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Original Research Article

Stem Cells Proliferation and Induction with Laser Therapy: A Systematic Review

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Abstract: **Objective**: to accomplish, through systematic review, if laser therapy can enhance proliferation of stem cells. **Methods**: the search was made in PUBMED Central, BVS/BIREME, Web of Science, Science Direct, Higher Level Personnel Improvement Coordinator (CAPES) Periodic Door (Portal de Periódicos da CAPES, The Cochrane Library and PROSPERO). Criteria: random clinical trials which used *in vivo* stem cells. No language restrictions, neither publication time. **Results:** two hundred ninety nine articles matched the criteria and constituted collection 1. Fully eligible papers counted 16 articles and constituted collection 2. The final sample of this work counted 3 articles that were the basis of this study. **Conclusions:** more *in vivo* clinical trials are necessary to assess the Low-Level Laser Therapy on stem cells.

Keywords: PUBMED Central, BVS/BIREME, CAPES,.

INTRODUCTION

Stem cells have become known because of their promising possibilities for applications in different dental and medical areas. Depending on the site which they come from, they can be designated as totipotent; able to give rise to all kind of cells; pluripotent when they can originate almost all kinds of cells and finally multipotent, when they can differentiate into more than one type of cell. Bone marrow stem cells are the main representatives for having been used for a longer time and many other experiments, especially when they are collected from the spinal cord, promoting a great amount of cells to be cultivated.

With the advances in locating and researching their behavior in vitro and in vivo, new sorts of stem cells were discovered and studied, especially adult stem cells which would not require polemical ethical considerations. In this context, Human Pulp-derived Stem cells appeared as an interesting option for the possibility of being easily collected, in a relative less expendable process with plenty of oral sites where they could be obtained, with high proliferative activity associated with ability for the formation of colony forming units. These are characteristics of Dental Stem Cells from Human Exfoliated Deciduos teeth (SHED), Stem Cells from Apical Papilla (SCAP), Dental Pulp Stem Cells (DPSCs) and Human Supranumerary Tooth-derived Stem Cells (SNTSCs). However; one of the main problems when working with these cells is the need to expand them to their minimum number of viable cells for experiments. Human dental pulp stem cells (HDPSCs) have self-renewing properties and are able to differentiate in many cell types, and therefore researchers are focusing on ways to stimulate their multiplication and differentiation in order to promote stimulation.

More recently yet, phototherapy with low intensity laser irradiation has been used to induce proliferation, with conflicting results. Low-level laser therapy (LLLT) or low level laser irradiation (LLLI) seems to promote photostimulatory and photomodulatory effects on the cells; observed in *in vivo* and *in vitro* experiments. With special interest for researchers is the fact that laser therapy has also been reported to be able to increase stem cells in numbers and to induce differentiation when used in culture trials. Therefore, the aim of this systematic review is to

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evaluate if laser therapy is able to enhance the induction and proliferation of stem cells.

Literature Review

The use of biostimulation with laser in medicine is not new as the first trials took place in the sixties, when the functional working laser was used for the first time. Ever since researchers have been trying to test its benefits in many human body tissues using different sources of light: lasers, characterized by coherent-light and LEDs (light-emitting diodes), characterized by noncoherent light. The former has thee special properties which lead to its usefulness in many health area applications: coherence, monochromaticity, and collimation. The latter, on the other hand, is a kind of light that is normally produced by gas discharges and blackbody sources; the range of wavelengths spanned by LEDs depends on some factors such as gas pressure, but it can be described as finite and could well be 0.1 nm in wave width.

Among the three properties of lasers, coherence seems to be the most important one for the fact that it states that all the protons that are emitted from a laser are disposed exactly at the same phase, that is to say, protons are at the same wavelength or very close to it; and must as well be directional at an specific target. Monochromaticity is related to a well defined wavelength which is a requirement for coherence. Some lasers are known to be able to produce multiple output lines, such as the argon-ion laser, which produces up to 10 lines in the blue–green region of the visible spectrum. A standard HeNe laser is able to produce a red light centered at 632.8 nm with a linewidth of only 0.002 nm, narrower than any incoherent source can produce. Therefore, the wavelength of a laser promotes a higher degree of light penetration that exerts an effect on animal cells, including human stem cells.

One of the first experiences using animals and laser took place in 1967, accomplished in mice with the aim of testing if laser radiation could cause cancer after shaving the dorsal hair of the experimental group. For so, a low powered ruby laser was used at a wavelength of 694-nm. Surprisingly to the authors, not only did the laser not cause cancer, as it also made the hair in the animals tested grow much faster than did the control animals (Mester, E. *et al.,* 1968). As time passed by, the use of lasers gained designations such as LLLT, LLLI, also named “cold laser”, “biostimulation” of “photobiomodulation” (Roelandts, R. 2002), and nowadays in the scientific world its unquestionable whether light exerts biological effects on living mammals cells, but how the mechanisms of energy emitted from therapeutic lasers work at them, more specifically the amount of light parameters used for different experiments. The study of Moore *et al.,* (2005) conducted with fibroblasts concluded that the proliferation rate reached its maximum when irradiated at 665-nm and 675-nm light, while 810-nm light seemed to inhibit their proliferation. With the advent of tissue regeneration with the use of stem cells, it was only natural that laser was chosen as a viable therapy for stimulating proliferation. On the other hand, the study of Tuby *et al.,* (2007) concluded that the best results can be obtained with wavelengths of 600-700nm, while Hawkings & Abrahanse (2007) concluded that the infrared light spectrum of 800-830nm might be associated with inhibition of proliferation.

Stem cells appeared in the literature nearly one century ago, but acquired improved scientific investigation in 1924 by Alexander Maksimov (Nerem, R.M., & Sambanis, A. 1995). Nowadays they are expected to be the solution for many of the problems affecting mankind, more specifically those concerning tissue regeneration, neurology and bioengineering, mainly. In this context, the oral region seemed to be an appropriate donor, since these cells can be collected in a relatively easy and less expensive way, without the polemical and ethical matters concerning embryonic stem cell.

Among the many different kinds of stem cells scattered in the human body, human Dental Pulp Stem Cells (hDPSCs) were firstly isolated in 2000 and opened possibilities for their use in medicine and odontology. Because they were the first kind of human dental stem cells investigated in scientific researches, there have been a vast amount of articles available in the current literature describing their characteristics, such as self-renewal, high proliferative activity, colony forming unity (CFU) ability and multipotency (Silva, L. B. *et al.,* 2016). The fact that teeth may be extracted for a number of reasons, such as orthodontic therapy and surgery indication, made the dental pulp easier to collect when compared to other stem cells available in the oral region, such as Periodontal Ligament Stem Cells (PDLSCs) (Kaku, M. *et al.,* 2012; Liu, W. *et al.,* 2014; Song, J. S. *et al.,* 2012), Stem Cells from the Apical Papilla (SCAP)(Ruparel, N. B. *et al.,* 2013; Huang, G.T. *et al.,* 2009), Human Supernumerary Tooth-Derived Stem Cells (SNTSCs), Dental Follicle Stem Cells (DFSCs) (Yang, B. *et al.,* 2012; Peng, L. *et al.,* 2009), Gingiva-derived Mesenchimal Stem Cells (GMSCs) (Zhang, Q. *et al.,* 2009) and Oral Mucosa CTS Cells (Marynka-Kalmani, K. *et al.,* 2010).

Despite the advantages of human dental stem cells’ collection, cultivating them in such a way to obtain the number necessary for their experimental use has been a fact that sometimes may jeopardize or restrain some experiments . Low Light Level Therapy (LLLT) then seemed to be an interesting alternative, mainly for the existence of available literature describing the effects on adult traditional cells and different tissues of the human body, such as pain reduction, healing promotion of damaged tissues and nerves, and stimulation of mitosis.

The healing process of soft tissues in mammals is complex and involves series of cell interactions and signaling, but generally follows hemostasis, inflammation, cell destruction, proliferation and finally maturing, which in general solves the problem (Robbins, S. L. *et al.,* 2001). The posterior inflammation determines the liberation of the cytokine family components for the cell-to-cell communication and recruitment. This family is vast, and includes Interleukins (IL), Interferon (IFN), Tumor Necrosis Factor (TNF), Coloning-stimulating Factor (CSF), Chemokines (CKs), and Growth Factor (GF). This chemical mediators associated with inflammatory cells such as polymorphonuclear leukocytes and macrophages, activate lymphocytes to accomplish the removal of necrosed and infected tissues (Alam, R., & Gorska, M. 2003; Chaplin, D.D. 2010). Following the predictable order of events, proliferation of cells are necessary for reepithelization and colonization of fibroblasts to enhance scar formation and finally the establishment of vascularization that will be responsible for blood flow with all nutrients and oxygenation necessary for the living of these newly formed matrix and tissues (Mandelbaum, S.H. *et al.,* 20003; Tanaka, A. *et al.,* 2004). In this context, laser therapy has been reported as being able to enhance proliferation by stimulating calcium release into the cytoplasm of the cells irradiated, triggering ATP production and mitosis, by exciting the mitochondrial respiratory chain (Friedman, H. *et al.,* 1991; Karu, T. 1989). Another thing that LLLT seems to be able to do is to induce the acceleration of electron transfer along with nitric oxide (NO) liberation from cytochrome c oxidade, and also to cause changes in the biochemical activity that takes place after the transient heating of chromophores (Ailioaie, L.M. *et al.,* 2005), leading to increased ATP and protein synthesis which triggers the expressions of growth factors such as cytokines, finally enhancing proliferation (Hawkins, D., & Abrahamse, H. 2006; Hu, W.P. *et al.,* 2007).

There have been many mechanisms speculated for the mitogenic effect caused by laser irradiation on the cells, such as ligand-free dimerization and activation of specific receptors that leads to autophosphorylation in the mytochondria of the cells irradiated, leading to increased intracellular concentration of calcium (Karu, T. 1999; Cohen, N. *et al.,* 1998; Lavi, R. *et al.,* 2003).

The work of Peplow *et al.,* (2010) claims that establishing a comparison between the results of previously published studies is not reliable because of the wide range of irradiation parameters, cell types and methods used in different experiments, which may lead the researchers to conflicting and contradictory results.

METHODS

This systematic review is registered in PROSPERO under the number CRD42016033673. For this review study, we searched the following electronic bibliographic databases:

PUBMED Central, BVS/BIREME, Web of Science, Science Direct, Higher Level Personnel Improvement Coordinator (CAPES) Periodic Door (Portal de Periódicos da CAPES, The Cochrane Library and PROSPERO).

The search strategy used Medical Subject Headings (MeSH) in all specified databases. For MEDLINE (Pubmed Central), the final strategy was (“DentalPulp”[Mesh]) AND (”Lasers”[Mesh] OR “Laser Therapy”[Mesh] OR “Low-Level Light Therapy”[Mesh]) AND (“Stem Cells”[Mesh] OR “Stem Cell Research”[Mesh] OR “Adult Stem Cells”[Mesh])). The search terms was adapted for use with other bibliographic databases.

The criteria used in this review were random clinical trials (RCT), which used *in vivo* cells. There was no language restrictions, neither publication time.

RESULTS

Three hundred fifty five (355) registries were initially found, which were reduced to 299 after the detection of duplicated results, forming Collection 1. After the analysis of the titles and the abstracts the researchers gathered 16 studies for the reading and scrutinizing of their full texts (Collection 2). Thirteen of them were excluded because of different reasons depicted in TABLE 1. Thus, a number of 3 articles constituted the basis of this work.

Two researchers accomplished the whole analysis of the texts by using the inclusion and exclusion criteria. The analysis was also registered in a form for the identification of the exclusion reasons. Conflicting cases was solved by a third researcher. Figure 1 summarizes the design of this study.

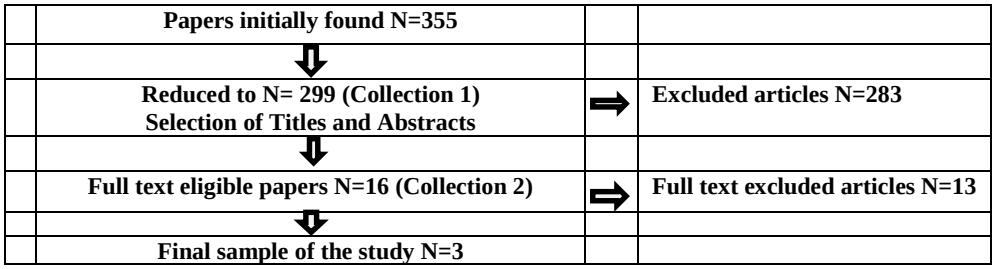

Fig.1. Flowchart of the searching method to detect eligible studies

Table 1: Articles excluded after full text reading and analysis

Author	Reason for exclusion
Light coaxes stem cells to repair teeth	Newspaper issues
Laser Light Encourages Tooth Regeneration	Newspaper issues
AlGhamdi *et al.*, 2012	Design study (literature review)
Arany *et al.*, 2014	Study development in mice
Arany *et al.*, 2011	Study used pulp capping
Chan *et al.*, 2011	Regenerative medicine
Eduardo *et al.*, 2008	*In vitro* study
El Alami *et al.*, 2014	*In vitro* study
Emelyanov e Kiryanova, 2015	Design study (systematic review)
Ginani *et al.*, 2015	Design study (systematic review)
Li Cui *et al.*, 2014	Cell cultivation without light source
Marchiori-Silva *et al.*, 2010	Congress presentation
Morad *et al.*, 2013	Design study (literature review)

Table 2 shows the differences in the light source applied in the included articles of this study. The lowest dose applied [REF] was 0.1J/cm^2 with a wavelength of 810nm, being the highest 42 J/cm^2 at a wavelength of 660nm.

Table 2: Parameters of the radiation sources used in the studies

Author	Light source	Wavelenght (nm)	Power (mW)	Energy density (J/cm^2)	Range of irradiation
Pereira *et al.*, 2015	LLLI	660	28	0.05, 0.30,7 and 42	Two irradiations, for 10 and 60 seconds.
Tabatabaei *et al.*, 2015	LLLI	810	60	0.1,0.2 and 0.3	Single irradiation
Zaccara *et al.*, 2015	LLLI	660	30	0.5 and 1	Two irradiations for 0 and 48 hours

DISCUSSION

All the clinical trials collected in this systematic review used hDPSCs to assess the effects of LLLT, with conflicting results. Zaccara used these cells isolated from two healthy fully erupted permanent third molars extracted due to orthodontic therapy, and the cells were cultivated until they reached the third passage. Pereira used hDPSCs collected from normal and inflamed dental pulps collected from different patients between 17-43 years of age, while Tabatabaei used hDPSCs from impacted third molars. The stability of these adult mesenchymal stem cells, as well as their low rate of teratoma formation and their being easily collectable was propably the reason that them more prone to their clinical trials[4]. Zaccara concluded that LLLI contributed to the growth of DPSCs and to the maintenance of their viability, particularly at the dose of 1.0J/cm^2; while Pereira claimed in the head of their article that LLLI did not increase the proliferation or the differentiation of DPSCs collected from normal and inflamed pulps, contrary to Tabatabaei who concluded that LLLI might be a novel approach for preconditioning of these cells *in vitro* before being used to bone tissue engineering. Both of the experiments who used non inflamed Dental Pulp Stem Cells responded positively to LLLT, while the experiment that used normal and inflamed stem cells in the same experiment did not show any effect on proliferation or osteogenic differentiation. According to the work of Moore (Moore, P. *et al.*, 2005), who claimed that the proliferation rate is at a peak in the presence of 665nm and 675nm with fibroblasts, the work of Pereira used red low level laser with 660nm of wavelength and did not obtain positive results for proliferation, although they hypothesized that the first three degrees would promote positive biostimulatory effects. Nevertheless, their work used the lowest power level of 28mW of all three works, with four therapeutic ranges of irradiation between 0.05 and 10J/cm^2, and another variable was the use of DPSCs from different patients with different pulp conditions (inflamed and non-inflamed) and this variance may have influenced their results simply because different cell lines, even belonging to the same cell type and species, may have different degrees of sensitivity to laser irradiation.

On the other hand, Tabatabaei used a power level of 60mV with a wavelength of 810nm, the latter being described by Hawkings and Abrahanse (2007) as associated with inhibition of proliferation, and obtained encouraging results for proliferation, using just one single irradiation due to higher wavelength.

The work of Zaccara *et al.*, relates with the works of Friedman *et al.*, (1991) and Karu (1989) who reported the stimulation of calcium release into the cytoplasms of the cells irradiated with laser and the consequent increase in ATP production due to mitochondrial excitement. In their work they show the increase in the numbers of DPSC viability along time

after irradiation, in all groups, suggesting the stimulatory effect LLLT on such cells.

Dental Pulp Stem Cells can be induced for differentiation by the addition of ascorbic acid dexametasone (Dexa) and b- glycerophosphate (b-gly) during cultivation when applied with the use of fetal bovine serum (Langenbach, F., & Handschel, J. 2013). Low-level Laser Therapy however, seems not only able to increase their number and to induce them to differentiate into different cell types (Peplow, P.Y. *et al.*, 2010), but also to stimulate their growth (Eduardo, F. D. P. *et al.*, 2008).

CONCLUSION

The studies matched in the inclusion criteria of this work, although few, indicate the need of more *in vivo* clinical trial experiments to made in humans as well as a parameter standardization searching for accurate comparison of the results obtained.

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