Review

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The effects of low level laser irradiation on proliferation of human dental pulp: a narrative review

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Abstract

Mesenchymal stem cells (MSCs) have the capability for self-renewal, proliferation, and differentiation in various types of specialized cells, so they are very important in cellular therapies. MSC from dental pulp are simply obtainable and have high proliferative capability. Among the therapies that can stimulate the proliferation of certain cell types, low-level laser therapy (LLLT) stands out. The target of this study is to perform a literature review to investigate these effects of low-level laser irradiation on proliferation of human dental pulp.The electronic search of scientific papers was conducted in the Lilacs, Scielo, Medline and PubMed databases through scientific articles published in national and international journals in the past 20 years.The results of this review suggest that LLLT may be a useful and important tool for future advances in cell therapy and tissue engineering associated to stem cells. Studies on cell therapy for regenerating dental tissues has already been done, and shows promising results. ***Clin Ter 2017; 168(5):e320-326. doi: 10.7417/CT.2017.2028***

Key words: *Low level laser therapy, mesenchymal stem cells, proliferation*

Introduction

The stem cells, used in tissue regeneration, are among the main topics of interest to the scientific community; being associated with the treatment of a wide range of diseases (1) Mesenchymal stem cells (MSCs) have the capacity for self-renewal, proliferation, and differentiation in various types of specialized cells, so they are very important in cellular therapies (2). MSC have been isolated already from many tissues such as bone marrow, (3) umbilical cord, (4,5) dental pulp, (6) periodontal ligament, (7)and adipose tissue (8).

Stem cells can be simply found in many peripheral tissues; however, it is not easy to isolate these cells without damaging the tissue of origin.The stem cells can be isolated from the dental pulp, being derived from dental pulp of adult subjects (dental pulp Stem Cells - DPSC) or derived from an exfoliation of a tooth deciduous (stem cells of human exfoliated deciduous teeth - SHED). These tissues are simply obtainable, have a high proliferative capacity and an important cellular plasticity, which are relevant characteristics for possible therapeutic applications (9).

Among the therapies that can stimulate the proliferation of certain cell types, low-level laser therapy (LLLT) stands out. Lasers (Light amplification by stimulated emission of radiation) are devices that generate electromagnetic radiation relatively uniform in wavelength, phase, and polarization (9). The concept of using LLLT is to supply direct biostimulative light energy to body cells. Absorbed laser energy causes stimulation of molecules and atoms of cells (10). This therapy has been reported to be responsible for promoting and stimulating effects photobiomodulation in vivo and in vitro, stimulate cell development, growing cell metabolism and enhance the regeneration of triggering cells and also an anti-inflammatory response (11). The positive effect of LLLT was studied on the proliferation of certain cell types, but not much is known about these effects on stem cells from dental pulp (DPSCs).

The target of this study is to perform a literature review to investigate these effects of low-level laser irradiation on proliferation of human dental pulp.

Materials and methods

The methodology chosen for this article was the narrative review. Narrative literature review articles are publications that describe and discuss the state of the science of a specific topic or theme from a theoretical and contextual point of view. This modality of review differ from systematic reviews in many ways, tend to be mainly descriptive, do not involve a systematic search of the literature, and thereby often focus on a subset of studies of a certain topic chosen based on availability or author selection (12).

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Narrative literature review articles have an important function in continuing education because they provide readers with up-to-date knowledge about a specific topic or theme (13).

Search strategy

Eligible papers were signalized as in vitro experimental studies which were evaluated the use of laser therapy on stem cells in proper to promote their proliferation. The electronic search of scientific papers was conducted in the Lilacs, Scielo, Medline and PubMed databases through scientific articles published in national and international journals in the past 20 years. The descriptors below (keywords) used by it self and in combination, were surveyed: low-power laser therapy, mitosis, cell proliferation, laser therapy, stem cells and dental pulp. As inclusion criteria, the articles needed to have availability of access to full text.

Eligibility Criteria: The selection criteria were original articles, use of statistical methods; intervention (the effects of LLLT on proliferation of human dental pulp) (Table 1).

Articles that propound, to evaluate the biomodulator properties of the laser, additionally classic works on the biological properties of laser and stimulating stem cells were selected. The authors, in the selected articles, used research methods in vitro and in vivo to low intensity laser study, and other types of laser, comparing their inductive results between experimental and control groups.

Data Extraction and Quality Assessment: Data extraction was carried out with the same strategy of the selection of the studies.

A quality assessment was performed according to the INSA (International Narrative Systematic Assessment) tool (14,15).

Different parameters of use that phototherapy with LLLT has shown to be efficacioust in promoting the proliferation of different cells. Soares et al. (16) obtained good results in his work using an InGaAlP laser, power of 30 mW, wavelength of 660 nm, and energy densities of 0.5 and 1.0 J/cm^2, which promoted the biostimulation of DPSCs.

Eduardo et al. (6) conducted an in vitro study to evaluate the potential effect of laser phototherapy on DPSC proliferation and found a elevated proliferative activity of stem cells from human dental pulp subjected to laser irradiation (InGaAlP) of 660nm and 20mW an energy density of 3.0 J/cm compared to non-irradiated control under the same conditions of nutrition. Cells cultured under nutritional deficit were either irradiated or not (control group) using two different power settings (20 mW/6 seconds to 40 mW/3 seconds), with an InGaAIP diode laser. The cell growth was indirectly assessed by measuring the cell mitochondrial activity.

Barboza et al. (17) acquired good results in his work with mesenchymal stem cells derived from bone marrow and adipose tissue. It was used two applications (T0 and T48 hours) of LLLT (660nm; doses of 0.5 and 1.0J/cm²). Growth curves were used to evaluate proliferation at zero, 24, 48, and 72 hours. Higher cell growth was observed when the cells were irradiated with a dose of 1.0J/cm², particularly after 24 hours ($p<0.01$). Adipose derived mesenchymal stem cells reacted better to a dose of 1.0J/cm², but higher cell proliferation was observed after 48 hours ($p<0.05$) and 72 hours ($p<0.01$). It was not detected nuclear alterations or significant change in cell viability.

Leonida et al. (18) in a study with marrow bone showed the effects of Nd:Yag laser (15 Hz, 100 mJ, 1.5 W and 15 Hz, 150 mJ, 2.25W) irradiation on proliferation and differentiation of MSCs. MSCs were collected from adult human marrow bone, isolated, and cultured in complete medium (α MEM). Afterwards, they were treated with osteogenic medium, seeded in three dimensional collagen scaffolds, and incubated. Evaluations were performed at 7 and 14 days. After 7 days, proliferation was significantly increased in samples treated with laser. After 14 days, laser irradiation did not appear to have any additionaleffect on cell proliferation.

Zaccara et al. (19) conducted a study in which DPSCs were divided into three groups according to treatment: control group (no irradiation); laser 0.5 (cells irradiated with an energy density of 0.5 J/cm^2); and laser 1.0 (cells irradiated with 1.0 J/cm^2). An InGaAlP diode laser, operating at the following parameters was used: power of 30 mW, wavelength of 660 nm, continuous action mode, and a tip diameter of 0.01 cm^2. The cells were irradiated at 0 and 48 h. The autohor obtained great results in his work, and was constatad an important increase of cell proliferation.

Ginani et al. (20) analyzes that in relation to the wavelength, although both red and infrared light have been descriptied to be effective for wound healing, pain attenuation, and cellular response in general, the literature reports that the spectrum of visible light (red), ranging from 600 to 700 nm, provides more impressiveresults.

The authors also highlight the need for more studies using various associations of wavelengths and doses in different cell lines isincontestable, in order to standardize the experimental designs and then to allow the correlationof results, making it possible to establish the more appropriate protocol for each cell type (20).

Results

Stem Cells

Stem cells aredescribed as undifferentiated cells that can proliferate indefinitely and have the capacity for self-renewal and differentiation to one or more types of specialized cells (8). De Sá Silva et al (1) affirms that in terms of clinical safety for the patient, choosing the stem cells that will be used in cellular therapy is decidedly important since the scientific literature has been reporting the teratogenic potential of embryonic stem cells and iPSCs (human induced pluripotent stem cells). The hematopoietic origin cells are the cells most commonly reported in scientific studies.

The International Society for Cellular Therapy has recommended the following minimum criteria for defining multi potent human MSCs: adherence to plastic under standard culture conditions; positive for expression of CD105, CD73, and CD90 and negative for expression of the hematopoietic cell surface markers CD34, CD45, CD11a, CD19 or CD79a, CD14 or CD11b, and histocompatibility locus antigen (HLA)-DR; under a specific stimulus, dif-ferentiation into adipocytes, osteocytes, and chondrocytes *in vitro*. To date,

Table 1. Studies about the effects of LLLT on proliferation of human dental pulp.

Author	Laser	Wavelength	Parameters	Time	Results
Eduardo et al. (2008)	InGaAlP	660nm	20mW/3J/cm²	6 seg	The group irradiated with the 20 mW setting presented significantly higher MTT activity at 72 hours than the other groups. After 24 hours / first irradiation: cultures grown under nutritional deficit (10% Fetal Bovine Serum) and irradiated presented significantly higher viable cells than the non-irradiated cultures grown under the same nutritional conditions. ↑
			40mW/3J/cm²	3 seg	
Soares et al . (2013)	InGaAlP	660nm	30mW/0.5 J/cm²	16s	The group irradiated with 1.0 J/cm² presented significantly higher MTT activity and improved the cell proliferation when compared to the energy density of 0.5 J/cm². ↑
			30 mW/1 J cm²	33 s	
Barboza et al. (2014)	InGaAlP	660nm	30 mW /0.5 J/cm²	16 s	Higher cell proliferation in the group treated with 1.0J/cm, but this difference was only significant after 24 hours. ↑
			30 mW /1 J cm²	33 s	
Leonida et al. (2012)	Nd:Yag	1,064 nm using 100 µs pulsed time for each sample irradiated.	15 Hz,100mJ, 1.5 W	3 Irradiations cicles/ 30 s each intervals.	7 days after irradiation: biostimulating effect on bone marrow-derived MSCs. Different energies of laser irradiation (100 or 150 mJ) seem to generate no differences between groups. LLLI is able to accelerate cell proliferation also in a tridimensional structure. ↑
			15 Hz,150 mJ,2.25 W		
Zaccara et al. (2015)	InGaAlP	660nm	0.5 J/cm²	Continuous action mode at 0 and 48 h	Significant increase of cell proliferation particularly a dose of 1.0 J/cm². Growth of DPSCs and maintenance of its viability ↑
			1 J /cm²		
Ginani et al. (2015)	Lit. review	600-700 nm	-	-	↑ cellular proliferation
Peplow et al. (2010)	Lit. review	600-700 nm	-	-	↑ cellular proliferation
		810-830 nm			↓ inhibitory effect
Giuventu (2014)	Nd:YAG	1064-10600 nm	4-50 Hz -27-90 mJ-0.5-10 W	Continuous action mode	Theproliferation rate of cells isolated from whole cryopreserved teeth (in which the holes with the laser have not been practiced) is very low, both in the presence and absence of the cryopreservation. Biostimulating effect. ↑

human bone marrow represents the majorsource of MSCs and has been applied for cell based therapies. However, some findings have suggested that epithelial cancers can be originated from marrow bone derived of MSCs.Thus, undoubtedly, a study of carcinogenic potential sources of many adults MSCs is very important (1).

The greatest challenge in the use of stem cells in cellular therapy is the ability to maintain genetic integrity and their ability to differentiate during long-term cultivation. By successive passages in vitro, the karyotype needs to be intact, conferring genomic stability to the cells that will beused in cellular therapy (1,21,22).

The decision between cell self-renewal and differentiation is influenced by a specialized microenvironment called the 'stem cell niche'. In the tooth, stem cell niches are formed at specific anatomic locations of the dental pulp and regulate how dental pulp stem cells participate in tissue maintenance, repair, and regeneration. Stem cells can be identified by their expression of certain genes and proteins. One such protein expressed on surface membranes of stem cells is Thy-1 that is used as a marker for a diversity of stem cells, including MSCs (23).

MSCs are most widely used in tissue engineering and can be obtained from a wide variety of sources, including bone marrow, adipose tissue, umbilical cord, and dental pulp.In the dental pulp, MSCs are known to play an important role during dental pulp tissue healing. In fact, the dental pulp holds cells in multiple stages of commitment, assisting in tissue homeostasis(1,16, 24). Many cytotypes can be obtained from the DPSCs. These cells can be obtained from exfoliated teeth or after extraction teeth and their collection can be madeeasily (25).

Saito et al. 2015 (26) affirms that dental tissues have also been investigated as niches of MSCs, and many toothderived stem cells have been identified, including dental pulp stem cells, stem cells from human exfoliated deciduous teeth, periodontal ligamentstem cells, dental follicle progenitor cells, and stem cells from apical papilla. As extracted/exfoliated teeth represent a waste product of dental procedures, dental tissues are an accessible source of MSCs that can be obtained with limited morbidity and without additional risks to the donor.

Recent studies demonstrate that DPSCs can differentiate into adipocyte cells when supplements are added to the adipogenic induction medium. (26,27,28) When transplanted *in vivo*, some DPSC differentiate into odontoblast-like cells and dentin-like structures (26,27,29).

According to D'aquino et al. (30), the dental pulp is an outstanding site of stem cells. These cells can be collected by a non-invasive practice that can be performed after surgical extraction of wisdom teeth, a common dentalpractice. Tissue sacrifice is very low when collecting DPSCsand these cells can be cryopreserved and stored for long periods. Several cytotypes can be obtained from DPSCs owing to their multipotency. Transplantation of new formed bone tissue obtained from DPSCs leads to the formation of vascularized adult bone and integration between the graft and the surrounding blood supply. Dental pulp is ideal for tissue engineering, for clinical use in several pathologies requiring bone tissue growth and repair. The authors also proposedthat 'if bone marrow is the site of first choice for hematopoietic stem cell collection, dental pulp must be considered as one of the major sites for mesenchymal cell collection'. The use of the laser to stimulate DPSCs is a promising tool to obtain viable cells, especially for the simplicity of the technique and accessibility (25,30).

According to Saito et al. (26), the best method to select the most appropriate MSC type for regenerating dental tissues is not yet clear. Although stem cells derived frommarrow bone, dental pulp, human exfoliated deciduous teeth, periodontal ligament, dental follicle progenitor, and apical papilla present a common marker profile, they differ in their proliferative ability, clonogenicity and differentiation potential in vitro and in vivo, suggesting that these properties are related to the microenvironments of origin of each cell lineage. Supplementary it has been noted that, even in the same population of MSCs, there are heterogeneous cell subpopulations with distinct differentiation potentials. Further advances in understanding the regulation of MSCs during differentiation and dental development are required in order to develop new approaches for dental tissue regeneration with predictable results (26).

In a study in dogs, Iohara et al. shows the complete pulp regeneration was achieved when $CD105^{+}$ DPSCs with stromal cell-derived factor-1 were transplanted into pulp (31,26). The pulp CD105(+) cells highly manifested angiogenic/ neurotrophic factors compared with other cells and localized in the vicinity of newly formed capillaries after transplantation, demonstrating its potent trophic effects on neovascularization.It was demonstrated that the qualitative and quantitative protein and mRNA expression patterns of the regenerated pulp were similar to those of normal pulp. (31) This study suggests a promising and revolutionary future for the functional reconstruction of dental pulp tissue in next generation of dentistry. Dental regenerative medicine has made the most progress and is the most useful model for the consideration of strategies in future bioengineering therapies (32).

Low Level Laser Irradiation and Dental Pulp Stem cells.

The principle of using LLLT is to supply direct biostimulative light energy to body cells. Absorbed laser energy causes stimulation of molecules and atoms of cellswithout significantly increasing the tissue temperature (33).

Pinheiro et al. (34) recommended lower doses for the irradiation of mucosa and skin because the absorption and spreading of light are greater due to the lack of an optical barrier. The authors also highlights that lower doses should be used in cases of biostimulation and proliferation of stem cells (17).

The LLLT has been reported to be responsible for promoting and stimulating effects of photobiomodulation, stimulate cell growth, increase cell metabolism and enhance the regeneration of triggering cells and also an anti-inflammatoryresponse (11).

Low-level laser irradiation stimulated the proliferation of mouse MSCs without causing nuclear alterations.This energy has been shown to induce stem cell activity by increasing cell migration, proliferation, and viability, activating protein expression, and inducing the differentiation of progenitor cells (35). The biostimulation of MSCs using laser therapy might be an important tool for regenerative therapy and

tissue engineering (17). Aspects of the laser can influence the desired results, such as the ideal light spectrum, energy density, power level, and wavelength (16).

Peplow et al. (36) affirms that comparison between the previous studies is difficult due to the wide range of irradiation parameters, methodologies and cell types used. Recent studies have shown that the best results are obtained when the visible light spectrum (600 to 700 nm) is used (37). In contrast, the infrared light spectrum (810 to 830 nm) has been associated with the inhibition of proliferation (19, 38).

Soares et al. (16) perceived a biostimulatory effect of LLLT on periodontal ligament stem cells using a lower energy density (1.0 J/cm^2) and advocates that the energy density is an eminent factor that influences the results of cell proliferation. According to Karu (39), an increase in energy density may damage the photoreceptors, with a resultant reduction in the biomodulatory effect of LLLT (19).

Zaccara et al. (19) conducted a survey in which human DPSCs were isolated from two healthy third molars. The cells were irradiated with an InGaAlP diode laser at 0 and 48 h using two different energy densities (0.5 and 1.0 J/cm²). Cell proliferation and viability and mitochondrial activity were evaluated at intervals of 24, 48, 72, and 96 h after the first laser application, and compared with control group (no irradiated). The group irradiated with an energy density of 1.0 J/cm² exhibited a significant increase of cell proliferation when compared to the control group at 72 and 96 h. No indicative changes in cell viability were observed throughout the experiment. LLLT, particularly a dose of 1.0 J/cm², contributed to the development of DPSCs and maintenance of its viability. This fact indicates this therapy to be an important tool for tissue engineering and regenerative medicine involving stem cell.Wu et al.(40) reported that LLLT suppresses inflammatory response of human adipose-derived stem cells. Those results indicatethat LLLT can potentially be applied in anti-inflammatory therapy followed by stem cell therapy.

Barbosa et al. (17) uses growth curves to establish the proliferation of cells that were submitted to laser therapy. In this study, BMSCs responded to laser therapy in a dose-dependent manner, and a similar cell proliferation curve at the two doses tested (0.5 and 1.0J/cm^2) and a cumulative action of these doses over time was noticed. Anyhow, a higher proliferation rate was observed for cells irradiated with 1.0J/cm^2, especially after 24 hours of culture. The authors showed that LLLT promoted the proliferation of bone marrow-derived MSCs and adipose tissue-derived MSCs. It can be concluded that the use of laser phototherapy can improving the cell growth of other types of stem cells.

Eduardo et al. (6) conducted a study in which DPSC cell are used. The group irradiated with the 20 mW setting presented significantly higher mitochondrial activity at 72 hours than the other two groups (negative control and lased 40 mW with 3 seconds exposure time). After 24 hours of the first irradiation, cultures grown under nutritional deficit and irradiated presented significantly higher viable cells than the non-irradiated cultures grown under the same nutritional conditions. It can be concluded that the DPSC responds positively to laser phototherapy by improving the cell growth when cultured under nutritional deficit conditions.

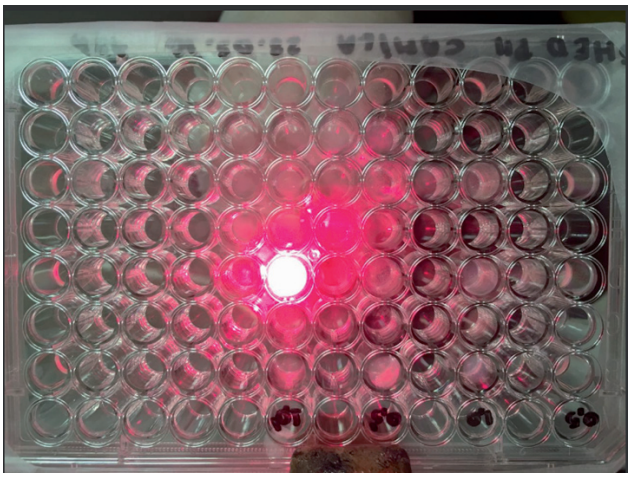

Fig. 1. Use of the LLLT to stimulate DPSC- Dental Pulp Biobank -UFJF- Brazil.

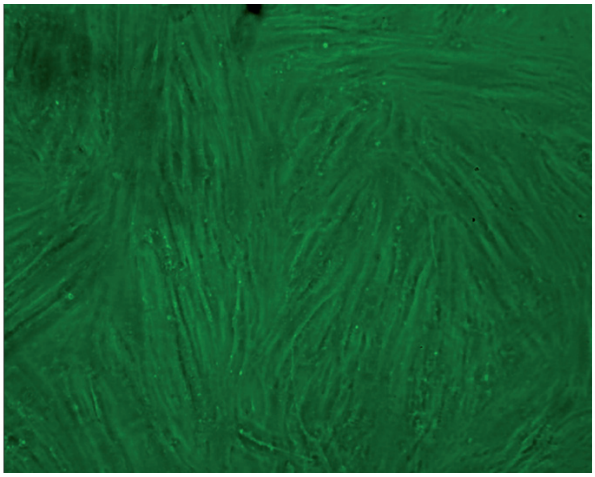

Fig. 2. Mesenchymal stem cells (MSCs) cultures- Dental Pulp Biobank-UFJF-Brazil.

Conclusions

In the recent years, the development of laser technology has led to significant progress in dentistry. Laser devices have been tested and used in all the branches (41-43).

The results of this review suggest that LLLT may be a useful and important tool for future advances in cell therapy and tissue engineering associated to stem cells (Fig. 1,2). Studies about cell therapy for regenerating dental tissues has already been done, and shows promising results.

The phenotypic and genotypic differences of cell lines, the lack of standardization of the experimental conditions, and multiple combinations of laser therapy parameters may explain the divergent results obtained after cell irradiation. Negative results may be due to the use of very low doses or even inadequate treatment techniques (20).

DPSC have emerged as a very promising tool with a great potential to be used in tissue engineering models aimed at the functional reconstruction of different organs and more research are needed to optimize parameters and explore this significant scientific advance.

References

1. De Sa Silva F, Almeida PN, Rettore JV, et al. Toward personalized cell therapies by using stem cells: seven relevant topics for safety and success in stem cell therapy. J Biomed Biotechnol 2012; 2012:758102-758102
2. Horvát-Karajz K, Balogh Z, Kovács V, et al. In vitro effect of carboplatin, cytarabine, paclitaxel, vincristine, and low-power laser irradiation on murine mesenchymal stem cells. Lasers Surg Med 2009; 41(6):463-9
3. Foldes A, Kadar K, Keremi B, et al. Mesenchymal stem cells of dental origin-their potential for anti-inflammatory and regenerative actions in brain and gut damage. Curr Neuropharmacol. 2016 (in press)
4. Soleimani M, Abbasnia E, Fathi M, et al. 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
5. Kern S, Eichler H, Stoeve J, et al. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells 2006; 24(5):1294-301
6. FP Eduardo, Bueno DF, Marques MM, et al. Stem cell proliferation under low intensity laser irradiation: a preliminary study. Lasers Surg Med 2008; 40(6):433-8
7. Seo BM, Miura M, Gronthos S, et al. Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet 2004; 364(9429):149-55
8. Mvula B, Moore TJ, Abrahamse H. Effect of low-level laser irradiation and epidermal growth factor on adult human adipose-derived stem cells. Lasers Med Sci 2010; 25(1):33-9
9. Gioventu S. New Frontiers for Stem cells: Banking methodology and cryopreservation of dental pulp (doctoral thesis)-Milano: Università degli studi di Milano.Departament of biomedical, surgical and dental sciences, 2014
10. Maiman TH.Stimulated optical radiation in Ruby. Nature 1960;187:493-94
11. Ebrahimi T, Moslemi N, Rokn A, et al. The influence of low-intensity laser therapy on bone healing. J Dent 2012; 9:238-48
12. Uman LS. Systematic Reviews and Meta-Analyses. Journal of the Canadian Academy of Child and Adolescent Psychiatry 2011; 20(1):57-59
13. Rother ET. Systematic literature review X narrative review. Acta Paulista de Enfermagem 2007; 20(2), v-vi
14. La Torre, Backhaus G, Mannocci A. Rating for narrative reviews: concept and development of the International Narrative Systematic Assessment tool. Senses Sci 2015; 2(2), 31-5
15. Di Murro F, Smaldone G, Pacella E, et al.Glaucoma and cigarette smoking: a review of narrative reviews. Senses Sci 2016; 3 (4):308-316
16. Soares DM, Ginani F,Henriques AG, et al. Effects of laser therapy on the proliferation of human periodontal ligament stem cells. Lasers in medical science 2015; 30(3),1171-4
17. Barboza CAG, Ginani F, Moura SD, et al. Low-level laser irradiation induces in vitro proliferation of mesenchymal stem cells. Einstein 2014; 12(1):75-81
18. Leonida A, Paiusco A, Rossi G, et al. Effects of low-level laser irradiation on proliferation and osteoblastic differentiation of human mesenchymal stem cells seeded on a three-dimensional biomatrix: in vitro pilot study. Lasers Med Sci 2013; 28(1):125-132
19. Zaccara IM, Ginani F, HG Mota-Filho, et al. Effect of low-level laser irradiation on proliferation and viability of human dental pulp stem cells. Lasers in Medical Science 2015; 30:2259-9
20. Ginani F, Soares DM, Portela M, et al. Effect of low-level laser therapy on mesenchymal stem cell proliferation: a systematic review. Lasers in Medical Science 2015; 30:2189-8
21. Roschke AV,Stover K, Tonon G, et al. Stable karyotypes in epithelial cancer cell lines despite high rates of ongoing structural and numerical chromosomal instability. Neoplasia 2002; 4(1):19–31
22. Rebuzzini PT, Neri T, Mazzini G, et al. Analysis of the euploid cell population of a mouse embryonic stem cell line revealed a high incidence of chromosome abnormalities that varied during culture. Cytogenetic and Genome Research 2008; 121(1):18–24
23. Masson NM, Currie IS, Terrace JD, et al. Hepatic progenitor cells in human fetal liver express the oval cell marker Thy-1. Am J PhysiolGastrointest Liver Physiol 2005; 291:G45–G54
24. Caplan AL, Bruder SP. Mesenchymal Stem cells: Building blocks for molecular medicine in the 21 st century. Trends Mol Med 2001; 7:259–64
25. Verma K, Bains R, Bains VK, et al. Therapeutic potential of dental pulp stem cells in regenerative medicine: An overview. Dental Research Journal 2014; 11(3):302-8
26. Saito MK, Silvério KG, Casati MZ, et al. Tooth-derived stem cells: Update and perspective.World J Stem Cells 2015; 7(2):399-407
27. Gronthos S, Brahim J, Li W, et al. Stem cell properties of human dental pulp stem cells. J Dent Res 2002; 81:531-535
28. Patil R, Kumar BM, Lee WJ, et al. Multilineage potential and proteomic profiling of human dental stem cells derived from a single donor. Exp Cell Res 2014; 320:92-107
29. Gronthos S, Mankani M, Brahim J, et al. Human dental pulp stem cells (DPSCs) in vitro and in vivo.Proc NatlAcadSci USA 2000; 97:13625-13630
30. D'Aquino R, De Rosa A, Laino G, et al. Human dental pulp stem cells: from biology to clinical applications. Journal of Experimental Zoology Part B: Molecular and Developmental Evolution 2009; 312(5):408-415
31. Iohara K, Imabayashi K, Ishizaka R, et al.Complete pulp regeneration after pulpectomy by transplantation of CD105+ stem cells with stromal cell-derived factor-1.Tissue Eng Part A 2011; 17: 1911-20
32. Ikeda E,Takashi T.Growing bioengineered teeth from single cells: potential for dental regenerative medicine. Expert opinion on biological therapy 2008; 8(6):735-744
33. Ebrahimi T, Moslemi N, Rokn AR, et al. The influence of low-intensity laser therapy on bone healing. J Dent (Tehran) 2012; 9(4):238-48
34. Pinheiro ALB, Brugnera Jr A, Zanin FAA. Application of laser in odontology In: Pinheiro ALB. Tissue interaction. Publishing Company Santos, Sao Paulo, 2010; 77-89
35. De Villiers JA, Houreld NN, Abrahamse H.Influence of low intensity laser irradiation on isolated human adipose derived stem cells over 72 hours and their differentiation potential into smooth muscle cells using retinoic acid. Stem Cell Rev 2012;7(4):869–882
36. Peplow PV, Chung TY, Baxter GD. Laser photobiomodulation of proliferation of cells in culture: a review of human and animal studies. Photomed Laser Surg 2010; 28:S3–S40
37. Tuby H, Maltz L, Oron U. Low-level laser irradiation promotes proliferation of mesenchymal and cardiac stem cells in culture.Lasers Surg Med 2007; 39:373–8
38. Hawkins DH, Abrahamse H. Time-dependent responses of

wounded human skin fibroblasts following phototherapy. J Photochem Photobiol 2007; 88:147–155
39. Karu TI.Photobiological fundamentals of low-power laser therapy. J Quantum Electron 1987; 23:1703–817
40. Wu Y, Wang J, Gong D, et al. Lasers Med Sci 2012; 27: 509.
41. Ierardo G, Di Carlo G, Petrillo F et al. Er:YAG laser for brackets bonding: a SEM study after debonding. Scientific World Journal 2014; 2014:935946
42. Romeo U, Russo C, Palaia G, et al. Biopsy of different oral soft tissues lesions by KTP and diode laser: histological evaluation. ScientificWorldJournal. 2014; 2014:761704
43. Romeo U, Palaia G, Tenore G, et al. Excision of oral mucocele by different wavelength lasers. Indian J Dent Res 2013; 24(2):211-5