. Evaluation of the Effect of Near Infra-red Photobiomodulation on Buccal Fat Pad-Derived Stem Cells. *Int J Dentistry Oral Sci.* 2020;7(12):1164-1171. **doi:** http://dx.doi.org/10.19070/2377-8075-20000231

laser or LED light have been tested for their photobiomodulatory effects in many *in vitro* and *in vivo* studies.

Previous studies on the biomodulatory effects of Laser therapy-have reported positive effects on cell proliferation, tissue regeneration and anti-inflammatory potentials [6-9]. However, due to inconsistencies in laser settings, cell types and treatment protocols and lack of well controlled clinical trials of photobiomodulation in different fields it has not yet become practical in the medical field and a clear protocol or guideline does not exist [6, 9, 10].

A biphasic dose response has been observed in many reports described as Arndt-Schulz curve showing that only irradiation doses within a specific range may have biostimulatory results and very low or very high doses may even lead to inhibitory or negative results [11]. Irradiation factors other than energy density or fluence (J/cm^2) which influencing the results of photobiomodulation. include power density, irradiation durations and number of applications and also, the continuous or pulsed mode of emission. The mechanisms behind PBM and the results observed on tissue and cell has also been an interesting topic of many researches [5, 12] Some accepted biological mechanism of PBM include the primaryabsorption by cellular chromophores such as enzymes likemitochondrial cytochrome c oxidase, porphyrin and flavoproteins and membrane photoreceptors. Secondary effects of this photon absorption may result in increases in ATP, reactive oxygen species (ROS), increase in nitric oxide, and modulation of calcium levels. Tertiary effects include activation of transcription factors resulting in changes in cell survival, proliferation and migration, and new protein synthesis [10, 13, 14].

Tissue engineering has revolutionized oral and maxillofacial and periodontal regenerative therapies and many different stem cell sources such as cells with craniofacial and dental origins seem to be promising for this novel field of cell therapy treatments [15-17]. Methods that are capable of increasing cell survival and proliferation and differentiation used as adjunctive either in *ex-vivo* expansion of cells or *in-vivo* on the treated area is of great value for regenerative medicine. PBM is a suitable adjunctive tool for this purpose. Despite the great number of researches on the effect of PBM on cell such as mesenchymal stem cells (MSC) used in tissue engineering there are still no clear optimal parameters and irradiation protocolsdefined. These effects seem to depend on the cell type and the irradiation settings [6, 7].

In search for ideal and easily accessible sources of stem cells for craniofacial tissue engineering Farre-Guasch et al., isolated adipose-derived stem cells (AdSCs) from Bichat's fat pad or the buccal fat pad (BFP). This is a highly vascularlized fat mass which has been an attractive graft, in oral surgery for the repair of bone and periodontal defects. It is located on both sides of the face between the buccinator muscle and other superficial muscles and is easily accessible through the oral cavity with minimal discomfort and donor site morbidity [18]. These cells are phenotypically similar to AdSC from abdominal subcutaneous adipose tissuein cell yield, morphology, and multilineage differentiation [18-20]. They have also been reported to proliferate faster and is more prone to producing colonies compared to other AdSCs. These cells were demonstrated to be capable of reliably forming engineered bone in an invivo study by Shiraishi et al., [21]. The clinical application of these cells in bone regeneration has also been positively reported in some studies [22-24]. Regarding the effect of PBM on adipose derived stem cells(BFPSCs), there are a few investigating the effects of phototherapy on proliferation and differentiation of these cells with varying light wavelengths and irradiation parameters [25-30]. The combination of laser photomodulation and adipose stem cells has been also studied for many different clinical applications with successful positive outcomes [31, 32]. Showing a promising potential for their applicability. However, up to our knowledge the effect of PBM of BFP -ASChas not been investigated previously and the effect of different pulsed and continuous irradiation settings of the near infra-red (NIR) wave length has not been investigated previously.

The near infra-red laser was chosen in this study since it has a more deeper penetration depth compared to red lasers making it a suitable choice for future translation of this technique to clinical practice in craniofacial bone tissue engineering. However, determination ofideal irradiation parameters is important to the standardization of a PBMfor achieving favorable results on proliferation and differentiation of cells.

Therefore,In the present study we aimed to comparatively evaluated the effect of different irradiation parameters ofpulsed and continuous 940nm near infra-red diode laser PBM on proliferation and osteogenic differentiation of Buccal fat pad derived Adipose stem cells.

Methods

BFPSCs isolation and characterization

BFP tissues were collected froma healthy individual who needed maxillofacial surgery after obtaining an informed consent. The isolated tissue (approximatly 10mm 3) was transferred to the cell culture laboratory in chilled phosphate buffer solution (PBS) (Life Technologies, Carlsbad, CA, United States). Then, tissues were minced and digested in 3 mg/mL collagenase type I (Life Technologies, Carlsbad, CA, United States) for one hour at 37 °C. The suspended cells, were cultured in Dulbecco's Modified Eagle Medium (DMEM, GIBCO BRL, Grand Island, NY), 15% fetal bovine serum (FBS) (Life Technologies, Carlsbad, CA, United States), and 1% Penicillin-Streptomycin 10,000 u/ml (Life Technologies, Carlsbad, CA, United States). Primary cells were passed upon confluency using 0.25% trypsin-EDTA (Life Technologies. Carlsbad, CA, United States). Cells at passage two were characterized for mesenchymal stem cell surface markers. Briefly, cells were trypsinized andthen, they were incubated in darkness for one hour at 4 °C with specific antibodies of CD90, CD73, CD105 markers as mesechymal stem cell markers and CD34, CD45 markers as a hematopoietic cell marker. (EXBIO Praha,Vestec, Czech Republic) at 2 µg/ml for each. Finally, expression of these molecules were analyzed by FACSCalibur Flow cytometer (Becton Dickinson, San Jose, CA), and the data were analyzed using FlowJo (Tree Star, Ashland, OR) software.

Laser PBM

BFPSCs were seeded in 96-well plates at a density of 2×10^3 per well and cultured in DMEM, 15% FBS, 1% Penicillin-Streptomycin. Then, the next day, cells were subjected to irradiation according to Table 1.

Table 1. Irradiation Parameters for MTT Assay.

Wavelength	940 nm							
Mode of irradiation	Continuous Wave (CW) Pulsed (P) (50%Duty cycle) – 20millisec pulse duration							
Power	0.1W 0.3 W							
Energy density	6J/cm^2				3J/cm^2			
Laser irradiated groups	CW0.3	CW0.1	P0.3	P0.1	CW0.3	CW0.1	P30.3	P30.1
Average Power(W)	0.3	0.1	0.3	0.1	0.3	0.1	0.3	0.1
Peak Power(W)	0.3	0.1	0.6	0.2	0.3	0.1	0.6	0.2
Irradiation Time(sec) 96 wells	7	19	7	19	3	10	3	10
Beam spot size at target (cm^2)	0.32 Cm^2							
Number and frequency of PBM	2 x daily for 3 days (every 12h)							

A 940nm InGaAsP Semi-conductor diode laser (Biolase, USA) was used in continuous and pulsed mode of irradiation with two energy densities of 3 and 6 J/cm^2 and out put powers of 0.1 or 0.3W. Cells were irradiated from underneath the wells by fixing the laser handpiece in a position perpendicular to the bottom of the plates in a distance to create a spot size equal to the diameter of a single well. The transmitted output power through the transparent bottom of plates was measured using a power meter (Nova II, Ophir photonics) to be sure of the correct power reaching the cells from the base of the plates. Cells were seeded every other well and wells not being irradiated were covered by dark cardboard during laser irradiation to avoid unintentional dispersion of light between the wells. The control groups were processed under the same conditions, except without laser irradiation. Irradiation was performed every 12h for three consecutive sessions. For each group 6 wells were considered and the test was repeated three times (n=18) for better reliability and reproducibility of the results.

The best setting was chosen for evaluation of its effect on osteogenic differentiation of cells for this test cells were cultured in 6 well plates and a therapy handpiece was used to irradiate each well from underneath. This hand piece was also fixed perpendicular to the plates at a distance(1cm) creating a spot size equal to the size of a single well of a 6 well plate (9.6cm^2). The cells were seeded in a osteogenic induction medium irradiated with 3J/cm2 0.3W pulsed mode for 96sec every 12h for three consecutive days. (OIM-3). A group with no irradiation served as a positive control (OIM-0) and we also cultured cells in non-osteogenic medium without any laser irradiation as a negative control (C_).

Proliferation assay

Immediately after irradiation, cells were returned to incubator providing 50% CO_2 and 37°C. After 24, 48, and 72h, cell viability/proliferation were evaluated using 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) solution (Sigma, St. Louis, Missouri, USA) (5g/L). Briefly, 10 µl of MTT solution (5 mg/ml) dissolved in 90 µl of medium was added to each well and the plates were incubated for 2 h at 37°C. The absorbance was measured at 570 nm by ELIZA reader (BioTek, Winooski, VT, USA). The best irradiation setting based on viability results were used for following osteogenic differentiation test.

Osteogenic differentiation

BFPSCs were seeded in 6-well plates at a density of 5 × 10^3 per well and cultured in DMEM, 15% FBS, 1% Penicillin-Streptomycin for 48h. Then, cells were irradiated with the 3J/cm^2 0.3W Pulsed for 96 sec and incubated in osteogenic medium containing DMEM, 10% FBS, 100 nM dexamethasone, 0.2 mM ascorbic acid, and 10 mM β-glycerophosphate (Sigma, St. Louis, Missouri, USA). After 14 and 21 days, the capability of cells osteogenic differentiation were measured using Alizarin Red staining (Sigma, St. Louis, MO, USA), which stains the precipitated calcium in the matrix.

Stained cells were imaged using optical microscopy. For quantitative analysis, cell layer was covered by mixing 15% acetic acid and 20% methanol for 45min.Then, the optical density of the solution was read at 405 nm.

Statistical analysis

All experiments were conducted in 6 wells for each group repeated at three different time points. First, the normal distribution of MTT data was tested using a k-s sample test. three-way analysis of variance (Three way ANOVA) and Tukey HSD was used for between group comparisons of the different laser settings. One sample t-test was also used to significantly compare the rate of MTT changes in groups compared to the control group. Data were analyzed by GraphPad Prism software version 8.0.1. Kolmogorov Smirnov test was used to examine the data normality. Mean values were compared by independent samples t-test for data with normal distribution; otherwise, Mann-Whitney U-test was used. P values of <0.05 (*), <0.01 (**), and<0.001 (***) were considered significant at different levels.

Results

Proliferation assay

Figure 1 shows the results of MTT assay at different time points. Also, the results of multiple comparison at different time points-cis presented in Table 2. There was no statistically significant dif-

ference between the two different output powers ant any of the time points, Pulsed mode of irradiation results showed statistically significant differences with better outcomes for pulsed mode (P<0.05) In terms of energy density as it shown in Figure 1, the highest proliferation capability was observed at irradiation of 3J 0.3W P at 24h and 48h, however, after 72h the highest proliferation rate was related to 6J 0.1W P. Considering the effect of 3J 0.3W P on cell proliferation at an earlier time, irradiation with this setting was used for osteogenic differentiation assay.

Osteogenic differentiation assay

Both microscopic and quantitative analysis (Figure 2 a and b) of Alizarin Red staining showed that cell subjected to 3J 0.3W P irradiation had statistically increased mineralization compared to the negative control group (p<0.05). However, there was no statistically significant difference between the quantitative evaluation of mineralized tissue deposition after 14 and 21 days between the OIM-3 and OIM-0 group with Pvalues of 0.53 and 0.097, respectively.

Figure 1. Proliferation evaluation of BFPSCs following irradiation at different time points.

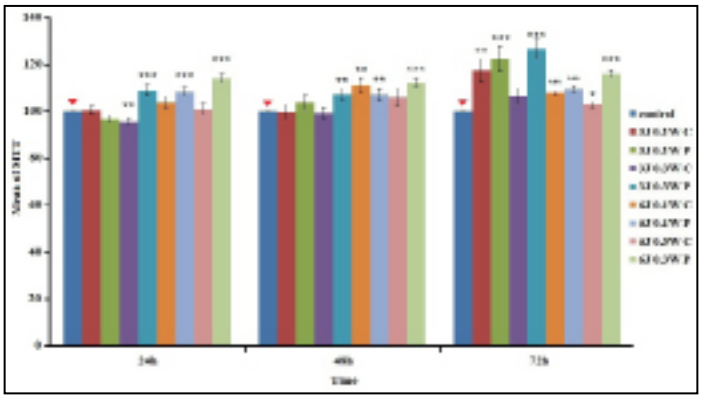
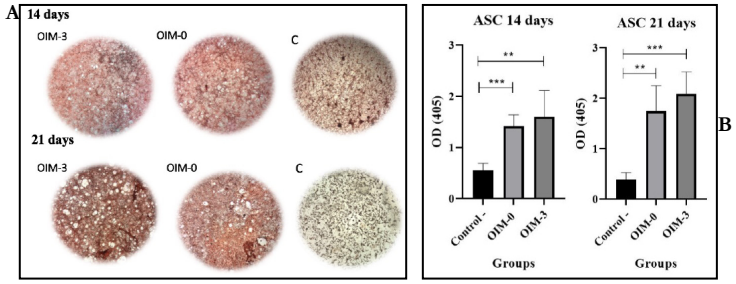


Table 2. Between group comparison results usingTukey HSD analysis.

Time point		24h	48h	72h
Multiple Comparison groups		P values	P values	P values
3J 0.1W C	3J 0.1W P	0.896	0.969	0.964
	3J 0.3W C	0.607	1	0.283
	3J 0.3W P	0.061	0.539	0.532
	6J 0.1W C	0.931	0.089	0.489
	6J 0.1W P	0.102	0.578	0.700
	6J 0.3W C	1.000	0.743	0.056
	6J 0.3W P	.000*	.038*	1.000
3J 0.1W P	3J 0.1W C	0.896	0.969	0.964
	3J 0.3W C	1.000	0.948	.020*
	3J 0.3W P	.001*	0.987	0.988
	6J 0.1W C	0.203	0.603	0.053
	6J 0.1W P	.002*	0.991	0.119
	6J 0.3W C	0.822	0.999	.002*
	6J 0.3W P	.000*	0.39	0.875
3J 0.3W C	3J 0.1W C	0.607	1.000	0.283
	3J 0.1W P	1.000	0.948	.020*
	3J 0.3W P	.000*	0.471	.001*
	6J 0.1W C	0.058	0.069	1.000
	6J 0.1W P	.000*	0.509	0.998
	6J 0.3W C	0.494	0.679	0.997
	6J 0.3W P	.000*	0.028	0.46
3J 0.3W P	3J 0.1W C	0.061	0.539	0.532
	3J 0.1W P	.001*	0.987	0.988
	3J 0.3W C	.000*	0.471	.001*
	6J 0.1W C	0.618	0.982	.003*
	6J 0.1W P	1.000	1.000	.010*
	6J 0.3W C	0.094	1.000	.000*
	6J 0.3W P	0.609	0.912	0.342

6J 0.1W C	3J 0.1W C	0.931	0.089	0.489
	3J 0.1W P	0.203	0.603	0.053
	3J 0.3W C	0.058	0.069	1.000
	3J 0.3W P	0.618	0.982	.003*
	6J 0.1W P	0.746	0.974	1.000
	6J 0.3W C	0.968	0.916	0.967
	6J 0.3W P	.010*	1.000	0.688
6J 0.1W P	3J 0.1W C	0.102	0.578	0.700
	3J 0.1W P	.002*	0.991	0.119
	3J 0.3W C	.000*	0.509	0.998
	3J 0.3W P	1.000	1.000	.010*
	6J 0.1W C	0.746	0.974	1.000
	6J 0.3W C	0.151	1.000	0.876
	6J 0.3W P	0.474	0.891	0.863
6J 0.3W C	3J 0.1W C	1.000	0.743	0.056
	3J 0.1W P	0.822	0.999	.002*
	3J 0.3W C	0.494	0.679	0.997
	3J 0.3W P	0.094	1.000	0.000
	6J 0.1W C	0.968	0.916	0.967
	6J 0.1W P	0.151	1.000	0.876
	6J 0.3W P	.000*	0.768	0.120
6J 0.3W P	3J 0.1W C	.000*	.038*	1.000
	3J 0.1W P	.000*	0.39	0.875
	3J 0.3W C	.000*	.028*	0.460
	3J 0.3W P	0.609	0.912	0.342
	6J 0.1W C	.010*	1.000	0.688
	6J 0.1W P	0.474	0.891	0.863
	6J 0.3W C	.000*	0.768	0.120

Figure 2. Osteogenic differentiation capability of BFPSCs following irradiation with 3J 0.3W P. (a) Microscopic evaluation, (b) Quantitative assessment.



Discussion

Positive effects of PBM on cellular biological behaviors, including cell proliferation and differentiation of various cell types such have been reported previously…ref, however, the optimal parameters for effective bio stimulation of cells needs further well-designed evaluations.

In the present study we aimed to study the effects of pulsed and continuous near infra-red laser irradiation with two different energy densities and output powers on proliferation and osteogenic differentiation of BFPSCs which is considered as a potentially suitable stem cell source for craniofacial tissue engineering.

According to the results of the present study a pulsed mode of irradiation resulted in significantly better outcomes for the proliferation of BFPSCs at all time points.($p<0.05$) however, the different powers did not have statistically significant differences.

72 h after irradiation the highest MTT results were observed in the 6J 0.1W Pulsed group, however the 3J 0.3W Puled mode of irradiation was chosen as the best irradiation setting since it resulted in significantly better MTT result at all time points of (24,48,72h) compared to controls and higher viability results at earlier time points of 24 and 48 h compared to the 6J 0.1W Pulsed group.

Due to the great number of studies on the effect of PBM on stem cells we mostly have focused on studies which have been conducted on Adipose stem cells (ASCs) for comparisons. Up to our knowledge there is no study on the PBMof BFPSCs until now. However, the effect of PBM on ASC has been the topic on some studies, although many different wavelengths and irradiation parameters have been employed, which makes precise comparisons difficult [6, 7].

In a study by Anwer et al., the green 532nm laser light with energy densities of 5, 6.8, 9.2, 28 and 45 J/cm^2 were used and they observed that high energy densities with longer exposures resulted in significant decrease in proliferation which is in accordance with the Arndt-Schulz law [11, 33].

Other studies have mostly studied the effects ofred laser for PBM [26, 31, 34-36]. However, they have all observed a continuous mode of irradiation of a red laser is able to improves proliferation and cell viability of ASC. However, many different laser energy densities and output powers have been used.

In a recent study by De Andrade et al., PBM with 660nm red laser with an energy of 0.56 and 1.96 J promoted proliferation of ASCs, but a higher energy setting of 5.04 J was found to be harmful. In this study they used a 660-nm laser and power of 40 mW [28]. This might be due to the fact that the corresponding energy densities applied for the energies utilized in their study were 20, 70, 180 J/cm^2 which were much higher than the energy densities used in the present report.

Wang et al., comparatively investigated the effect of four different wavelengths of 420, 540, 660, 810nm on AdSCs. They showed that blue/green irradiation had inhibitory effects on proliferation and reduced cellular Adenosine Tri Phosphate (ATP) while red/NIR stimulated proliferation, all at 3J/cm^2 and also increased ATP in a biphasic manner [37].

Similarly, we have shown thatpositive result with observed with the 3J/cm^2 energy density.

However, none of these studies have evaluated the effect of pulsed mode of irradiation on ASCsIn a different study by Wang et al two different wavelengths of the near infra-red spectrum, 810nm and 980nm were comparatively evaluated their effects on ASCs. Interestingly they reported that although the wavelengths showed a biphasic dose response, but 810 nm had a peak dose response at 3 J/cm^2 for stimulation of proliferation at 24 h, while the peak dose for 980 nm was 10-100 times lower at 0.03 or 0.3 J/cm^2 [38].

Based on these findings it seems that PBM studies are very complicated and need detailed study of each wave length of the electromagnetic spectrum needs to be evaluated individually.In this study we used a 940nm which is potentially be a more suitable adjunctive laser wavelength for clinical application in craniofacial bone regenerative treatments due to its deeper penetration depth compared to green or red lasers.

According to the finding of the present study the laser with that wavelength and settings utilized had different effects on the proliferation and differentiation of BFPASC. While cell proliferation was significantly increased the biominelalization results did not show a statistically significant effect for the same laser irradiation. This difference of effect of PBM settings and differences in effects of wavelengths on stem cell proliferation and differentiation has also been previously reported by some researchers [37, 39, 40]. Which could be attributed to the difference in underling signaling pathways that needs to be further elucidated in future studies.

There have not been many studies investigating the effect of PBM on osteogenic differentiation of ASCs. In a recent report on PBM of ASCs by Ates et al., both red 635nm and 809nm near infra-red lasers were studied on their effect on ASC proliferation and osteogenic differentiation with three energy densities of 0.5, 1.5 and 2 J/cm^2 in continuous mode [25]. According to their resultscell proliferation was not changed significantly which was different to our results and might be due to the lower energy densities used in their studyand the use of a continuous wavelength. Another difference that might be the reason for this in significant change might be that they have evaluated MTT levels after 7 and 14 days. However according to their alizarin red staining results for evaluation of mineralization at day 14 the 809 nm irradiation at all energy densities increased mineralization and in the 2 J/cm^2 group of 635 nm laser also resulted in significantly increased results of mineralization based on normalized optical absorbance results. In the present study we similarly observed biomineralization of ASC in OIM compared to the control as shown by Alizarin red staining resultsafter 14 and 21 days. However, our results did not indicate a statistically significant difference between the laser irradiated group in OIM and the OIM without laser irradiation at any of these time points.

Near infra-red wavelength PBM either by laser or LED has been reportedto have positive effectson proliferation and differentiation of other types of stem cell [41, 44]. Looking at the results of studies with similar wavelengths to the one used in the present study only a few was found.

Paschalidou et al have used a similar 940nm laser device to ours in order to evaluate the effect of 4,8,16J/cm^2 irradiation on viability/proliferation, migration, odontogenic differentiation, and biomineralization of stem cells from human exfoliated deciduous teeth (SHED). Their results were consistent with ours and they reported an increase in proliferation with overall higher results for 4 J/cm2 and 16 J/cm^2. They also evaluated *in vitro* biomineralization potential by alizarin red staining and found significantly higher mineral deposition in the 8j/cm^2 group [41].

Although the results of the present study confirm the results of previous reports of PMB using near-infra red irradiation. The majority of previous studies have focused on the effect of energy densities in PBM with only a continuous mode of irradiation [6, 7].
In the present study, we found no difference between power densities and both energy densities of 3 and 6 j which are regarded as within the biostimulatory, range were capable of increasing proliferation. However interestingly our results revealeda statistically significant positive effect with the pulsed mode of irradiation and no statistically significant difference in the continuous mode irradiated groups was observed compared to controls.

Continuous wave or pulsed modes of irradiation may have different biological effects. Some reports have also indicated even better biological effects of Pulsing in Low-Level Light or PBM Therapy, which is consistant with the findings of the present report [45]. This might be explained by the fact thatthe pulse-off times may allow a rest time for the irradiated tissue and also the higher peak powers produced which might result in the differences observed. This high peak powers production is while the total energy is kept the same,which leads to less thermal effects and deeper penetration [45, 46].

There have been a few *in vitro* studies of PBM with pulsed mode of diode lasers ona variety of cell lines such asbone marrow stem cells, osteoblasts, fibroblasts, normal human neural progenitor cells, [47-51] While only a few studies have comparatively studied pulsed and continuous irradiation modes on cells [46, 52, 53].

Kim et al have reported an interesting pulse frequency dependency of PBM in the differentiation of hDPSCs by applying 810nm LED and evaluating the effects of different frequencies of pulsed mode. Ueda et al have demonstrated that low-frequency pulsed 830nm laser irradiation significantly stimulates bone formation compared to continuous irradiation [53]. Pulsed near infra-red irradiation has also attracted a lot of attention as shown effective results as a therapeutictool of in wound healing and neurology with more beneficial results compared to continuous wave specially in deep tissue repair [54-58].

It is believed that pulsed PBM can promote light-biological system interactions.This can be explained with the fact that some fundamental frequencies in biological systems have some fundamental frequencies that are in the range of tens to hundreds Hz, are similar to the pulsing frequencies used in pulsed PBM. This time period could be for instance the half-life of an ion channel in the mitochondrial membrane. Another reason for improved biological effects of pulsed irradiation could be its effect on the cellular levels of mechanisms of action of PBM for instance pulsed mode multiple photodissociation of nitric oxide from a protein binding site may be possible which can prevent its rebound observed in continuous mode. More research is needed for understanding the exact mechanisms involved with pulsed irradiations in PBM [45, 55].

Howeverpulsation frequency, pulse duration, duty cycle, duration of dark period between pulses, peak and average intensities all are important parameters when comparing pulsed and continuous modes of the same wavelengths which need to be considered and evaluated in future studies [59].

Conclusion

According to the results of this study a pulsed mode of irradiations showed better viability results. Although the 3J/cm^2 0.3W, Pulsed irradiation showed significantly better results for viability and proliferation, however no statistically significant effect was observed in osteogenic differentiation. Further investigations are needed to optimize the settings of this adjunctive treatment technique and effectively translate it into clinical application of bone tissue engineering.

Acknowledgments

This study was supported by Research Institute for Dental Sciences, Shahid Beheshti University of Medical Sciences.

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