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The biostimulative effectiveness of photobiomodulation therapy application on thawed dental pulp stem cells

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This study was conducted to understand the cellular proliferative effect of Photobiomodulation Therapy (PBMT) on thawed dental pulp stem cells (DPSCs) stored for 2 years. For this purpose, cells were exposed to PBMT for short period of time to evaluate the most appropriate PBMT parameter for stimulating cellular proliferation that can be used for future tissue engineering therapies. Fully characterized DPSCs were seperated into three groups according to the laser energy densities (5 J/cm^2 or 7 J/cm^2) applied and a group was served as control in which cells did not receive any laser irradiation. The cells in laser-irradiated groups were further divided into two subgroups according to the period of application (24 h and 0 h) and exposed to Gallium–Aluminum–Arsenide diode laser irradiation. Cell viability and the proliferation rate of the cells were analyzed with the 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay, any PBMT related cellular cytotoxicity were determined by performing a lactate dehydrogenase assay (LDH) and statistical analysis of data were performed. The percentage of proliferation seemed to increase upon laser therapy in both different doses of irradiation (5 J/cm^2 and 7 J/cm^2). DPSCs showed significantly higher proliferation rate upon 7 J/cm^2 irradiation in

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both 0 h and 24 h when compared to control groups. However, DPSCs irradiated with 5 J/cm^2 dose induced relatively lower proliferation rate when compared to 7 J/cm^2 dose of irradiation. According to the LDH data, PBMT exposure did not show any significant cytotoxicity at both energy densities in all different time periods. PBMT at 7 J/cm^2 should be an effective parameter to stimulate proliferation of long-term cryopreserved DPSCs in a short term time period. Photobiomodulation therapy may be an upcoming tool for future tissue enngineering and regenerative dentistry applications.

Keywords: PBMT; tissue engineering; proliferation; short-term; cytotoxicity.

1. Introduction

Tissue engineering is dependent on three major necessities that can be used to regenerate tissue in this pathway.[1] Scaffold formation, stem cells and growth signals are main components of this triad.[2] Therefore, factors that provide the growth rate of dental mesenchymal stem cells (DMSCs) are enormously important for dentistry field.[3] DMSCs exhibit high proliferative ability via their multiple differentiation capacities such as osteoblasts, chondrocytes, adipocytes and odontoblasts.[4–6] Generally DMSC can be isolated from adult dental pulp tissue (DPSCs),[7] exfoliated deciduous teeth (SHEDs),[8] periodontal ligaments (PDLSCs)[9] and apical papilla of immature teeth (SCAP).[10]

Nowadays, researchers in tissue engineering field have been spending time expressing the main mechanism of stem cell proliferation and differentiation for future clinical use of them in cellular therapies.[11] In this sense, a few studies indicated that undifferentiated stem cells of dental pulp (DPSCs & SHEDs) have great potential to differentiate into another cell types and usage in the treatment of necrotic immature permanent teeth.[12,13] These cells are required to be cryopreserved for cellular therapies. However, thawing process was reported to decrease cellular efficiency remarkably.[14] Thus, alternative approaches gain importance to this extent and one of these are *in vitro* biostimulation of cells leading a remarkable increase in cellular expansion. Low-level laser therapy (LLLT) or using the new terminology "*photobiomodulation therapy* (PBMT)" is one of these therapies and PBMT first improved by Marques *et al.* in 2016 as the fourth component of tissue engineering.[15] PBMT has numerous biostimulative effects on proliferation and differentiation of stem cells, regeneration and wound healing via light sources such as light emitting diodes (LEDs), lasers or those that spread light in the visible red to near infrared (NIR) area.[16] Besides the biostimulative effects of PBMT on cellular response and growth, this therapy also promotes bone tissue formation and increasing the expression of growth factors and genes that are related with the differentiation of bone cells stimulating cell proliferation for dentin-pulp complex.[17] However, there is a little knowledge about the cytotoxicity and the proliferative capacity of PBMT on the cryopreserved DPSCs when applied short period of time. This study aimed to assess the possible proliferative and cytotoxic effects of the short term PBMT on long term cryopreserved DPSCs and also to evaluate the most appropriate PBMT protocol for stimulating cellular proliferation that can be used for future tissue engineering approaches.

2. Materials and Methods

2.1. *Cell culture*

The present experimental study had been approved by Ethics Committee of Near East University (NEU/2015/34-243) and followed the principles of Declaration of Helsinki. All subjects gave their informed consent for inclusion before they participated in the study. The experiments were performed at the laboratories of Near East University, Research Center of Experimental Health Sciences. The previously characterized DPSCs (Positive for CD44, CD73, CD90, and CD105; negative for CD11b, CD14, CD19, CD34, CD45, CD79a, and HLA-DR via flow cytometric analyses) that were cryopreserved by participating a fast freezing protocol were used in the experiment. In this fast freezing protocol, cells were trypsinized and followed by centrifugation (1000 rpm/5 min). Cells were then transferred into freezing medium [90% FBS, 10% Dimethylsulphoxide (DMSO)] and transferred to −80°C for 2 years time period. DPSCs at third passage incubated in −80°C for two

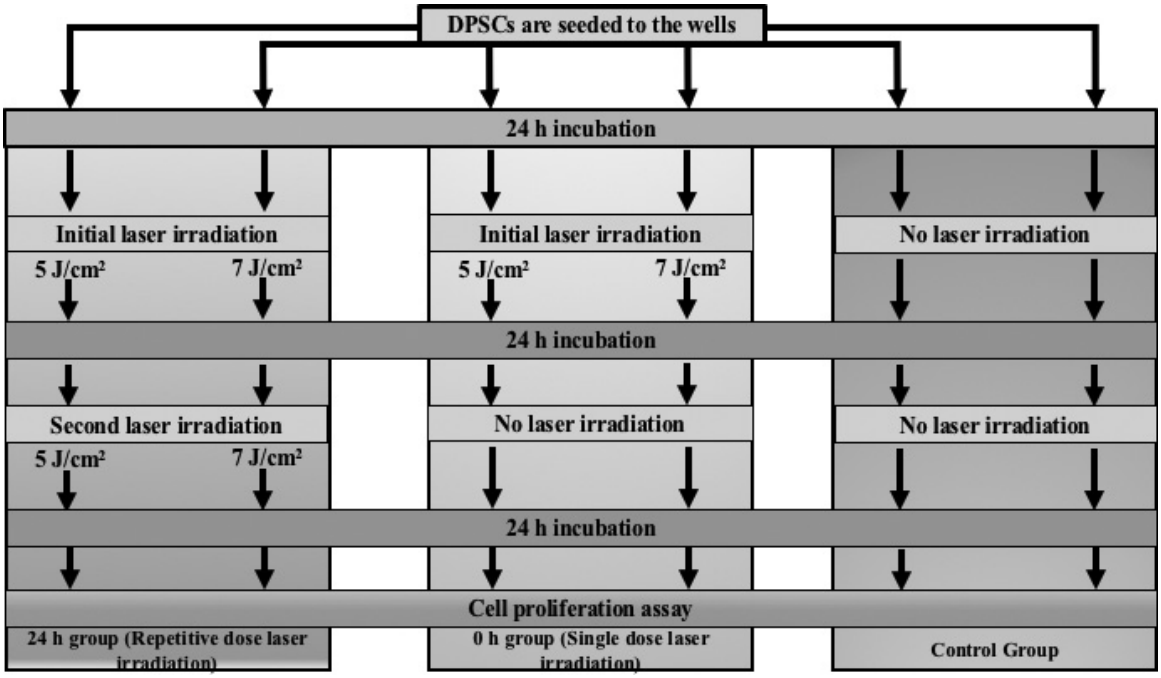

Fig. 1. Graphical representation of experimental design for different PBM therapy used study groups.

years were used. Cells were thawed and maintained in Dulbecco's modified Eagle's medium F12 (DMEM/F12) (Gibco, Switzerland), supplemented with 10% fetal bovine serum (FBS) (Gibco,Switzerland), 2 mM glutamine, 1% of penicillin and streptomycin (Gibco, Visp, Switzerland). The cells were incubated at 37°C, 5% CO_2 incubator and sub-cultured in every second days.

Cells grown at 80% confluence rate were used in the experiments. For this purpose, DPSCs were trypsinized, counted and 2×10^4 cells/ml were transferred to 96-well plates. Cells were maintained in an incubator at 37°C in a humidified atmosphere of 5% CO_2 overnight. After overnight incubation, photobiomodulation therapy was conducted to investigate cell viability and proliferative capacity in all 5 different groups. All assays were studied as triplicates.

2.2. *Experimental design*

The experimental set up aimed to asses the effect of short term PBMT on cellular growth. For this purpose, diode laser irradiation was performed in two different energy densities. The experimental set up was designed to measure the effect of PBMT therapy at two different time points (0 h and 24 h). Fully characterized DPSCs were seperated into three groups according to the laser energy densities (5 J/cm^2 or 7 J/cm^2) applied and a group was served as control in which cells did not receive any laser irradiation.The cells in laser-irradiated groups were further divided into two subgroups according to the period of application (0 h and 24 h). Cells belonging to 0 h groups were subjected to only single dose of laser irradiation; whereas, 24 h groups were subjected to repetitive laser irradiation in every 24 h. In this manner, 0 h groups and 24 h groups were irradiated 1 and 2 times, respectively. The summary of experimental design is represented in Fig. 1.

2.3. *Laser irradiation*

PBMT was applied to four different experimental groups by using a Gallium–Aluminum–Arsenide (GaAlAs) diode laser (Medency Primo, Vicenza, Italy) whereas no laser irradiation was applied to control (untreated) groups. Two different energy densities of diode laser irradiation and two different time intervals (0 h and 24 h) were applied on DPSCs. The desired irradiation densities were performed by applying average power of 0.1 W in continuous mode for 20 s and 28 s at 980 nmwavelength, in order to achieve 5 J/cm^2 and 7 J/cm^2, respectively. The laser probe was measured to be

$0.4\,\text{cm}^2$ diameter. PBMT application was conductedat a height of 0.5 cm to each cell seeded well. Cells were put back into the incubator (37°C) after each PBMT application.

2.4. *MTT assay*

The effect of PBMT treatment on cells was measued by conducting a cholorimetric MTT assay (Acros Organics, New Jersey, USA). In this respect, cells seeded were incubated for 24 h in 96-well microplates with an initial concentration of 5×10^4 cells/ml in incubator (5% CO_2, 37°C). As a detailed and explanatory expression, following overnight incubation, cells in 0 h group were subjected to single laser irradiation and incubated for 24 h. For 24 h group, laser irradition was repeated at the end of each 24 h and then cells were put into incubator again. In this manner, the cells in 0 h and 24 h were subjected to irradiation for 1 and 2-times, respectively. Each cell group was incubated for 24 h after laser irradiation and the end of this incubation periods, the rates of cellular proliferation were analyzed by performing an MTT assay in order to determine the short-term effect of PBMT on cellular proliferation. was added to cells ($1\,\mu$g/ml) and incubated for 4 h. After 4 h incubation, $50\,\mu$l DMSO were added to each well. Cholorimatric differences formed between different groups were measured at $\lambda = 570$ nm by using UV–Visible spectrophotometer (Thermo Multiskan Spectrum). The percentage proliferation of cells was determined according to the following formula:

$$\%\text{proliferation} = \frac{[(\text{absorbance of PBMT appliedcells}) - (\text{Absorbance of blank})]}{[(\text{absorbance of control}) - (\text{absorbance of blank})]}.$$

2.5. *LDH assay*

Cytotoxicity mediated by PBMT on DPSCs was determined by performing an LDH assay (Cayman). Cytosolic lactate dehydrogenase catalyzes the reaction that converts pyruvate to lactate in the presence of NADH. Cells damaged by PBMT were assumed to secrete LDH enzyme into extracellular medium. For this purpose, 5 × 104 cells/ml were transferred to 96 well plates. Laser irradiation protocols were performed likewisely with the MTT study. To determine the maximum release, 10% Triton X 100 were subjected to the cells, without application of any laser irradiation. DPSCs belonging to control group were added spontaneus release. Cells were then incubated for 4 h at 37°C. Cell culture plate (96 well) was centrifuged at 1200 rpm and 100 ml of cell culture medium aliquots was collected and transferred into a new 96-well plate. A 100 ml of substrate was added to each well and incubated for 30 min at room temperature. Plate was read at 490 nm by using spectrophotometer and cytotoxicity of PBMT on DPSCs were evaluated by the following formula:

$$\%\text{Cytotoxicity} = \frac{[(\text{Experimental value A 490}) - (\text{Spontaneous release A 490})]}{[(\text{Maximum release A 490}l) - (\text{Spontaneous release A 490})]} \times 100.$$

2.6. *Statistical analysis*

The data were calculated with mean standard errors. The statistical analysis was performed by using One-way ANOVA, and Tukey's test for multiple comparisons between each group by using GraphPad Prism 5.0 and SPSS. Significance level was determined as 5% ($P < 0.05$).

3. Results

3.1. *MTT assay*

The proliferative effect of short-term PBMT application in two different energy densities ($5\,\text{J/cm}^2$ and $7\,\text{J/cm}^2$) and time intervals (0 h and 24 h) was determined by participating MTT assay. Among the control and laser irradiated groups, $7\,\text{J/cm}^2$ energy densities were reported to have significant proliferative effect in both periods of time 0 h and 24 h ($P < 0.001$) whereas $5\,\text{J/cm}^2$ energy density were found to induce significant proliferation at 24 h only ($P < 0.05$) (Fig. 2). DPSCs belonging to same groups indicated a stronger proliferative effect in 24 h compared to 0 h ($P < 0.01$) upon irradiation with $7\,\text{J/cm}^2$ energy density, whereas no significant differences were detected when cells are irradiated with $5\,\text{J/cm}^2$ energy densities in both 0 h and 24 groups (Fig. 2). Application of $7\,\text{J/cm}^2$ energy density indicated significant proliferation compared to

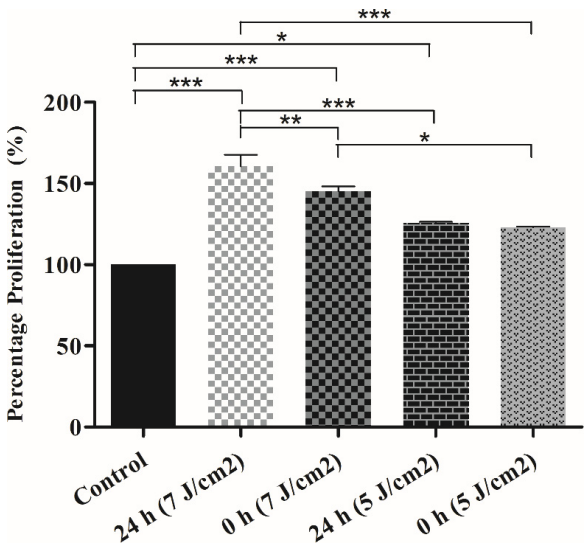

Fig. 2. Proliferation of dental pulp stem cells incubated with two different doses of laser irradiation ($5\,J/cm^2$ and $7\,J/cm^2$) for short period of time (0 h, 24 h) evaluated by MTT assay. Values are the mean percentage viabilities ± standard deviation ($*p < 0.05$, $**p < 0.001$, $***p < 0.0001$ One-way ANOVA, and Tukey's test for multiple comparisons).

$5\,J/cm^2$ ($P < 0.001$) at both time intervals. Intergroup comparisons of DPSCs also indicated that irradiation with $7\,J/cm^2$ energy density revealed more significant proliferative effect than $5\,J/cm^2$ at 0 h ($P < 0.05$) (Fig. 2).

3.2. *LDH assay*

Level of cellular damage was investigated via participating LDH assay. Data obtained indicated that there is no significant cellular damage caused by diode laser irradiation regarding energy densities ($5\,J/cm^2$ and $7\,J/cm^2$) and time periods (0 h and 24 h) when compared to nonirradiated control group (Table 1).

4. Discussion

Stem cells have self-renewable and multiple differentiation capabilities. Dental pulp tissue is regarded as a rich adult mesenchymal stem cell (MSC) source in dentistry field. Stem cells originated from dental pulp exhibit a great interest for tissue regeneration and stem cell therapies due to their simple harvesting and high proliferation properties.[7,8,18,19] Besides all advantages of MSCs, controllability of their viabilities, proliferative capacities, differentiation abilities and the loss of their high clonogenicity

Table 1. Measuring the percentage cytotoxicity of DPSCs irradiated with two different doses of laser irradiation ($5\,J/cm^2$ and $7\,J/cm^2$) for short period of time (24 h, 0 h) via LDH assay (One-way ANOVA, and Tukey's test for multiple comparisons).

Group	*Percentage Cytotoxicity* (%) ± *Std Dev*
Control (**A**)	8 ± 5
24 h ($7\,J/cm^2$) (**B**)	8 ± 3
0 h ($7\,J/cm^2$) (**C**)	11 ± 1
24 h ($5\,J/cm^2$) (**D**)	13 ± 4
0 h ($5\,J/cm^2$) (**E**)	14 ± 1
Inter Group Comparisons	*P values*
A-B	Ns
A-C	Ns
A-D	Ns
A-E	Ns
Intra Group Comparisons	*P values*
B-C	Ns
D-E	Ns

during cellular transfer is still acting as a barrier affecting the fate of tissue engineering applications.[20] Long-term cryopreservation and various types of cryopreservation protocols may also create different post-thaw problems such as decreased cell viability and proliferation rate of cells.[21] For handling with aforementioned problems, PBMT is regarded a potential candidate for supporting cellular and molecular inducement by new developments in biomedical technology. Hence, this study was conducted to assess the cellular proliferative and apoptotic effects of different PBMT parameters on long-term cryopreserved DPSCs. PBMT works out with the stimulation of mitochondrial molecules. For instance, cytochrome C (cyt C) in its intermediate structures acts as a major photoacceptors of PBMT under stress conditions. When cyt C is stimulated, reactive oxygen species (ROS) is generated by pumping of more hydrogen protons into the inner part of mitochondria. In another words, this process could be explained by the conversion of absorbed laser energy to metabolic energy.[22,23] Regarding that, PBMT could provide a growth of DPSCs and maintenance of their viability, this therapy might be a therapeutic tool for tissue engineering and regenerative dentistry.[24] Diode laser was the most popular light source used in PBMT. Several studies reviewed that diode laser

with different wavelengths; different types of stem cells, different energy densities ranged between 0.3–9 J/cm^2 can increase the cellular viability, multi-lineage differentiation and proliferation of MSCs.[25,26] Proper application of an optimal laser irradiation and selection of correct parameter for PBMT in clinical settings are very important issues due to lack of adequate studies. Our previous research about the therapeutic effect of GaAlAs diode laser suggests that the therapeutic energy densities of GaAlA diode laser ranged between 2 J/cm^2 and 10 J/cm^2.[27] Review of data in the literature revealed that Helium–Neon (HeNe) lasers and diode lasers such as Gallium–Aluminum–Arsenic (GaAlAs), Arsenic–Gallium (AsGa), and Indium–Gallium–Aluminum–Phosphide (InGaAlP) (wavelengths of 660 nm, 810 nm, and 980 nm) with energy densities of 0.1–3 J/cm^2 were effective in increasing the proliferation of DPSCs under without stress conditions (such as long-term cryopreservation, oxidative stress). However, it has been reported that DPSCs started to exhibit biostimulatory cellular proliferation response following PBM therapy at 5 J/cm^2 under stress conditions.[28] Thus, the short-term (0 h and 24 h) proliferative potential of PBMT on long-term cryopreserved DPSCs with different low-level energy densities (5 J/cm^2 and 7 J/cm^2) of diode laser was investigated in this study to present more exact biostimulatory energy densities. First, the cytotoxic effect of PBMT on cells was determined using LDH assay analysis. LDH is a cytoplasmic enzyme that is naturally present in human body tissues and is secreted extracellulary in case of cell damage or death caused from certain drugs, ischemia, excessive heat/cold, dehydration, trauma, bacterial toxins. Hence, extracellular LDH secretion acts as an indicator of cell-membrane destruction.[29,30] In this study, no cytotoxicity (extracellular LDH release) was detected in each experimental group's culture medium after subjected to PBMT with both energy densities. Even though no cytotoxicity was determined, it could be better to support this result with a different cytotoxic assay. Since different cytotoxic concentrations may be taken by numerous cytotoxic assays. Additionally, in this study, long-term cryopreserved DPSCs were preferred instead of using freshly isolated DPSCs because to the best of our knowledge, there is a clear indication over the biostimulative effect of PBM therapy on fresh DPSCs. But there was no data analyzing the effect of PBM treatment on DPSCs that were thawed after long time period (24 months). However, in the future, we could plan another study to compare the effect of PBM therapy with a wide range of laser fluence and/or time of irradiation on freshly isolated DPSCs and thawed DPSCs. All of these points could be accepted the major limitations of this study.

The main MTT assay results of this study demonstrated that DPSCs that irradiated with energy density of 7 J/cm^2 for both of time intervals (0 h and 24 h) showed higher cellular proliferation rate compared to nonirradiated control group. But the same manner was not observed on DPSCs irradiated with energy density of 5 J/cm^2 for 0 h. Only the cells irradiated with energy density of 5 J/cm^2 for 24 h exhibited higher cell growth than nonirradiated group. The cellular growth and proliferation abilities of DPSCs used in this study might be affected adversely with uncontrolled rapid freezing protocol. Another important issue is related with intracellular ice formations during rapid cooling. In the context of rapid cooling, water cannot leave the cell as they were directly put into −80°C. This condition might lead to formation of supercooling effect on cellular cytoplasm hence building up of intracellular ice crystals. Following the formation of these ice crystals in confluent monolayers of DPSCs may damage the membrane integrity, the cells lose the ability for proliferation.[31] In this study, ineffective stimulation of energy density of 5 J/cm^2 on long-term cryopreserved DPSCs for proliferation was observed. Most probably, effective dose threshold of PBMT on long-term cryopreserved DPSCs could not be reached and it's not able to create stimuli on cell growth of DPSCs. Additionally, the inadequate biostimulatory effect of energy density of 5 J/cm^2 on long-term cryopreserved DPSCs for proliferation at 0 h time points could be correlated with repeated and a single dose irradiation protocols because the prolonged cumulative effects of laser therapies with repetitive dose irradiations were distinctly shown by Huang *et al.*[32,33] In this study, the clinical significance second irradiation after 24 h could be clarified by cumulative–prolonged effect of PBM therapy and using lower power outputs on vital tissues. The data of MTT and LDH assays suggest that cellular viabilities of DPSCs could be increased by PBMT without creating any cytotoxic effect on cells.

As a result, according to the findings of this study, it may conclude that PBMT at 7 J/cm^2 should be an effective parameter in case of necrotic

immature teeth to stimulate proliferation of DPSCs in a short time period without affecting cell viability and without causing cellular cytotoxicity. Photobiomodulation therapy may be an upcoming tool for future tissue enngineering and regenerative dentistry applications by representing a therapeutic opportunity. That could describe the reason behind why PBMT should be applicated with different parameters and microenvironment protocols that near clinical conditions.

Clinical Significance

The repeated application of photobiomodulation therapy might be an useful tool to ensure a prolonged positive biostimulatory effect on long-term cryopreserved DPSCs prior to usage of cells in regenerative dentistry and tissue engineering field.

Conflict of Interest

The authors declare that there is no conflict of interest relevant to this article.

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References

1. U. Ripamonti, "Soluble, insoluble and geometric signals sculpt the architecture of mineralized tissues," *J. Cell Mol. Med.* **8**, 169–180 (2004).
2. E. Alsberg, E. E. Hill, D. J. Mooney, "Craniofacial tissue engineering," *Crit. Rev. Oral Biol. Med.* **12**, 64–75 (2001).
3. H. Jin, J. Y. Park, H. Choi, P. H. Choung, "Inhibitor trichostatin A promotes proliferation and odontoblast differentiation of human dental pulp stem cells," *Tissue Eng. Part A.* **19**(5–6), 613–624 (2013).
4. Y. Cao, M. Song, E. Kim, W. Shon, N. Chugal, G. Bogen, L. Lin, R. H. Kim, N. H. Park, M. K. Kang, "Pulp-dentin regeneration: Current state and future prospects," *J. Dent. Res.* **94**, 1544–1151 (2015).
5. G. T. Huang, S. Gronthos, S. Shi, "Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine," *J. Dent. Res.* **8**, 792–806 (2009).
6. J. Liu, F. Yu, Y. Sun, B. Jiang, W. Zhang, J. Yang, G. T. Xu, A. Liang, S. Liu, "Concise reviews: Characteristics and potential applications of human dental tissue-derived mesenchymal stem cells," *Stem Cells* **33**, 627–638 (2015).
7. S. Gronthos, M. Mankani, J. Brahim, P. G. Robey, A. Shi, "Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo," *Proc. Natl. Acad. Sci. USA* **97**(25), 13625–13630 (2000).
8. M. Miura, S. Gronthos, M. Zhao, B. Lu, L. W. Fisher, P. G. Robey, S. Shi, "SHED: Stem cells from human exfoliated deciduous teeth," *Proc. Natl. Acad. Sci. USA.* **100**(10), 5807–5812 (2003).
9. B. M. Seo, M. Miura, S. Gronthos, P. M. Bartold, S. Batouli, J. Brahim, M. Young, P. G. Robey, C. Y. Wang, S. Shi, "Investigation of multipotent postnatal stem cells from human periodontal ligament," *Lancet.* **364**, 149–155 (2004).
10. W. Sonoyama, Y. Liu, T. Yamaza, R. S. Tuan, S. Wang, S. Shi, G. T. J. Huang, "Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: A pilot study," *J. Endod.* **34**(2), 166–171 (2008).
11. I. Kerkis, A. I. Caplan, "Stem cells in dental pulp of deciduous teeth," *Tissue Eng. Part B Rev.* **18**(2), 129–138 (2012).
12. S. Corbella, G. Ferrara, A. El Kabbaney, S. Taschieri, "Apexification, apexogenesis and regenerative endodontic procedures: A review of the literature," *Minerva Stomatol.* **63**(11–12), 375–389 (2014).
13. D. E. Martin, J. F. De Almeida, M. A. Henry, Z. Z. Khaing, C. E. Schmidt, F. B. Teixeira, A. Diogenes, "Concentration dependent effect of sodium hypochlorite on stem cells of apical papilla survival and differentiation," *J. Endod.* **40**(1), 51–55 (2014).
14. N. Pilbauerová, J. Suchánek, "Cryopreservation of dental stem cells," *Acta Medica (Hradec Králové).* **61**(1), 1–7 (2018).
15. M. M. Marques, I. M. Diniz, S. P. De Cara, A. C. Pedroni, G. L. Abe, R. S. D'Almeida-Couto, P. L. Lima, T. K. Tedesco, M. S. Moreira, "Photobiomodulation of dental derived mesenchymal stem cells: A systematic review," *Photomed. Laser Surg.* **34**(11), 500–508 (2016).
16. R. O. Poyton, K. A. Ball, "Therapeutic photobiomodulation: Nitric oxide and a movel function of mitochondrial cytochrome c oxidase," *Discov. Med.* **11**, 154–159 (2011).
17. F. Mangione, M. EzEldeen, C. Bardet, J. Lesieur, M. Bonneau, F. Decup, B. Salmon, R. Jacobs, C. Chaussain, S. Opsahl-Vital, "Implanted dental pulp cells fail to induce regeneration in partial pulpotomies," *J. Dent. Res.* **96**(12), 1406–1413 (2017).
18. S. Gronthos, "The therapeutic potential of dental pulp stem cells: more than pulp fiction?," *Cytotherapy.* **13**(10), 1162–1163 (2011).

19. N. Kawashima, "Characterisation of dental pulp stem cells: A new horizon for tissue regeneration?," *Arch. Oral Biol.* **57**(11), 1439–1458 (2012).
20. C. Xinaris, M. Morigi, V. Benedetti, B. Imberti, A. S. Fabricios, E. Squarcina, A. Benigni, E. Gagliardini, G. Remuzzi, "A novel strategy to enhance mesenchymal stem cell migration capacity and promote tissue repair in an injury specific fashion," *Cell Transplant.* **22**(3), 423–436 (2013).
21. E. Ratcliffe, R. J. Thomas, D. J. Williams, "Current understanding and challenges inbioprocessing of stem cell-based therapies for regenerative medicine," *Brit. Med. Bull.* **100**(1), 137–155 (2011).
22. T. Karu, "Primary and secondary mechanisms of action of visible to near-IR radiation on cells," *J. Photoche. Photobiol. B.* **49**(1), 1–17 (1999).
23. V. J. Thannickal, B. L. Fanburg, "Reactive oxygen species in cell signaling," *Am. J. Phys. Lung Cell Mol. Phys.* **279**(6), L1005–L1028 (2000).
24. I. M. Zaccara, F. Ginani, H. G. Mota-Filho, A. C. Henriques, C. A. Barboza, "Effect of low-levellaser-irradiation on proliferation and viability of human dental pulp stem cells," *Lasers Med. Sci.* **30**(9), 2259–2264 (2015).
25. I. M. Zaccara, L. B. Mestieri, M. S. Moreira, F. S. Grecca, M. D. Martins, P. M. Poli Kopper, "Photobiomodulation therapy improves multi-lineage differentiation of dental pulp stem cells in three dimensional culture model," *J. Biomed. Optics.* **23**(9), (095001-1)–(095001-9) (2018).
26. R. Fekrazad, S. Asefi, M. Allahdadi, K. A. M. Kalhori, "Effect of photobiomodulation on mesenchymal stem cells," *Photomed. Laser Surgery.* **34**(11), 533–542 (2016).
27. H. Y. Yılmaz, B. K. Seker, H. Bayındır, T. F. Tözüm, "Low-level laser therapy in the treatment of mucous membrane pemphigoid," *J. Periodontol.* **81**(8), 1226–1230 (2010).
28. A. B. Farahani, "Effect of low-level laser irradiation on proliferation of human dental mesenchymal stem cells; a systemic review," *J. Photochem. Photobiol. B.* **162**, 577–582 (2016).
29. M. Drent, N. A. M. Cobben, R. F. Henderson, E. F. M. Wouters, M. V. Diejen-Visser, "Usefulness of lactate dehydrogenase and its isoenzymes as indicators of lung damage or inflammation," *Eur. Respir. J.* **9**, 1736–1742 (1996).
30. L. Yu, J. Yang, X. Wang, B. Jiang, Y. Sun, Y. Ji, "Antioxidant and antitumor activities of Capparis spinosa L. and the related mechanisms," *Oncol. Rep.* **37**(1), 357–367 (2017).
31. M. Zhurova, E. J. Woods, J. P. Acker, "Intracellular ice formation in confluent monolayers of human dental stem cells and membrane damage," *Cryobiology* **61**(1), 133–141 (2010).
32. Y. Y. Huang, A. C. H. Chen, J. D. Carroll, M. R. Hamblin, "Biphasic dose response in low level light therapy," *Dose Response.* **7**(4), 358–383 (2009).
33. A. Mirzaei, M. A. R. Ladiz, F. J. Jouni, S. S. Hendi, R. N. Vosough, H. H. Zehi, L. Gholami, "Effects of 980 nm diode laser ırradiation on gingival fibroblasts and periodontal ligament stem cells," *Acta Scientific Microbiol.* **3**(1), 50–54 (2020).