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REVIEW ARTICLE

The effect of photobiomodulation on human dental pulp–derived stem cells: systematic review

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Abstract

This systematic review assessed if photobiomodulation of human dental pulp tissue improved cell viability, proliferation, and/or differentiation compared with a placebo. This systematic review was conducted in line with PRISMA. PICO question was established; inclusion and exclusion criteria were established before a search had begun. A literature search was conducted through PubMed, Scopus, and Cochrane. Studies were included if published within the last 20 years in English language, or where translation was available; laser parameters were mentioned; human dental pulp tissue was studied in vitro. Studies were excluded if non-human dental pulp tissue was studied and where the study was an in vivo study. Out of the total 121 studies found, 109 were excluded. Of the twelve included studies, three full-text articles were not available despite attempts made to contact the respective authors, leaving nine studies. Four of the included studies reported the use of stem cells derived from human deciduous teeth (SHEDs), and five used those from human permanent teeth (DPSCs). Most included studies utilized InGaAlP laser with wavelengths 660 nm, and one study with 610 nm. Other types of lasers included LED InGaN, and GaAlAs. Out of all included studies, two had a moderate risk of bias, and the rest had a low risk of bias. All studies confirmed positive effects on proliferation. One study also found improved osteogenic differentiation of the stem cells derived from stem cells of deciduous teeth. After assessing SHEDs and DPSCs separately, it is found that photobiomodulation improved cell proliferation in both subgroups. Due to heterogeneity in design protocols and laser parameters, it was not possible to compare the studies together. However, this study indicated that cell viability and proliferation did improve with photobiomodulation.

Keywords DPSCs · Laser phototherapy · Mesenchymal stem cells · SHEDs · Tissue engineering

Introduction

Tissue defects in the craniofacial region have serious costs financially, psychologically, and physiologically. Reconstruction, therefore, is highly desired. There have been significant developments over the last few decades in this area of tissue engineering, both in terms of research and clinical protocols [1, 2]. It now focuses on three factors: regenerative cells, cell scaffolds, and bioactive substances. In terms of regenerative cells, the focus has been on those derived from bone marrow. However, stem cells make up 0.001–0.01% of all cellular components in bone marrow, and the patient is still required to undergo an invasive procedure to acquire these cells. Therefore, when Gronthos et al. in 2000 isolated and cultured human dental pulp stem cells (DPSCs) from permanent teeth, and later in 2003 Miura et al. did the same from deciduous teeth, there have been a growing number of studies looking into proliferative and differentiation properties of these cells [1, 2].

There are multiple types of stem cells derived from the dentoalveolar tissues—dental pulp stem cells (DPSCs), dental pulp tissue of deciduous teeth (SHEDs), and periodontal ligament (PDLSCs). The difference in proliferative and differentiation properties between these three types of stem cells has not yet been conclusively elucidated. It has, however, been shown that these cells are more proliferative than human BMSCs (hBMSCs). They are capable of differentiating into osteoblasts, adipocyte chondrocytes, and neurons [3]. There is also some in vivo evidence to suggest that bone regeneration occurs with SHEDs and DPSCs on par with BMSCs [3].

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Upon transplantation, stem cells have demonstrated a low percentage of viability and proliferation [1]. This has been attributed to multiple drawbacks in the current tissue engineering process. One of the main drawbacks is nutritional deficiency, identified as lack of blood supply. Photobiomodulation therapy has previously successfully demonstrated its effectiveness in improving proliferation, migration, and differentiation of cells, and activation of growth factors, as well as the acceleration of protein synthesis. Therefore, photobiomodulation has been applied in tissue engineering to improve cell viability, proliferation, and differentiation of stem cells. All three processes, improving cell viability, improved proliferation, and induction or acceleration of differentiation of stem cells can dictate the success of repair of tissue defects [1, 2]. Hence, there is a need to conclusively prove that photobiomodulation can improve the said processes in DPSCs and SHEDs.

Two recent systematic reviews in 2016 and one narrative review in 2017 have considered photobiomodulation and dental-derived mesenchymal stem cells [4–6]. The narrative review conducted in 2017 did not include studies identified in the previous systematic review, and only included studies that the authors identified, without justification, as important [5]. This reduces the external validity of the narrative review. The systematic review by Farahani (2016) identified studies with the outcome measure to be proliferation of human dentoalveolar–derived stem cells alone, and excluded studies that considered cell viability and differentiation of the stem cells [5]. This exempts the consideration of the complete effect of Photobiomodulation on dental-derived stem cells [5]. Another criticism of this systematic review, and that of Marques et al. (2016), was that they considered more than one type of stem cell for comparison—dental pulp stem cells (DPSCs), dental pulp tissue of deciduous teeth (SHEDs), and periodontal ligament (PDLSCs) [4, 5]. The resulting conclusion therefore does not point unequivocally towards the synthesis of current evidence for photobiomodulation and DPSCs or SHEDs.

Farahani (2016) did not conduct a risk of bias assessment, and only included one comment about the possible source of bias in a table dedicated to outcomes [5]. Marques et al. (2016) did conduct a risk of bias assessment; however, the constructed tool in the review was deemed inadequate [4]. Risk of bias assessment evaluates internal validity. This is a test of study design and its credibility to link the exposure and outcome. Inadequate risk of bias assessment leads to a reduction in the level of evidence possible from the systematic review. In addition, comment on external validity was found lacking in the two systematic reviews [7–9]. Furthermore, the 2016 systematic review also included one study without a complete description of laser parameters despite the recommendation by the World Association for Laser Therapy (WALT) [4]. This undermines reproducibility as well as comparison and synthesis of data. These problems compromise the level of evidence present from the existing evidence.

In addition, multiple new in vitro studies have been identified since 2016 dictating more concrete evidence and future direction for research. The need for an update was therefore identified. This systematic review aims to answer the null hypothesis that photobiomodulation does not improve the cell viability, proliferation, and/or differentiation of dentally derived stem cells, under the question:

> *In human dental pulp tissue* (*population*), *does photobiomodulation* (*intervention*) *improve cell viability*, *proliferation*, *and/or differentiation* (*outcome*) *compared with a placebo* (*control*)?

Methods

This systematic review was written and conducted to comply with Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) [10]. A systematic search strategy was conducted using Medical Subject Heading (MeSH) terms [dental pulp] and [low-level laser therapy]. The search was conducted through PubMed, Scopus, and Cochrane up until 11 September 2019.

Inclusion and exclusion criteria were decided prior to searching in accordance with the PRISMA protocol and were registered on PROSPERO to avoid duplication of results. These criteria were as follows:

Inclusion criteria:

- Laser parameters are mentioned
- Published in English language or English translation is available
- Published in the last 20 years
- Human dental pulp tissue is studied
- In vitro study

Exclusion criteria

- Non-human dental pulp tissue is studied
- Non–in vitro study

Risk of bias assessment

As no formal risk of bias assessment tool was identified for these in vitro cell culture studies, the last used tool in the 2016 systematic review was critiqued and built upon to construct the tool as displayed in Table 1 [4]. The systematic review identified some parameters to assess the study design and

Table 1 Risk of bias assessment

	Montoro et al. [11]	Eduardo et al. [12]	Zaccara et al. [13]	Tabatabaei et al. [14]	Moura-Netto et al. [15]	Marques et al. [16]	Kim et al. [17]	Pinheiro et al. [18]	Sivakumar et al. [19]
Cell type	Y	N	Y	Y	Y	Y	Y	Y	Y
Were laser parameters reported for all studied groups?	Y	Y	Y	Y	Y	Y	N	Y	Y
Was cell passage reported?	Y	N	Y	Y	Y	Y	N	Y	Y
Was cell characterisation reported to be done?	Y	Y	Y	Y	Y	Y	N	Y	Y
Has cell culture method been reported?	Y	Y	Y	Y	Y	Y	Y	Y	Y
Have number of wells lost been reported with reason?	Nil lost	Nil lost	Nil lost	Nil lost	Nil lost	Nil lost	Nil lost	Nil lost	Nil lost
Has handling of cell culture between establishment and measurement been reported?	Y	Y	Y	Y	Y	Y	Y	Y	Y
Have all outcomes been reported?	Y	Y	Y	Y	Y	Y	Y	Y	Y
Outcome	Low	Moderate	Low	Low	Low	Low	Moderate	Low	Low

comment on risk of bias. However, these were deemed inadequate.

The key features of the study design were identified using the list recommended by the Cochrane Handbook for Systematic Reviews Chapter 13 [7]. The following parameters were identified as being imperative for assessment of internal validity and hence were added to the risk of bias assessment table: handling of cell culture between establishment and measurement, reporting of all outcome measures as stated, number of lost wells with reason, and reporting of all laser parameters. Two independent researchers evaluated the studies and reported 'Y' if the study had reported that parameter, and 'N' if the study did not. More than five 'N's were considered to have a high risk of bias, between 2 and 5 'N's were considered to have a moderate risk of bias, and 1 or 0 'N's were considered to have a low risk of bias.

Results

Once the systematic search strategy was constructed, and the said databases were searched, duplicates were identified and eliminated. Two independent researchers (S. K., R. G.) reviewed the titles and abstracts to allocate the studies to 'included' and 'excluded' folders in the citation manager Endnote X8® (Clarivate Analytics, Boston, MA, USA). Any that were left in the 'unsure' folder, were later discussed with a third researcher (M. M.) to include or exclude it. This selection process can be seen in Fig. 1.

The study characteristics have been reported in Tables 2 and 3. The source of stem cells is reported in Table 2. Four of the included studies reported the use of stem cells derived from human deciduous teeth (SHEDs), and five used those from human permanent teeth (DPSCs) [11–19]. No comparative study was found to analyse the different effects of photobiomodulation between SHEDs and DPSCs. One study found higher proliferative activity of SHEDs compared with bone marrow mesenchymal cells and DPSCs without any stimulation, as well as greater expression of runt-related transcription factor 2 (Runx2) and alkaline phosphatase (ALP) genes [3]. Runx2 expression indicates osteoblastic differentiation and ALP is a matrix mineralisation marker, both indicating possibly greater osteoblastic differentiation potential compared with DPSCs [3]. Due to this difference, studies with SHEDs were compared amongst themselves, separately to those with DPSCs.

Laser parameters in the included studies (Table 3)

Most included studies utilized InGaAlP laser with wavelengths 660 nm, and one study with 610 nm [12–16, 18]. Other type of lasers included LED InGaN, and GaAlAs [14, 19].

This may not be of great significance as a recent study found no difference in cell viability on myoblasts when InGaAlP was compared with GaAlAs and control [20]. However, no comparative study on dentally derived stem cells was found to confirm this finding. It is important to note that there are some differences between LED and lasers. LEDs have a larger bandwidth, meaning they can be applied to a

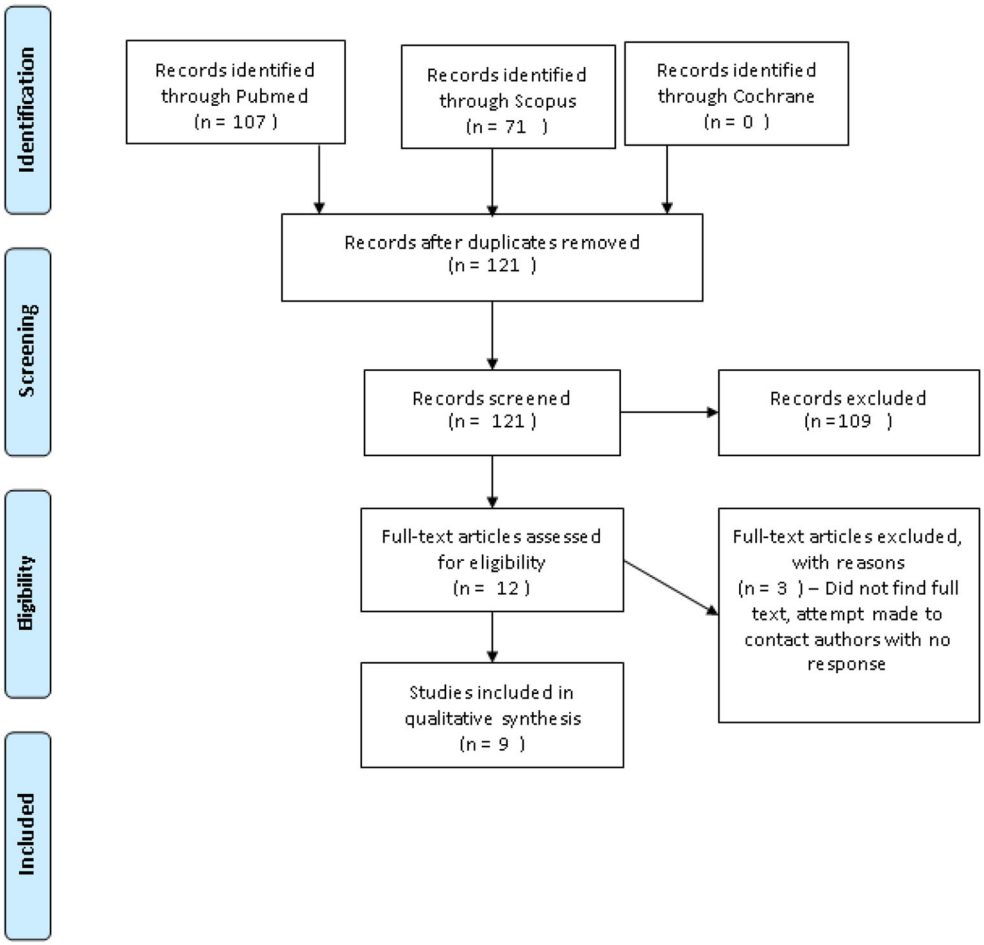

Fig. 1 Selection process for inclusion of studies according to PRISMA protocol

larger tissue size. The coherent characteristic of the lasers, unlike other LED-based light sources, could be the difference in the photobiomodulated stimulatory effect on cells. The coherent light from laser can result in an interference pattern due to tissue imperfections, and the resultant stimulation is theorised to affect mitochondrial activity [21]. Similarly, it is reported that pulsed lasers have different photobiomodulatory effects compared with continuous wave therapy [22]. Given these differences, there is a potential source of heterogeneity between the studies that used LED and those that used lasers.

In addition, Kim et al. (2017) did not state the duration of exposure, which determines the energy induced into the cells; therefore, that study does not remain comparable [17].

Risk of bias and study methodology assessment

Out of all included studies, two had a moderate risk of bias, and the rest had a low risk of bias [12, 17] (Table 1). All included studies reported cell culture techniques, handling techniques, laser parameters, cell passages, and also reported on all outcomes as specified prior in their methods section. One study did not report all laser parameters, specifically the duration of each laser session [17]. They also did not report the stem cells passage, nor did they report whether the characterisation was done [17]. Cell passage is the number of times the cells were subcultured and although this does not have an impact on the effect of photobiomodulation, lack of recording and reporting of cell passage questions the handling of cells during the experiment. This, if not recorded or reported, adds to increase the risk of bias. In addition, the lower the number of cell passages, the closer it is to the primary cell culture. A higher number, on the other hand, means the cells might have a finite life span and more prone to changes in their proliferative and differentiation ability [23]. Cell characterisation, on the other hand, can be conducted using flow cytometry and is important to determine as this allows comparison of cells of the same lines. Failure to report the cell line and whether characterisation was done adds to the increased risk of bias.

Table 2 Outcome measures and characteristics of included studies

Author(s)	Source of human dental pulp	Cell type	Studied characteristic/outcome measure	Control	Main finding(s)
Montoro et al. [11]	Healthy deciduous teeth	SHED (5th and 6th passage)	Cell metabolic activity, NO production, and ROS production measured with or without LPS induced oxidative stress 24 h after irradiation	Non-irradiation and non-LPS induced oxidative stress	Irradiation reduced the amount of NO and ROS produced in both LPS and non-LPS groups, and increased cell viability in non-LPS groups.
Eduardo et al. [12]	Not mentioned	DPSC (no detail of cell passage)	Cell viability and proliferation measured at 20, 24, 48, and 72 h after first laser irradiation	Negative control (grown in nutritional deficit), and positive control (non-irradiated)	Group irradiated with the 20 mW setting presented significantly higher MTT (cell metabolic) activity at 72 h
Zaccara et al. [13]	Healthy third permanet molars	DPSC (3rd passage)	Cell viability and proliferation measured at 24, 48, 72, and 96 h after laser irradiation	Non-irradiated	More viable cells at 72 in irradiated groups than control
Tabatabaei et al. [14]	Healthy third permanent molars	DPSC (3rd passage)	Proliferation of 0.1, 0.2, and 0.3 J/cm^2 irradiated DPSCs measured 24 and 168 h after laser irradiation	Non-irradiated	Cell proliferation not significantly different at 24 h, but significantly increased in groups exposed to 0.1 or 0.2 J/cm^2. No difference in 0.3 J/cm^2 and control.
Moura-Netto et al. [15]	Healthy deciduous teeth	SHED (6th and 7th passages) in nutritional deficiency	Effect of PBM on cell proliferation	Non-irradiated	Significantly higher proliferation
Marques et al. [16]	Healthy deciduous teeth	Fibroblasts (4th and 8th passages)	Effect of changing power and duration of PBM on cell proliferation	Non-irradiated	All PBM groups showed no statistical difference. But more viable than non-irradiated.
Kim et al. [17]	Permanent teeth (healthy or otherwise not mentioned)	DPSC (passage not mentioned)	Osteogenic differentiation of cells subjected to pulse wave photobiomodulation	Cells subjected to continuous wave photobiomodulation	PW-PBM induced more significant changes in the differentiation of hDPSCs and created longer-lasting DL from the cells than CW-PBM.
Pinheiro et al. [18]	Healthy deciduous teeth	SHED (passage 4)	Osteogenic differentiation of cells	Non-irradiated	Most cell differentiation and calcium deposition noted for irradiated group with 20 J of energy
Sivakumar et al. [19]	Healthy permanent premolar	DPSC (passage 3)	Assessing proliferation and osteogenic potential of N-acetylcysteine-loaded hydroxyapatite scaffold on DPSC with irradiation	Non-irradiated	Double irradiation group (L2) showed an increase in the calcium nodule formation as evaluated on day 5 when compared with single exposure group (L1) and control group

Table 3 Laser parameters of included studies

Author(s)	Laser type	Wavelength	Duration/frequency of sittings	Power (mW)	Energy density (J/cm^2)
Montoro et al. [11]	Infrared light emitting diode (LED) InGaN	855	1 irradiation session [50 s, 1 min 20 s, 3 min 20 s, 6 min 15 s, and 12 min 30 s, to achieve required energy density 2, 4, 8, 16, 30 J/cm^2]	40	2, 4, 8, 16, and 30
Eduardo et al. [12]	InGaAlP	660	2 irradiations (6 h apart) 3 s and 6 s	40 and 20	3
Zaccara et al. [13]	InGaAlP	660	2 irradiations [48 h apart]	30	0.5 and 1.0
Tabatabaei et al. [14]	InGaAlP	810	7 irradiation sessions every 24 h, 60 s	60	0.1, 0.2, and 0.3
Moura-Netto et al. [15]	InGaAlP	660	1 irradiation session [8 and 14 s]	10	3 and 5
Marques et al. [16]	InGaAlP	660	Group 1 [5 mW for all subgroups with varying duration]: 1.25 J/cm^2 for 10 s, 2.50 J/cm^2 for 20 s, 3.75 J/cm^2 for 30 s, 5.00 J/cm^2 for 40 s, and 6.25 J/cm^2 for 50 s	5	1.25, 2.50, 3.75, 5.00, 6.25
			Group 2 [10 s for all subgroups with varying power]: 1.25 J/cm^2 for 5 mW, 2.50 J/cm^2 for 10 mW, 3.75 J/cm^2 for 15 mW, 5.00 J/cm^2 for 20 mW, and 6.25 J/cm^2 for 25 mW	5, 10, 15, 20, 25	1.25, 2.50, 3.75, 5.00, 6.25
Kim et al. [17]	LED pulsed: 1, 3, 30, 300 Hz and 3 kHz.	810	1 session, *duration not stated*	426 $\mu W/cm^2$	38 mJ/cm^2
Pinheiro et al. [18]	InGaAlP	660	Duration not stated, every 24 h for 21 days	20 mW	12.5, 25, and 50
Sivakumar et al. [19]	GaAlAs	810	Group 1 had 1 irradiation session, group 2 had 2 48 h apart, 5 s	800 mW	4

Eduardo et al. (2008) failed to report the source of the cells, posing a significant problem in validating the study [12]. This means that the cell type was not clearly identified in this study, making it difficult to conclude on the effect of PBM on the cells in relation to other studies.

Outcome measures for SHEDs

Although all four studies using SHEDs studied the main effect of proliferation under the influence of photobiomodulation, two studies added specific conditions. One study assessed the effect on nitric oxide production [11]. Another study created a nutritionally deficient environment as a simulator for stress. All studies confirmed positive effects on proliferation [11, 15, 16, 18]. One study also found improved osteogenic differentiation [18]. A study evaluated the effect of varying energy densities and output power on the photobiomodulatory effects on cell viability and proliferation in SHEDs [16]. It found that any energy density between 0.5 and 4 J/cm^2 improved cell viability. Higher energy density has been reported to potentially reduce the photobiomodulatory effect by damaging the photoreceptors. However, that study did not find any statistically significant difference by varying output power [16].

Outcome measured for DPSCs

Five studies used permanent teeth as the source of dental stem cells [12–14, 17, 19]. One study, although agreeing with other studies that photobiomodulation improves proliferation, found that the proliferation rate of cells improved as time passed from 1 to 2 weeks [14]. Kim et al. (2017) went on to test pulse frequency dependency of photobiomodulation on biostimulation and concluded that 300 Hz was more effective for enhanced alkaline phosphatase activity, compared with 3 Hz. This, and a new testing mechanism of biostimulation with detection of biophoton emission, adds to the new areas of research but does not form part of our question and systematic

review. The new testing mechanism used in this study is called delayed luminescence, where emissions of photons are determined after the light source is switched off. This is based on the theory that oxidative metabolic reactions in cells emit these photos, and photobiomodulation increases these reactions. Therefore, testing for these emitted photos allows testing the effectiveness of light sources on cells [24]. Eduardo et al. (2008) found that 20 mW was more effective than 40 mW as output power in stimulating proliferation of DPSCs [12]. This was done under the 660 nm InGaAlP. Sivakumar et al. (2019) also concurred with the study by Eduardo et al. (2008) and showed that there might be more benefits with two sessions of laser therapy instead of one [12, 19]. Comparing SHEDs and DPSCs was not done by any of the included studies.

Discussion

Tissue engineering has varied applications in the orofacial region from the repair of craniofacial defects to repair of dental tissues. Stem cell research has been of increasing interest over the last two decades. The aim of the present systematic review was to add to the existing systematic reviews upon finding new in vitro studies, as well as to expand on the flaws identified from previous systematic reviews. Out of three reviews, two were systematic, and one was a narrative review [4–6]. The narrative review had a high selection bias as studies were included without any justification [6]. Although the other two reviews were conducted with a systematic search strategy and inclusion and exclusion criteria, they had a few flaws. One of them did not report a risk of bias assessment, and when the other did report a tool, it was deemed inadequate as not all factors in the design of study were added to it [4, 5]. The present systematic review built on that tool to make it more comprehensive in its assessment for risk of bias. The systematic reviews also combined the results of different types of stem cells—DPSCs, SHEDs, as well as PDLSCs. This present systematic review separately analysed the two groups of studies, SHEDs and DPSCs.

One of the most significant findings is that there is a therapeutic window of laser parameters of varying energy densities [16], as evidenced by one study, and supported by other studies [4, 12, 19]. This is in line with the Arndt-Schulz law which states that small doses can stimulate, moderate doses inhibit, and large doses kill cells. The existence of an effective window could be the reason why the lowest (0.05 J/cm^2) and the highest (42 J/cm^2) energy densities reported no effects [4]. In this range of energy densities, positive effects of photobiomodulation therapy were observed, as has been demonstrated in other cell types [4, 12–14, 17, 19]. The effects of photobiomodulation analysed on cell activities relevant for tissue regeneration were mostly cell viability and proliferation and odonto/osteogenic differentiation. When in vitro survival, viability, and proliferation in response to photobiomodulation therapy was analysed in this systematic review, as was the case in the previous reviews, positive effects were obtained.

The red laser (660 nm) was the most used wavelength. The wavelength of laser used does not require a debate as a range of wavelengths from 660 to 855 nm have all shown to be effective, and this is in line with the absorption range of cells [11, 16, 18]. Beam divergence however is a major issue in all studies with laser therapy. Four studies performed irradiations from the top of the wells, which could have beam divergences that should be further considered in the calculation of energy density deposited on the cell monolayer [11, 13, 14, 25]. In some studies, the contact mode was used by irradiating the cells through the bottom of the wells, where the distance between the cell monolayer and the laser source is < 1 mm, and the beam divergence is negligible [11, 15]. In accordance, the calculations of energy densities were more accurate in these cases, as the irradiation spot sizes were the same as those of the laser tips. Although all studies found positive results, in future studies, it is important to note this design flaw of beam divergence to better evaluate photobiomodulation.

Additionally, some included studies used near-infrared lasers (780–1100 nm) [11, 14, 17, 19]. Both red and near-infrared lasers have some similar properties. They both have been found to increase intracellular ATP level, as well as increase cell proliferation, and display biphasic dose-dependent response. Literature also reports an increase in intracellular matrix metalloproteinase levels and reduced ROS levels [26]. This is consistent with an included study which found reduced ROS and NO levels when subjected to near-infrared radiation [11]. An important condition to consider when determining the effect of irradiation is the number of mitochondria in a cell as they tend to be the initial site of light absorption. Cytochrome c oxidase (a light absorbing enzyme in mitochondria) (CCO) is the most important chromophore in photobiomodulation effects and has two different absorption bands—one corresponding to red and another near-infrared laser wavelengths. Although both wavelengths produce positive effect, the depth of penetration is different. In a clinical situation, tissue penetration would determine which wavelength should be used. Red wavelengths can penetrate 0.5–1 mm and wavelengths in the range of 780–1100 nm can penetrate 2 mm before losing 37% of its intensity [27]. Therefore, deeper tissues such as bone have shown better response with near-infrared lasers [27].

Characteristics and gene expression related to the mineralisation processes, innervation, tissue formation, vascularization, and immune response are different in dental pulp cells from primary and permanent teeth. Thus, cells from these different sources may demonstrate different levels of effect to photobiomodulation. Therefore, this systematic review

compared SHEDs and DPSCs separately. The key outcome measure was cell viability and/or proliferation. In both groups of studies, positive evidence was found, with some distinct findings as detailed below.

SHEDs

It is known that children will lose their deciduous teeth between 6 and 12 years of age. Therefore, harvesting the stem cells from deciduous teeth is more sustainable and less painful for the patient. Studies have shown there is no difference in cell viability between stem cells from deciduous teeth and permanent teeth [4, 14]. Currently, to harvest stem cells from deciduous teeth, the teeth should have a pulp that appears red and at least two thirds of the root is remaining [26]. These teeth should be disease-free and extracted in a sterile environment. After rinsing with phosphate-buffered saline and alcohol, they are transferred for harvestation of cells. The pulp is harvested in the laboratory or in the dental clinic with a sterile barbed broach and then trypsinized and cultured to acquire different colonies of stem cells. After sorting the colonies of cells with fluorescence activated system, the identified mesenchymal stem cells are then stored under cryopreservation [28].

Four of the included studies used SHEDs [11, 15, 16, 18]. Although Montoro et al. (2014) studied different energy densities and their effect on nitric oxide production, it still concluded that all resulted in improved cell viability [11]. Nitric oxide is produced as a free radical and although beneficial in some quantity, initially for vasodilation, it is known to be cytotoxic and reduction in NO is therefore a desirable mechanism to improve cell viability. NO production also is known to increase in the presence of bacteria. Therefore, Montoro et al. (2014) demonstrated that photobiomodulation can be effectively used in the region of transplanted cells in tissue defects with present bacteria to allow for increased cell viability [11]. After creating nutritional deficiency as a simulator for stress, due to poor blood supply, in in vivo conditions, Moura-Netto et al. (2016) reported that photobiomodulation could effectively improve cell viability and proliferation for both their protocols (3 J/cm^2 and 5 J/cm^2) [15]. In the future, this simulated stress should be combined with a Montoro et al. style study on NO production and cell viability and proliferation testing [11]. Clinically, both of these situations present themselves whether through bacterial infiltration or reduced blood flow, and it is a key finding of these studies that photobiomodulation can be beneficial in those situations.

DPSCs

Kim et al. (2017) and Eduardo et al. (2008) did not state how the cell characterisation was done, nor what the cell passage was [12, 17]. Kim et al. (2017) also did not state the duration of their lesser sessions [17]. This was a problem with comparison as cell passages determine their proliferative and differentiation properties [29]. The duration of the laser session also has an impact on the effectiveness of laser—and this meant that the comparison in this subgroup of DPSCs was flawed in two studies with bias. This means that both the internal validity of those two studies was affected and so was the overall external validity of those studies, therefore leaving the conclusion for this subgroup weaker in strength than that for SHEDs.

Conclusion

This systematic review was conducted in line with PRISMA. PICO question was established; inclusion and exclusion criteria were established before a search had begun. However, the data limited the scope of the study. No previous study compared the effect of photobiomodulation on these two types of stem cells. In the subgroup of SHEDs, there was a low risk of bias, and cell viability and proliferation did improve with photobiomodulation. Similar results can be reached for the other subgroup DPSCs, but with a moderate level of bias. Due to heterogeneity in design protocols and laser parameters, it was not possible to compare the studies together.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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