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Effects of Two Protocols of Low-Level Laser Therapy on the Proliferation and Differentiation of Human Dental Pulp Stem Cells on Sandblasted Titanium Discs: An *In Vitro* Study

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Abstract

Introduction: Stem cell activities have different effects on tissue response and its outcomes. Low-level laser therapy (LLLT) can be considered a trigger to modify stem cell activities. The objective of the present experimental investigation was to study the effects of two protocols of LLLT on the proliferation and differentiation of human dental pulp stem cells (hDPSCs) cultured on sandblasted titanium discs.

Methods: Cells obtained from human dental pulp were seeded/cultured on titanium discs and were set in 2 main groups: (*i*) Radiated cells using the gallium-aluminium-arsenide (GaAlAs) diode laser at a continuous wavelength of 808 nm at 3 J/cm^2 for 12 sec or 5 J/cm^2 for 20 seconds, and (*ii*) Non-irradiated cells serving as control groups. The impact of LLLTs on hDPSC-proliferation and viability was investigated using the MTT assay after 24, 72 and 96 hours. The alkaline phosphatase activity was studied with p-nitrophenylphosphate after 14 and 28 days. The ability of hDPSCs to express osteocalcin was investigated using real-time polymerase chain reaction after 28 days, while their attachment was observed under a scanning electron microscope (SEM) after 14 and 28 days.

Results: Our study showed that LLLTs caused maximum cell proliferation in 96 hours ($P<0.001$) with 3 J/cm^2 resulting in a higher proliferation rate. The highest activity of alkaline phosphatase and osteocalcin expression was observed in the laser radiation groups after 28 days.

Conclusion: The outcomes of the current study showed that cultured hDPSCs on sandblasted titanium discs had a tendency towards increased cellular activity in response to LLLTs. Thus, LLLTs could regulate the activities of hDPSCs on bone repair surrounding the sandblasted titanium discs.

Keywords: Differentiation; Low-level laser therapy; Proliferation; Stem cells; Titanium.

Introduction

Nowadays, titanium implants are applied as replacements to restore function, phonetics and aesthetics in dentistry.[1,2] They are inserted in the alveolar bone after surgical intervention.[3] The stability of the dental implant is achieved via close contact with its surrounding alveolar bone during an interface formation called "osseointegration".[3] The clinical success of dental implants depends on the wound healing process, the formation of a new bone around the implant, the quality of the surrounding bone, the dimensions of the alveolar bone, the primary stability of the implant, the time of the masticatory loading, regional infections and implant surface characteristics.[4-8] Therefore, the main purpose of many treatments is to improve and accelerate bone formation around dental implants.[6]

Stem-cell-based therapeutic approaches can provide treatment alternatives in dentistry.[9] Stem cells are obtained from many different sources including dental pulp tissue.[10-12] The use of human dental pulp stem cells (hDPSCs) is one of the strategies in tissue engineering.[13] hDPSCs can be easily isolated and tend to expand in medium culture.[14] They have also shown the capacity of renewal and differentiation to one, or many types of specific cells, under controlled laboratory conditions.[5] Studies on DPSCs cultured on a porous surface showed

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osteoblast differentiation, production of bone-forming proteins and specific bone proteins.[15]

One treatment that seems to have a stimulatory effect on stem cells proliferation and differentiation is low-level laser therapy (LLLT).[16,17] LLLT is a simple and non-invasive technique that uses various wavelengths from 650-1000 nm.[6] This treatment is also known as "photobiomodulation", which improves tissue repair and reconstruction through the stimulation of cellular reactions such as proliferation and differentiation.[18] Therefore, it is an accepted clinical tool for reducing edema and pain[16,19]; the enhancement of mineralization in alveolar sockets, as another clinical effect of LLLT, has also been reported.[6]

However, there is not sufficient literature on the provision of LLLT impacts on hDPSCs and acceptable irradiation to increase the pace and quality of bone integration. The current study aimed to compare the effects of two protocols of LLLT on the activity of hDPSCs cultured on sandblasted titanium discs.

Materials and Methods

Titanium Discs

Sixty-three titanium discs (Servo-dental, Germany) were used in this study. The discs were made of titanium alloy grade 5 and measured 7 mm and 2 mm in diameter and thickness respectively. The surfaces of all discs were sandblasted and acid-etched.

Cell Culture

hDPSCs were obtained from the Iranian Biological Resource Center (IRIB C10896).[20] The culture medium was DMEM-F12 (Gibco, Grand Island, USA) and had 100 IU/mL penicillin, 100 mg/mL streptomycin solution, 2 mML L-glutamine and 20% fetal bovine serum. The cells were incubated at 37°C in humidified atmosphere of 5% Co_2 and 95% air. In alkaline phosphatase activity and differentiation tests, hDPSCs were cultured in a completed medium supplemented with 100 mML dexamethasone, 50 µg/mL ascorbic acid, and 10 mML β-glycerophosphate.

Laser Treatment

As it can be seen from Table 1; a photon-plus, gallium-aluminium-arsenide (GaAlAs) diode laser device (Konftec Corporation, Taiwan) was employed in the current investigation. The system operated with 250 mW output power and in the near-infrared spectrum at a continuous wavelength of 808 nm. Each disc was placed in the selected well of the chosen plates and seeded with hDPSCs. The laser was irradiated at 3 J/cm^2 for 12 seconds or 5 J/cm^2 for 20 seconds using a straight handpiece in a dark place. The tip of the laser handpiece was in constant contact with each well, making a 90° angle with the bottom of the plate. The control wells were maintained for the same period as each of the experimental groups; however, they were not exposed to irradiation.

Table 1. Laser Parameters

Type of laser	GaAlAs-diode-laser device (Konftec Corporation, Taiwan)
Emission mode	Continuous
Time on/Time off	-
Delivery system	straight handpiece
Energy distribution	-
Peak power	
Average power	250 mW
Spot diameter at the focus	0.5 cm^2
Focus spot area	-
Spot diameter at the tissue	
Focus-to-tissue	
Spot area at the tissue	
Peak power density at spot area	3 J/cm^2 and 5 J/cm^2
Peak power density at the tissue	-
Average power density at spot area	-
Average power density at the tissue	-
Beam divergence	-
Water irrigation	-
Air and aspirating airflow	-

Cell Proliferation and Viability (MTT Assay)

In this study, the methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay was used to evaluate and compare the effects of LLLT on the proliferation and viability of hDPSCs. After the cells reached ≥ 80% of confluence, the cells were separated from culture flasks using trypsin 0.25% in *Phosphate Buffered Saline* (PBS), containing EDTA 0.02%. The cells were washed with PBS 3 times. Then, they were centrifuged, suspended in a growth medium and placed at a density of 10^4 per well on twenty-seven titanium discs located at the bottom of 48-well plates.

Subsequently, the discs were divided into 4 groups: (*i*) non-irradiated cultured cells (without discs) which were considered the negative control group, *(ii)* non-irradiated cells cultured on discs and considered the positive control group, (*iii*) cells which received laser doses of 3 J/cm^2 after 24 hour- and 72 hour-culture, and (*iv*) cells which received laser doses of 5 J/cm^2 after 24 hour- and 72 hour-culture. Then, the cells were incubated at 37°C for 24, 72 and 96 hours. After incubation, 10 µL tetrazolium bromide solution (Sigma-USA) at a concentration of 5 mg/mL was added to wells, and then they were incubated at 37°C for 4 hours. The medium was discarded without washing and 100 µL of DMSO was used as a formazan crystal solvent. After 15 minutes of incubation, the optical density of each well was read using a spectrophotometer (Biotech, USA) at a wavelength of 570 nm.

Alkaline phosphatase activity (ALP)

Eighteen titanium discs were located at the bottom of two 24-well plates and seeded with hDPSCs at the density of 3×10^4 cells per well. Then, the discs were allocated randomly to one of the above-mentioned groups. After 24-hour culture, the experimental groups were irradiated using the GaAlAs diode laser at the dosages of 3 J/cm^2 or 5 J/cm^2 every 48 hours for 6 or 12 sessions. Culture media were collected after 14 or 28 days. A buffer, containing 150 mML NaCl, 0.5% sodium deoxycholate, 0.1% Triton X-100, 0.1% sodium dodecyl sulphate (SDS), 50 mML Tris-HCl and Protease inhibitors, was added at 4°C for 10 minutes to extract entire protein. The solution was then centrifuged for 10 minutes at 14000 rpm. The supernatant was collected containing the protein. The amount of protein was measured using the Bradford method, and the ALP activity (Unit/Litre) was balanced by the total protein (mg/dL). To evaluate the ALP activity, a kit containing paranitrophenyl phosphate substrate was used (Pars Azmun, Iran). 20 mL of the sample was thoroughly mixed with 1 mL of the reaction solution, and the difference in absorption was read between 0 min and 3 min at 405 nm using a spectrophotometer (Biotech, USA).

Osteocalcin Production

In this study, real-time quantitative reverse-transcription polymerase chain reaction (qRT-PCR) was used to observe/evaluate the effects of LLLT on osteocalcin gene expression in hDPSCs. The cells were placed on six titanium discs at a density of 3×10^4 cells per well. Then, the discs were randomly allocated to one of the four above-mentioned groups. After 24-hour culture, the experimental groups were irradiated using the GaAlAs diode laser at dosages of 3 J/cm^2 or 5 J/cm^2 every 48 hours for 12 sessions. Subsequently, the culture media were collected after 28 days. Then, total RNA from cultured cells in control and LLLT groups was directly isolated from the culture media using TRIzol reagent (Sigma-Aldrich, MO 63103 USA). After quantitative and qualitative RNA extractions, complementary DNA (cDNA) synthesis was performed by reverse transcription reaction. The used primers for the amplification were forward 5'-TGAGAGCCCTCACACTCCTC-3' and reverse 5'-CGCCTGGGTCTCTTCACTAC-3' for osteocalcin (150 bp), and forward 5'-CATGAGAAGTATGACAACAGCCT-3' and reverse 5'-AGTCCTTCCACGATACCAAAGT-3' for the internal control gene; "glyceraldehyde 3-phosphate dehydrogenase" (GAPDH) (113 bp). The final volume (20 µL) was used to examine gene expression; it was composed of 10 µL of Master Mix Kit (Takara, Japan), 2 µL of cDNA solution, 1.6 µL of progressive/retrogressive primers and 6.4 µL of water. The real-time PCR reaction was performed at 95°C (30 seconds), 40 cycles at 95°C (5 seconds) and 60°C (30 seconds). In each process, and for each primer pair, a cDNA-free sample was used to ensure proper functionalisation. After the normalisation of mean cycle threshold (C_t) values (two repetitions), the changes in the level of osteocalcin gene expression were evaluated in comparison with the expression of GAPDH using the comparative C_t ($\Delta\Delta C_t$) formula. The osteocalcin gene expression rate was set to 1 in the negative control group (Non-irradiated cells).

Cell Morphology

To verify cell morphology, hDPSCs at the density of 3×10^4 (cells/well) were placed on twelve titanium discs located at the bottom of two 24-well plates. After 24-hour culture, eight samples, representing experimental groups, were irradiated by the GaAlAS diode laser at the dosages of 3 J/cm^2 or 5 J/cm^2 every 48 hours for 6 or 12 sessions. The cells were then fixed using glutaraldehyde 2.5% after 14 and 28 days of laser irradiation and were controlled-dehydrated afterwards. The titanium discs were coated with gold to be observed under a scanning electron microscope (TeScan-Mira III, Czech) at 500X, 2500X, 5000X and 10000X.

Statistical Analysis

GraphPad Prism Version 8.0.1 (GraphPad Software, San Diego, CA) was used for statistical analysis. To analyse the main effects of time and group on the proliferation and activity of alkaline phosphatase, One-way ANOVA was used. Two laser groups that were irradiated 3 J/cm^2 and 5 J/cm^2 as well as the control group without laser irradiation were compared using the Tukey HSD method. The relevant graph was plotted and the level of significance was set at $P<0.05$.

Results

Cell Proliferation and Viability

Figure 1 depicts the proliferation of hDPSCs seeded on titanium discs after 24, 72 and 96 hours. The results showed that time had a significant proliferative effect on hDPSCs ($P<0.05$). Additionally, the cell proliferation was higher in 96, 72 and 24 hours respectively. Moreover, the outcomes demonstrated that an increase in cell proliferation in all treatment groups after 24 hours was observed. However, the proliferation of the cells was significantly higher in the groups treated with 3 J/cm^2 in 72 and 96 hours. There was no difference in the cell viability of hDPSCs between the treatment groups, exceeding 80% in all the groups.

Alkaline Phosphatase Activity

The alkaline phosphatase activity of hDPSCs, cultured on titanium discs after 14 and 28 days, is presented in Figure 2. The results showed that ALP activity was higher in the 3 J/cm^2 irradiation group than that of the control groups ($P<0.05$). However, its activity was not different between 3 J/cm^2 and 5 J/cm^2 irradiated groups ($P>0.001$).

In addition, the ALP activity on day 28 was significantly higher than that of day 14 ($P<0.001$). Nevertheless, the pattern of ALP activity in response to LLLT was similar in all groups on days 14 and 28.

Osteocalcin Production

Figure 3 shows that LLLT caused significant up-regulation of osteocalcin gene expression compared to control groups ($P<0.05$). There was no significant difference between the irradiated groups with 3 J/cm^2 and that of 5 J/cm^2 in the gene expression of osteocalcin.

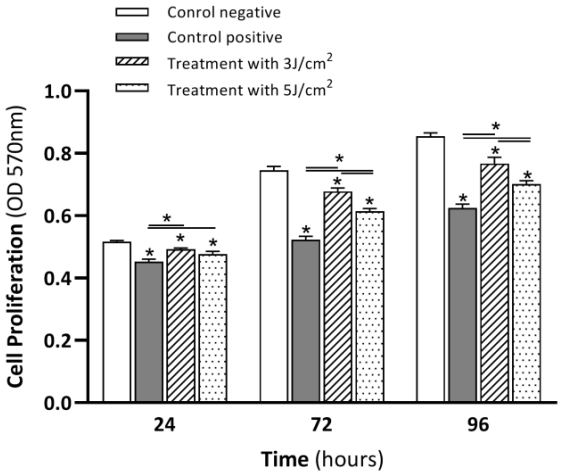

Figure 1. Effects of LLLT on the Proliferation of Non-irradiated Control Groups and hDPSCs Irradiated With the 3 J/cm^2 or 5 J/cm^2 GaAlAs Diode Laser. Proliferation was evaluated using the MTT assay at 24, 72 and 96 h after exposure. The graph shows 'mean ± SD' for four independent experiments. Stars on the top of columns represent $P<0.05$ compared with the corresponding negative control group. The stars between columns (connected with horizontal lines) indicate a significant difference between the experimental groups. There was an increase in cell proliferation in all treatment groups after 24 h.

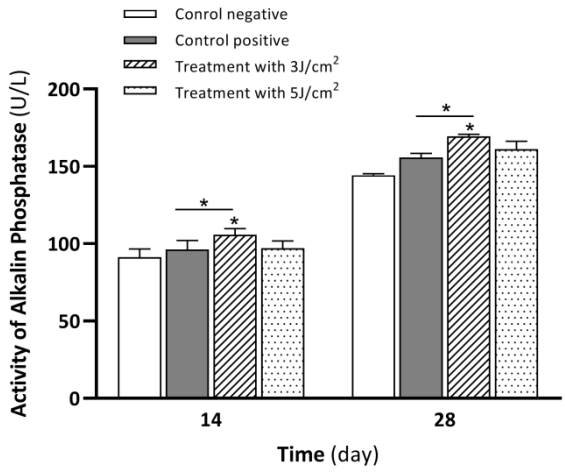

Figure 2. Effects of LLLT on the Alkaline Phosphatase Activity of the Untreated Control Groups and of the Cells Treated With the 3 J/cm^2 or 5 J/cm^2 GaAlAs Diode Laser. ALP activity was assessed 14 or 28 days after the treatment. The graph shows mean ± SD for four independent experiments. Stars on the top of columns indicate a statistically significant difference ($P<0.05$) from the negative control group. The ALP activity was higher in the laser-irradiated groups.

Observation of Cell Morphology

Sandblasted titanium discs cultured with hDPSCs were observed using a scanning electron microscope (SEM) (Figure 4). SEM micrographs showed attached-like cells on the specimens after 14/28 days and covered the surfaces of the titanium discs.

Discussion

The current study introduced LLLT, at two dosages of 3 J/cm^2 and 5 J/cm^2, for use as potential and promising biostimulants for further proliferation and/or differentiation of hDPSCs. This research showed that the investigated cells seemed to relatively proliferate and further differentiate into osteoblasts after being exposed in due time to LLLT, especially 3 J/cm^2.

Stem cells have an important role in tissue regeneration in the target site, that is, in different dental applications including the bone around dental implants.[21] Biostimulation using LLLT could increase the activity of stem cells.[22] However, there are reports that LLLT can have adverse effects on their activities.[17,23] This is supported by "Arndt-Schulz Law" stating that optimal stimulation creates proper cell activity; nevertheless, high/low stimulation might decrease, inhibit and/or result in lack of cell response.[24] Therefore, the determination of an ideal dose seems necessary. Several investigations have indicated that 1 J/cm^2 to 5 J/cm^2 irradiations are effective in inducing positive outcomes.[22,23,25] In the present study, 3 J/cm^2 and 5 J/cm^2 dosages of LLLT were used to stimulate hDPSCs, which were in line with similar investigations.

Our research showed that the proliferation of hDPSCs in response to LLLT increased with time and was higher at 96 hours in comparison to 72 and 24 h hours demonstrating the positive effect of time on cell

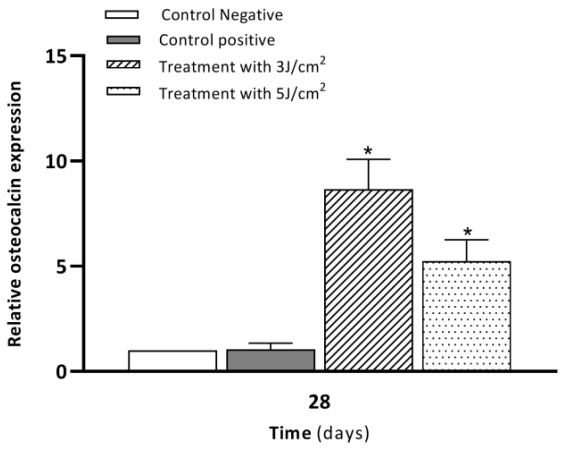

Figure 3. Effects of LLLT on the Expression of the Osteocalcin Gene in the Control Groups and hDPSCs Irradiated With the 3 J/cm^2 or 5 J/cm^2 GaAlAs Diode Laser. Gene expression was assessed using Real-Time PCR 28 days after the treatment. The graphs show mean ± SD for four independent experiments. Stars on the top of columns indicate a statistically significant difference ($P<0.05$) from the negative control group. LLLT caused significant up-regulation of osteocalcin gene expression compared to the control groups.

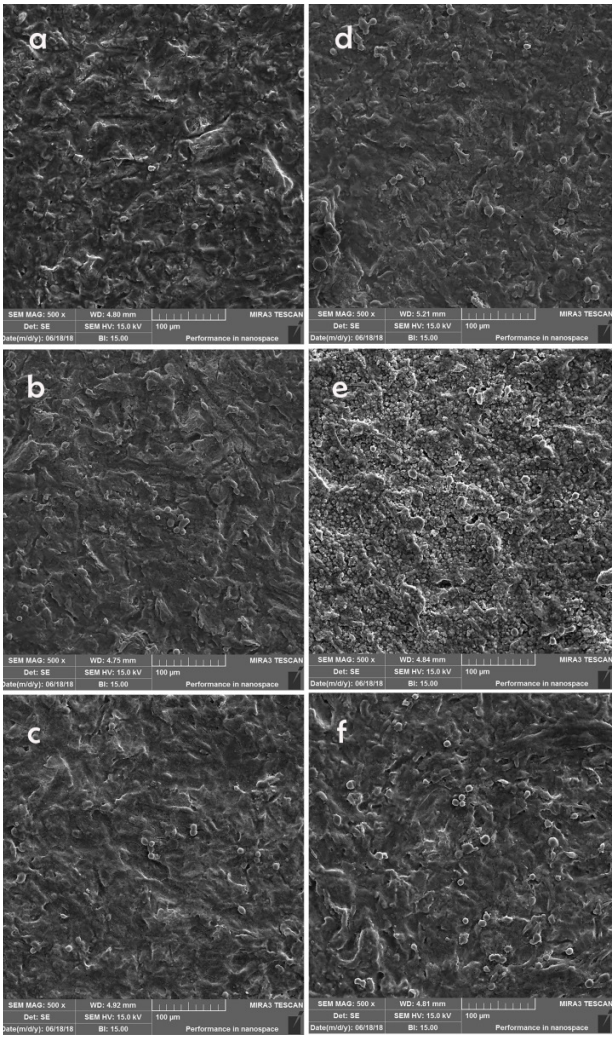

Figure 4. Scanning Electron Microscopy of hDPSCs (×500): (a) Non-irradiated group - positive control group - after 14 days, (b) Laser irradiation with 3 J/cm^2 after 14 days, (c) Laser irradiation with 5 J/cm^2 after 14 days, (d) Positive control group after 28 days, (e) Laser irradiation with 3 J/cm^2 after 28 days, (f) Laser irradiation with 5 J/cm^2 after 28 days. SEM micrographs showed attached-like cells on the specimens after 14 or 28 days.

proliferation. Furthermore, the proliferation was higher at the 3 J/cm^2 irradiation group than that of 5 J/cm^2 after 24 hours, expressing that higher irradiation doses (i.e. 5 J/cm^2) do not necessarily enhance proliferation. These outcomes are consonant with those of Eduardo et al[5] and Khadra et al[23] who showed that LLLT stimulates stem cells proliferation, and therefore can be used for improving cell growth. It has also been shown that proliferation can be increased with a single dose at 5 J/cm^2 and/or 7.5 J/cm^2 in comparison to the dose of 2.5 J/cm^2.[26] However, in a study by Bouvet-Gerbettaz et al., it was concluded that LLLT would not increase cell proliferation due to irradiation.[27] Moreover, cell viability of more than 80% was reported for all treated groups, exhibiting that LLLT using the GaAlAs diode laser did not cause cell damage. This result is in line with the studies conducted by Fernandes et al[28] and de Oliveira et al[29] whose research reported that LLLT at a specific parameter (0.8 J/cm^2 – 5 J/cm^2) did not cause loss of cell viability and modulated it.

The findings of the current investigation showed that ALP activity increased following the application of LLLT on days 14 and 28, with higher activity on day 28. Similar results were reported in other studies claiming that ALP shows more activity when affected with LLLT.[25,30] The mentioned outcome is in contrast with another study, which showed that LLLT, in both irradiation doses of 1/5 J/cm^2 and 3 J/cm^2, did not significantly affect ALP.[23] The difference might be due to different protocols of the irradiation and employed cells in the two studies.

One of the major purposes of our research was to analyse the osteocalcin synthesis by hDPSCs after irradiation with LLLT. Osteocalcin is considered a marker for osteogenic maturation and stem cells osteoblast differentiation.[31] Our investigation showed that osteocalcin synthesis was significantly higher in the groups irradiated by 3 J/cm^2 compared to 5 J/cm^2 after 28 days. Besides, it could be inferred that this stimulatory effect was not directly related to the amount of irradiation. However, the production of osteocalcin seems to be dependent upon multiple factors in LLLT, including wavelength, total dose of laser density, laser ability to accelerate cellular activity and synthesis of adenosine triphosphate.[9,23,32] In contrast, Bouvet-Gerbettaz et al[27] revealed that LLLT could not cause any alterations in the differentiation of stem cells in bone marrow. In addition, the potential of stem cells for osteoblastic differentiation, while adhering to biomaterial surfaces, makes them favourable for bone tissue engineering.[33] The present study showed that hDPSCs adopted an earlier spread-up morphology after irradiation with LLLT. Nevertheless, further/future investigations on morphology are recommended.

The present research showed that LLLT irradiation with the dose of 3 J/cm^2 on consecutive days could enhance proliferation, suggesting that the dose of 5 J/cm^2 presumably exceeded the optimal doses. In addition, the outcomes indicated that LLLT modulated the hDPSCs activity and could accelerate bone repair.

Conflict of Interests

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Ethical Considerations

The study was approved by the Bioethics Committee of research institute of dental sciences at Medical University of Shahid Beheshti (No. IR.SBMU.RIDS.REC.1395.361).

References

1. Petri AD, Teixeira LN, Crippa GE, Beloti MM, Oliveira PTd, Rosa AL. Effects of low-level laser therapy on human osteoblastic cells grown on titanium. *Braz Dent J.* 2010;21(6):491-498. doi: 10.1590/s0103-64402010000600003.
2. Albrektsson T, Johansson C. Osteoinduction, osteoconduction and osseointegration. *Eur Spine J.* 2001;10 Suppl 2(Suppl 2):S96-S101. doi: 10.1007/s005860100282.
3. Parithimarkalaignan S, Padmanabhan TV. Osseointegration:

an update. *J Indian Prosthodont Soc*. 2013;13(1):2-6. doi:10.1007/s13191-013-0252-z.
4. Mitsiadis TA, Orsini G, Jimenez-Rojo L. Stem cell-based approaches in dentistry. *Eur Cell Mater*. 2015;30:248-57. doi: 10.22203/ecm.v030a17.
5. Eduardo Fde P, Bueno DF, de Freitas PM, Marques MM, Passos-Bueno MR, Eduardo Cde P, et al. Stem cell proliferation under low intensity laser irradiation: a preliminary study. *Lasers Surg Med*. 2008;40(6):433-8. doi: 10.1002/lsm.20646.
6. Amid R, Kadkhodazadeh M, Ahsaie MG, Hakakzadeh A. Effect of low level laser therapy on proliferation and differentiation of the cells contributing in bone regeneration. *J Lasers Med Sci*. 2014;5(4):163-70.
7. Boonsiriseth K, Suriyan N, Min K, Wongsirichat N. Bone and soft tissue healing in dental implantology. *J Med Sci*. 2014;5(5):121-126. doi: 10.14303/jmms.2014.074
8. Raikar S, Talukdar P, Kumari S, Panda SK, Oommen VM, Prasad A. Factors Affecting the Survival Rate of Dental Implants: A Retrospective Study. *J Int Soc Prev Community Dent*. 2017;7(6):351-355. doi: 10.4103/jispcd.JISPCD_380_17.
9. Ginani F, Soares DM, Barreto MP, Barboza CA. Effect of low-level laser therapy on mesenchymal stem cell proliferation: a systematic review. *Lasers Med Sci*. 2015;30(8):2189-94. doi: 10.1007/s10103-015-1730-9.
10. Pierdomenico L, Bonsi L, Calvitti M, Rondelli D, Arpinati M, Chirumbolo G, Becchetti E, et al. Multipotent mesenchymal stem cells with immunosuppressive activity can be easily isolated from dental pulp. *Transplantation*. 2005;80(6):836-42. doi: 10.1097/01.tp.0000173794.72151.88.
11. Shi S, Gronthos S. Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. *J Bone Miner Res*. 2003;18(4):696-704. doi: 10.1359/jbmr.2003.18.4.696.
12. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proc Natl Acad Sci U S A*. 2000;97(25):13625-30. doi: 10.1073/pnas.240309797.
13. Tatullo M, Marrelli M, Shakesheff KM, White LJ. Dental pulp stem cells: function, isolation and applications in regenerative medicine. *J Tissue Eng Regen Med*. 2015;9(11):1205-16. doi: 10.1002/term.1899.
14. Ashri NY, Ajlan SA, Aldahmash AM. Dental pulp stem cells. Biology and use for periodontal tissue engineering. *Saudi Med J*. 2015;36(12):1391-9. doi: 10.15537/smj.2015.12.12750.
15. Iaculli F, Di Filippo ES, Piattelli A, Mancinelli R, Fulle S. Dental pulp stem cells grown on dental implant titanium surfaces: An in vitro evaluation of differentiation and microRNAs expression. *J Biomed Mater Res B Appl Biomater*. 2017;105(5):953-965. doi: 10.1002/jbm.b.33628.
16. Kushibiki T, Hirasawa T, Okawa S, Ishihara M. Low reactive level laser therapy for mesenchymal stromal cells therapies. *Stem Cells Int*. 2015;2015: ID 974864. doi: 10.1155/2015/974864.
17. Fekrazad R, Asefi S, Allahdadi M, Kalhori KA. Effect of Photobiomodulation on Mesenchymal Stem Cells. *Photomed Laser Surg*. 2016;34(11):533-542. doi: 10.1089/pho.2015.4029.
18. Borzabadi-Farahani A. Effect of low-level laser irradiation on proliferation of human dental mesenchymal stem cells; a systemic review. *J Photochem Photobiol B*. 2016;162:577-582. doi: 10.1016/j.jphotobiol.2016.07.022.
19. Kemmotsu O, Sato K, Furumido H, Harada K, Takigawa C, Kaseno S, et al. Efficacy of low reactive-level laser therapy for pain attenuation of postherpetic neuralgia. *Laser Therapy*. 1991;3(2):71-75. doi:10.5978/islsm.91-OR-10
20. Ganjibakhsh M, Shahzadeh Fa, Gohari Ns, Rahmati H, Elyasi Gz, Izadpanah M, et al. Isolation, characterization and standard storage of human mesenchymal stem cell derived from adipose and dental pulp tissue. *Razi Journal of Medical Sciences*. 2017;24(157):35-50.[Persian]
21. y Baena RR, Rizzo S, Graziano A, Lupi SM. Bone regeneration in implant dentistry: Role of mesenchymal stem cells.In: Kalantar Motamedi M. *A Textbook of Advanced Oral and Maxillofacial Surgery*. 2016;3269. doi: 10.5772/63346
22. AlGhamdi KM, Kumar A, Moussa NA. Low-level laser therapy: a useful technique for enhancing the proliferation of various cultured cells. *Lasers Med Sci*. 2012;27(1):237-49. doi: 10.1007/s10103-011-0885-2.
23. Khadra M, Lyngstadaas SP, Haanaes HR, Mustafa K. Effect of laser therapy on attachment, proliferation and differentiation of human osteoblast-like cells cultured on titanium implant material. *Biomaterials*. 2005;26(17):3503-9. doi: 10.1016/j.biomaterials.2004.09.033.
24. Chung H, Dai T, Sharma SK, Huang YY, Carroll JD, Hamblin MR. The nuts and bolts of low-level laser (light) therapy. *Ann Biomed Eng*. 2012;40(2):516-33. doi: 10.1007/s10439-011-0454-7.
25. Soleimani M, Abbasnia E, Fathi M, Sahraei H, Fathi Y, Kaka G. The effects of low-level laser irradiation on differentiation and proliferation of human bone marrow mesenchymal stem cells into neurons and osteoblasts--an in vitro study. *Lasers Med Sci*. 2012;27(2):423-30. doi: 10.1007/s10103-011-0930-1.
26. Almeida-Junior LA, Marques NCT, Prado MTO, Oliveira TM, Sakai VT. Effect of single and multiple doses of low-level laser therapy on viability and proliferation of stem cells from human exfoliated deciduous teeth (SHED). *Lasers Med Sci*. 2019;34(9):1917-1924. doi: 10.1007/s10103-019-02836-y.
27. Bouvet-Gerbettaz S, Merigo E, Rocca JP, Carle GF, Rochet N. Effects of low-level laser therapy on proliferation and differentiation of murine bone marrow cells into osteoblasts and osteoclasts. *Lasers Surg Med*. 2009;41(4):291-7. doi: 10.1002/lsm.20759.
28. Fernandes AP, Junqueira Mde A, Marques NC, Machado MA, Santos CF, Oliveira TM, et al. Effects of low-level laser therapy on stem cells from human exfoliated deciduous teeth. *J Appl Oral Sci*. 2016;24(4):332-7. doi: 10.1590/1678-775720150275.
29. Oliveira FA, Matos AA, Matsuda SS, Buzalaf MA, Bagnato VS, Machado MA, et al. Low level laser therapy modulates viability, alkaline phosphatase and matrix metalloproteinase-2 activities of osteoblasts. *J Photochem Photobiol B*. 2017;169:35-40. doi: 10.1016/j.jphotobiol.2017.02.020.
30. Medina-Huertas R, Manzano-Moreno FJ, De Luna-Bertos E, Ramos-Torrecillas J, García-Martínez O, Ruiz C. The effects of low-level diode laser irradiation on differentiation, antigenic profile, and phagocytic capacity of osteoblast-like cells (MG-63). *Lasers Med Sci*. 2014;29(4):1479-84. doi: 10.1007/s10103-014-1557-9.
31. Nakamura A, Dohi Y, Akahane M, Ohgushi H, Nakajima H, Funaoka H, et al. Osteocalcin secretion as an early marker of in vitro osteogenic differentiation of rat mesenchymal stem cells. *Tissue Eng Part C Methods*. 2009;15(2):169-80. doi: 10.1089/ten.tec.2007.0334.
32. Laakso L, Richardson C, Cramond T. Factors affecting Low Level Laser Therapy. *Aust J Physiother*. 1993;39(2):95-9. doi: 10.1016/S0004-9514(14)60473-6.
33. Chan C-W, Hussain I, Waugh DG, Lawrence J, Man H-C. Effect of laser treatment on the attachment and viability of mesenchymal stem cell responses on shape memory NiTi alloy. *Mater Sci Eng C Mater Biol Appl*. 2014;42:254-263. doi: 10.1016/j.msec.2014.05.022.