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Effect of Low-Level Laser Therapy on Differentiation and Proliferation of Human Dental Pulp Stem Cells: A Systematic Review

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Abstract

Introduction: Considering the positive impact of laser treatment on the proliferation of certain cell types, we opted to perform a systematic review aimed at evaluating the effects of laser therapy and photobiomodulation on the proliferation and differentiation of human dental pulp stem cells (hDPSCs).

Methods: We included all research studies examining the impact of laser therapy on hDPSCs, without limitations on publication dates or article languages. The major international databases, including PubMed, ISI Web of Science, and Scopus, were searched from inception to April 2022 by the relevant keywords.

Results: In total, 1886 studies were identified in the initial search from the mentioned databases and other sources. Finally, 17 relevant studies were included in the present systematic review after removing duplicates and non-relevant articles. The results indicated the useful effect of low-level laser therapy (LLLT) on the hDPSCs.

Conclusion: The findings of this systematic review indicate the useful role of LLLT in cell therapy, proliferation, and differentiation associated with hDPSCs.

Keywords: Low-level laser therapy; Low-level laser irradiation; Proliferation; Differentiation; Dental pulp stem cells.

Introduction

Stem cells are the body's natural reservoirs to restore damaged specialized cells. Stem cells have a significant potential to create different types of cells during life and hence play a key role in the growth and development period. In addition, their role in restoring lost tissues has received much attention. These cells can continuously generate a copy of themselves, which makes them unique. By dividing the stem cell, the new cells which are able to either remain a stem cell or produce another type of cell with more specific functions are generated, such as osteoblast cells, red blood cells, or brain cells.[1,2]

Dental pulp stem cells are one of the main sources of adult stem cells. These multipotential cells offer a wide range of advantages for clinical applications compared to other mesenchymal cells derived from bone marrow, adipose tissue, and cord blood. Pulp stem cells are non-distinct cellular cells that can be distinguished from a variety of specific cells.[3] Endodontic therapy is a treatment performed to address infections or damage to the tooth pulp. Optimal outcomes may be attained by employing tissue engineering techniques to regenerate damaged dental tissues including pulp, dentin, and cementum in the context of regenerative endodontics. In such scenarios, deficient dental structures may be replaced or regenerated with bioengineered tissues that closely mimic the properties of native tissues.[4]

The aim of using regenerative methods in endodontics is to restore tissues similar to pulp and dentin that have been lost due to various reasons such as caries and pathological or infectious lesions. In tissue engineering, stem cells, growth factors, and cell scaffolds are the three main components to create a new tissue or organ.[5]

In recent years, laser therapy has received much attention from researchers as an effective method for enhancing the proliferation and differentiation of

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tissue. Laser systems are categorized based on various parameters, such as their physical structure, energy density, radiation time, and degree of exposure. In clinical practice, lasers are further classified into three categories based on their energy output and tissue interaction: high, moderate, and low-energy levels. In tissue engineering, low-level laser therapy (LLLT) is usually used to stimulate cells.[6] LLLT modulates the tissue repair mechanism by triggering cellular reaction, including proliferation, migration, apoptosis, and tissue differentiation.[7] Therefore, considering the positive effects of laser therapy on the proliferation of certain cell types, this systematic review aimed to evaluate the available major international databases to assess the effects of laser therapy and photobiomodulation on the differentiation and proliferation of dental pulp stem cells.

Materials and Methods

In adherence to The Preferred Reporting Items for Systematic Reviews,[8] the initial phase encompassed the meticulous curation and systematic management of articles procured from diverse sources. This process was facilitated through the utilization of Endnote software version 19, developed by Clarivate Analytics. Subsequently, a comprehensive, collaborative review was undertaken by a pair of researchers to discern articles that deviated from the research focus and the predetermined inclusion criteria. Following this, the abstracts of the retained articles were subjected to rigorous, independent scrutiny by the same research duo.

The subsequent phase entailed an exhaustive examination of the full texts of the selected articles, conducted by the same research team, with the overarching objective of identifying articles that unequivocally conformed to the pre-established inclusion criteria. At every juncture of this methodical process, any disparities in judgment were diligently addressed through rigorous discussion and, when necessary, resolved by the deliberations of a third researcher, ultimately ensuring consensus.

Criteria for Including Studies

All studies investigating the effects of laser treatment on human dental pulp stem cells (hDPSCs) were included without any limitation on the data of publication and language of articles. The inclusion criteria encompassed articles evaluating the effects of LLLT intervention on hDPSCs' proliferation and differentiation, along with descriptions of the key parameters of the employed laser, such as energy level and duration of use. Moreover, case series, letters to editors, systematic reviews, narrative reviews, medical hypotheses, and studies of unrelated exposures were excluded.

Searching Techniques

We conducted a search across major global and inclusive databases, such as PubMed, ISI Web of Science, and Scopus, to assess the impact of laser therapy on the proliferation and differentiation of hDPcs from their inception up to April 2022. The keywords included low-level laser therapy, low-level laser irradiation, proliferation, differentiation, cell proliferation, cell differentiation, human dental pulp stem cells, and hDPSCs (Table 1). In order to enhance the sensitivity of the search, the reference lists of the related studies were also investigated to discover other conceivable sources.

Gathering Data and Evaluating Validity

Two researchers (SE and AD) independently evaluated the articles. Any discrepancies between the researchers were resolved through discussion involving all three

Table 1. Search Strategy in the Investigated Databases for Finding Relevant Studies

Scopus		PubMed		Web of Science	
#1	TITLE-ABS-KEY(low level laser therapy	#1	low level laser therapy [MeSH Terms]	#1	TS= low level laser therapy
#2	TITLE-ABS-KEY(low level laser irritation)	#2	low level laser therapy [Title/Abstract]	#2	TS=low level laser irritation
#3	TITLE-ABS-KEY(LLLT/I)	#3	low level light therapy[MeSH Terms]	#3	TS=LLLT/I
#4	#1 OR #2 OR #3	#4	LLLT[Title/Abstract]	#4	#1 OR #2 OR #3
#5	TITLE-ABS-KEY(proliferattion)	#5	#1 OR #2 OR #3 OR #4	#5 TS= proliferation	
#6	TITLE-ABS KEY(differentiation)	#6	proliferation [Title/Abstract]	#6 TS= differentiation	
#7	TITLE-ABS-KEY(dental pulp stem cells)	#7	differentiation [Title/Abstract]	#7 TS= cell proliferation	
#8	TITLE-ABS-KEY(hDPCs)	#8 cell proliferation [MeSH Terms]		#8 TS= cell differentiation	
#9	#5 OR #6	#9 cell differentiation [MeSH Terms]		#9 TS= human dental pulp stem cells	
#10	#7 OR #8	#10 adult stem cell [MeSH Terms]		#10 TS=hDPCs	
		#13 adult stem cell [Title/Abstract]		#11 #5 OR #6 OR #7 OR #8 OR #9 OR #10	
		#14 human dental pulp stem cells [Title/Abstract]			
		#15 hDPCs [Title/Abstract]			
		#16 #14 OR #15			
		#17 #6 OR #7			

authors (HK). Two authors independently extracted the pertinent variables from the studies, and the gathered information was then condensed and presented in a dedicated table.

Results

In total, 1886 studies were identified in the initial search through database searching and other sources. Of them, 643 duplicate articles were excluded. In total, 1153 studies were excluded after reviewing the studies by title and abstract, and 58 other studies were excluded after reviewing the full paper. In the end, our systematic review incorporated 17 pertinent studies (Figure 1).

Table 2 presents the characteristics, aims, and findings of the included articles regarding the effect of LLLT on the proliferation and differentiation of hDPSCs. As can be seen here, eight studies were conducted in Brazil. Results of the majority of the included studies indicated that LLLT offered a useful effect on the proliferation and differentiation of hDPSCs.

Discussion

This systematic review aimed to explore the impact of laser therapy on the growth and specialization of dental pulp stem cells, drawing upon findings from previously published studies within prominent international databases. Owing to heterogeneity in the design and characteristics of the lasers employed, it was not possible to combine study findings and report a summary measure or conduct inter-study comparisons. Most of the included studies in this systematic review have demonstrated the useful effect of LLLT on the cell therapy, differentiation, and proliferation of dental pulp stem cells.

Staffoli et al, in a narrative review on the effect of laser therapy on the proliferation of dental pulp stem cells, reported that laser therapy acts as a useful tool in the development of cellular treatments and tissue engineering.[6] In the present study, in addition to the Medline database, Scopus and Web of Science databases were searched with no language and time restrictions. In a systematic review and meta-analysis that was conducted by Kulkarni et al, nine related articles were reviewed, while the search was done until 2019 and the Web of Science database was not checked. In line with our findings, they concluded that photobiomodulation improves cell viability and proliferation.[25]

The primary aim of employing LLLT is to deliver biostimulating light energy directly to the body's cells. The energy absorption of the laser activates the molecules and atoms within the cell without including a substantial rise in tissue heat.[26] In the context of photobiomodulation, lower doses of light are associated with higher efficacy in promoting stem cell proliferation.[27] In using LLLT, different factors such as the energy density, light spectrum, power level, and wavelength can affect the results, and hence the contradictory results in different studies can be explained by these factors.[28]

However, more evidence is needed to draw conclusions

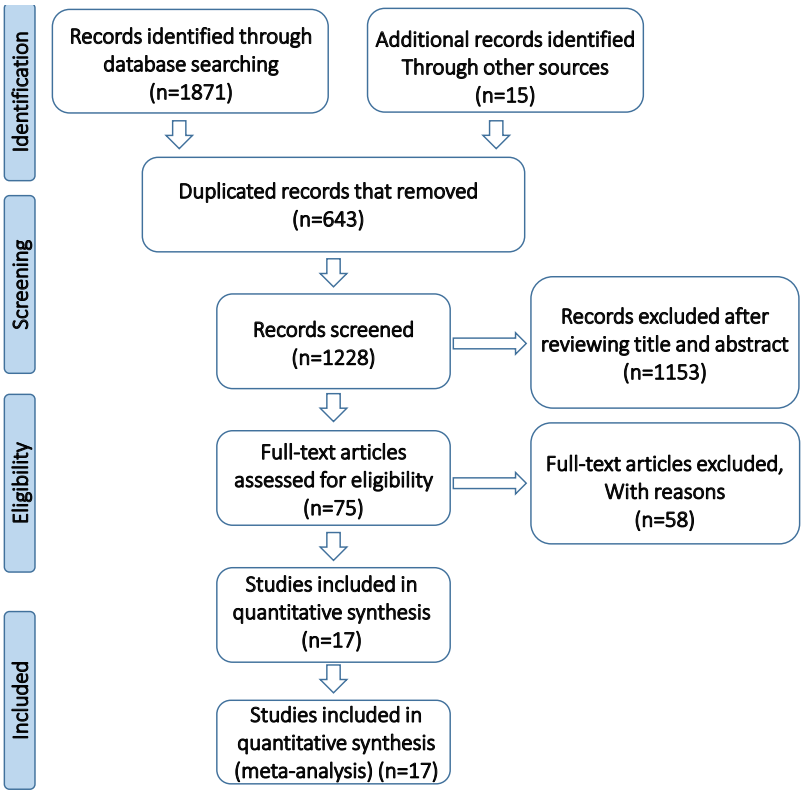

Figure 1. The Selection Process of the Included Articles

Table 2. Characteristics of the Articles in Relation to the Effect of LLLT on the Proliferation and Differentiation of hDPSCs

First author	Year	Country	Objective	Results
Fernandes et al.[9]	2021	Brazil	Evaluating the influence of different laser therapy energy densities on SHED viability and proliferation.	The LLLT energy densities used in this study did not cause loss of cell viability and stimulated SHED proliferation within the parameters described in this study.
Gutiérrez et al[10]	2021	Colombia	Evaluating the proliferation and osteogenic potential of the SCAPs stimulated with LLLT.	LLLT promotes proliferation, osteogenic differentiation, and VEGF and TGFβ2 expression on SCAPs. LLLT is a practical approach for the preconditioning of SCAPs *in vitro* for future regenerative therapies. More studies are needed to determine the underlying molecular processes that determine the mechanism of the LLLT.
Amid et al[11]	2022	Iran	Studying the effects of two protocols of LLLT on the proliferation and differentiation of hDPSCs cultured on sandblasted titanium discs.	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.
Abo El-Dahab et al[12]	2020	Egypt	Assessing the effect of diode laser irradiations on the proliferation of stem cells isolated from the human dental pulp.	There was no significant difference between the different groups (gp 1 control, gp II dose 0.5 J/cm^2, gp III dose 1 J/cm^2) on day 1, while on day 2, the optical density of DPSCs subjected to dose 1 was found to be significantly higher than that of those subjected to dose 2. This was also demonstrated on day 3.
Kunimatsu et al[13]	2015	Japan	Studying the effects of diode laser irradiation on the proliferation and osteogenic differentiation of hDPSCs. hDPSCs were cultured and exposed to an 810-nm diode laser at a dose of 0 or 1 J/cm^2.	Treatment of hDPSCs with laser irradiation also enhanced significantly ($P<0.01$) ALP activity and calcium concentration, resulting in enhanced mineralization, as evidenced by the high intensity of Alizarin Red staining. In conclusion, the present study showed that 810-nm diode laser irradiation may contribute to the regeneration of dental pulp by enhancing hDPC proliferation and differentiation.
Chang SY et al[14]	2021	South Korea	The ability of stem cells to self-renew and differentiate can contribute significantly to the success of regenerative medical treatments.	PBM stimulates different types of stem cells to enhance their migration, proliferation, and differentiation *in vitro* and *in vivo*. This review summarizes the effects of PBM on targeted differentiation across multiple stem cell lineages. The analytical expertise gained can help better understand the current state and the latest findings in PBM and stem cell therapy.
Zaccara et al[15]	2018	Brazil	Evaluating the PBM effect of hDPSCs differentiation and ALP activity using an agarose 3-D model under different nutritional conditions.	It can be concluded that hDPSCs cultured in 3-D agarose associated with PBM could be a promising tool for tissue engineering applications.
Zaccara et al[16]	2015	Brazil	Identifying the lowest energy density able to promote the proliferation of DPSCs and to maintain cell viability.	LLLI, particularly a dose of 1.0 J/cm^2, contributed to the growth of DPSCs and the maintenance of its viability.
Ginani et al[17]	2018	Brazil	Evaluating the effect of LLLI on the proliferation and viability of stem cells from human exfoliated deciduous teeth	The LLLI parameters used (660 nm, 30 mW, 1.0 J/cm^2) promote the proliferation of stem cells from human exfoliated deciduous teeth and the maintenance of cell viability
Pereira et al[18]	2012	Brazil	Investigating whether LLLT could increase the proliferation and differentiation potentials of hDPSC isolated from healthy dental pulps and from inflamed pulps.	No statistically significant differences were observed between the proliferation rates and the relative productions of mineralized nodules compared to the respective controls, either for hDPSC from normal or inflamed dental pulps
Sivakumar et al[19]	2019	India	Evaluating the biomodulatory effect of low-level laser dose on dental pulp stem cells in the presence of hydroxyapatite-based scaffold particle for osteogenic differentiation.	The result envisages the enhanced osteogenic potential of PBM therapy on the differentiation of DPSCs in NAC-HA scaffolds. Double exposure of PBM therapy expresses a better biomodulatory effect on DPSCs as compared with the single dose
Tabatabaei et al[20]	2015	Iran	Assessing the effect of LLLI on the proliferation and osteogenic differentiation of dental pulp stem cells	One week after the last day of laser irradiation, it significantly increased in groups exposed to the laser at 0.1 or 0.2 J cm^{-2} and decreased in groups containing OM ($P < 0.05$). Osteoblast marker expression was observed in groups containing OM. LLLI at 0.2 J cm^{-2} dramatically enhanced cell differentiation. The laser at 0.3 J cm^{-2} increased bone sialoprotein and decreased ALP.
Milward et al[21]	2015	United Kingdom	Assessing the modulation of DPC responses using two LLLT lasers with wavelengths of 660 nm and 810 nm.	Significantly higher MTT activity was obtained for both lasers ($P<0.05$) using the high and intermediate radiant exposure (5-20 J/cm^2). The MTT response significantly decreased ($P<0.05$) at lower radiant exposures with no statistical significance from the control ($P>0.05$).
Moura-Netto et al[22]	2016	Brazil	Evaluating the influence of laser phototherapy on the cellular growth of a dental pulp stem cell lineage originating from deciduous teeth	Laser phototherapy with 5 J/cm^2 can enhance the growth of SHEDs during situations of nutritional deficiency.

Table 2. Continued

First author	Year	Country	Objective	Results
Hou et al[23]	2018	China	Examining whether HF-LPLI is able to activate stem cells to promote regeneration in periodontitis by promoting inflammation resolution and evaluating the underlying mechanism of action if an effect is observed.	HF-LPLI stimulated stem cell proliferation and differentiation by promoting inflammation resolution subsequent to stem cell activation, providing a new strategy for the clinical treatment of periodontitis.
Neto et al[24]	2015	Brazil	Verifying the influence of different energy densities of LLLT on the viability and proliferation of pulp fibroblasts of human primary teeth	This study suggests that different energy densities of LLLT influence the viability and proliferation of pulp fibroblasts of human primary teeth.

Abbreviations: LLLT, Low-level laser therapy; hDPSCs, human dental pulp stem cells; hDPSCs, human dental pulp cell; DPC, dental pulp cell; LLLI, low-level laser irradiation; ALP, alkaline phosphatase; VEGF, vascular endothelial growth factor; transforming growth factor-beta 2; SCAPs, stem cells from the apical papilla; PBM, photobiomodulation; NAC-HA, N-acetylcysteine-labeled hydroxyapatite; OM, osteogenic medium.

about the effect of LLLT on increasing the proliferation of pulp stem cells and to reach a definitive conclusion. Moreover, further studies are required to determine the effective properties of LLLT, such as the wavelength, the energy density, and the output power of the laser associated with an increase in the proliferation of stem cells, and to evaluate the feasibility of its use in the differentiation and proliferation of dental pulp stem cells.

Conclusion

The results of this systematic review indicate the useful effect of LLLT on cell therapy, differentiation, and proliferation associated with dental pulp stem cells. It is recommended to conduct further studies to provide stronger evidence regarding the effect of LLLT on stem cells in restoring dental tissues.

Authors' Contribution

Conceptualization: Amin Doosti-Irani.
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Validation: Hamed Karkehabadi.
Visualization: Jaber Zafari.
Writing–original draft: Sogand Esmailnasab.
Writing–review & editing: Jaber Zafari.

Competing Interests

The authors declare that they have no conflict of interest.

Ethical Approval

The present study has been reviewed and approved by the Ethics Committee of Hamadan University of Medical Sciences (Code: IR.UMSHA.REC.1401.355).

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