Frontiers in Dentistry

Effects of 660 and 940nm Low-Level Laser Irradiation on Viability and Proliferation of Stem Cells from Human Exfoliated Deciduous Teeth

Samar Rahimpour[1], Arash Azizi[2], Mahshid Hodjat[3], Mohammad Vahedi[2]*

1. Department of Oral and Maxillofacial Medicine, School of Dentistry, Iran University of Medical Sciences, Tehran, Iran
2. Department of Oral and Maxillofacial Medicine, TeMS.C., Islamic Azad University, Tehran, Iran
3. Dental Research Center, Dentistry Research Institute, Tehran University of Medical Sciences, Tehran, Iran

Article Info

Article type:
Original Article

Article History:
Received: 07 Feb 2025
Accepted: 15 May 2025
Published: 10 Feb 2026

**** Corresponding author:***
Department of Oral and Maxillofacial Medicine, TeMS.C., Islamic Azad University, Tehran, Iran

Email: vahedi_md@yahoo.com

A B S T R A C T

Objectives: This study evaluated the effects of 660 and 940nm lasers on viability and proliferation of stem cells from human exfoliated deciduous teeth (SHED).

Materials and Methods: SHED were cultured and divided into six groups for laser irradiation: 660nm laser at 25mW with energy densities of 1 and 2J/cm^2 for 8 and 16 seconds, and 940nm laser at 100mW with the same energy densities and durations. Two additional groups served as controls with no laser exposure. Cell viability was evaluated by the methyl thiazolyl tetrazolium (MTT) assay, and cell proliferation was measured by cell counting with a Neubauer chamber and qualitatively evaluated by 4′,6-diamidino-2-phenylindole (DAPI) staining at 24 and 72 hours post- irradiation. Data were analyzed using one-way ANOVA, followed by Tamhane's and Tukey's tests.

Results: Irradiation with 940nm laser enhanced cell proliferation compared to both 660nm laser and control groups at 24 and 72 hours ($P<0.05$), as confirmed by DAPI staining. At 24 hours, all laser-treated groups showed increased cell viability relative to the control, although the increase in the 660nm/2J/cm^2 group was not statistically significant ($P>0.05$). At 72 hours, only 940nm laser group showed significantly higher cell viability and proliferation than the control group ($P<0.05$).

Conclusion: These findings suggest low-level laser therapy, particularly at the wavelength of 940nm enhances the viability and proliferation of stem cell, supporting its potential as a promising tool in regenerative medicine and tissue engineering.

Keywords: Stem Cells; Low-Level Light Therapy; Cell Proliferation; Cell Survival

➢ **Cite this article as:** Rahimpour S, Azizi A, Hodjat M, Vahedi M. Effects of 660 and 940nm Low-Level Laser Irradiation on Viability and Proliferation of Stem Cells from Human Exfoliated Deciduous Teeth. *Front Dent.* 2026:23:07. http://doi.org/10.18502/fid.v23i7.21236

INTRODUCTION

Regenerative medicine is an emerging field of medicine that focuses on tissue regeneration and the restoration of function in damaged tissues and organs, offering significant potential benefits for patients with severe injuries or chronic diseases [1]. Reconstruction of injured or defective maxillofacial structures using regenerative approaches is particularly important, as conventional dental materials have limited clinical longevity and often need to be replaced after a while. Tissue engineering, as an alternative treatment modality, aims to restore lost structures by preserving and promoting tissue viability. The mechanism of action of tissue engineering approaches is based on the capacity of undifferentiated cells to respond to specific biological signals, leading to their proliferation, migration, and

differentiation into defined cell lineage [2-4].
The oral cavity is a rich and accessible source of mesenchymal stem cells (MSCs), due to the ease of obtaining tissue sample. Since 2000, several types of MSCs have been isolated from the oral tissues, including dental pulp stem cells (DPSCs), stem cells from human exfoliated deciduous teeth (SHED), periodontal ligament stem cells, stem cells of the apical papilla, dental follicle cells, oral periosteum-derived cells, and more recently, MSCs from human periapical cysts [5,6]. Among tooth-derived MSCs, SHED exhibit a higher proliferation rate and greater population doubling rate compared to periodontal ligament stem cells, DPSCs, and stem cells of the apical papilla [7]. Moreover, the differentiation potential of SHED is not limited to odontoblasts, as they can also differentiate into various cell types, such as neurons, osteoblasts, adipocytes, and endothelial cells [8,9].
Given that SHED are isolated from the pulp of primary teeth- a transiently available tissue postnatally, and considering the natural exfoliation of these teeth during growth and development, their collection is relatively simple and non-invasive. They can also be isolated from carious primary further increasing their accessibility. Thus, ethical and legal concerns regarding the isolation of SHED are minimal [2,8-10]. Considering the high proliferative capacity, multi-lineage differentiation potential, easy access, preservation of biological, immunological, and therapeutic functions after long-term cryopreservation, and high plasticity, SHED represent a promising stem cell model for biomedicine research, and tissue engineering applications [2,10]. However, their proliferation rate in culture is relatively slow, and strategies to stimulate their in vitro growth are necessary to obtain an adequate number of cells.
Low-level laser therapy (LLLT) is increasingly used in dentistry to enhance cellular metabolism, promote epithelialization and vascularization, and stimulate collagen synthesis. It has been shown to effectively enhance cell proliferation and differentiation [2]. The key physiological effects of LLLT are associated with its ability to induce cell proliferation and migration, regulate protein expression, and promote differentiation of various cell lineages [10]. Evidence indicates that LLLT enhances the proliferation of fibroblasts, endothelial cells, and osteoblasts. However, its effects on cellular activity—whether stimulatory or inhibitory—depend on specific laser parameters, including wavelength, power, energy density, fluence, exposure time, and tip diameter [2,10,11].
Given the limited research on the effects of LLLT at different wavelengths on SHED [2,12], this study aimed to assess the impact of 660 and 940nm laser irradiation on viability and proliferation of SHED.

MATERIALS AND METHODS

The present in vitro study, approved by the Ethics Committee under the code IR.IAU.DENTAL.REC.1399.283, was conducted on SHED obtained from the Dental Research Center of Shahid Beheshti University of Medical Sciences.The cells met the ISCT criteria (expressing mesenchymal surface markers and demonstrating the potential to differentiate into adipocytes, chondrocytes, and osteoblasts).

Sample size calculation:

The required sample size for assessment of cell viability using the methyl thiazolyl tetrazolium (MTT) assay was calculated as 10 replicates per group across six groups, based on a study by Fernandes et al. [13]. The calculation assumed $\alpha = 0.05$, $\beta = 0.2$, an effect size of 0.51, and a standard deviation of 0.1 using one-way ANOVA power analysis feature of PASS 11.
The required sample size for assessment of cell proliferation using cell counting with Neubauer chamber and 4′,6-diamidino-2-phenylindole (DAPI) staining was calculated as three replicates per group across six groups, based on a study by Navaei-Nigjeh et al [14]. The calculation assumed $\alpha = 0.05$, $\beta = 0.2$, an effect size of 1.41, and a standard deviation of 2.8 x 104 using one-way ANOVA power analysis feature of PASS 11.

Cell culture:

SHEDs were cultured in high-glucose Dulbecco's modified Eagle's medium (Biochrom, Berlin, Germany) containing 10% fetal bovine serum (Biosera, France) and 1% penicillin-streptomycin (Biosera, France). The cells were incubated at 37 °C in a humidified atmosphere with 5% CO_2. Once the cultures reached approximately 90% confluence, the cells were detached using 0.5% trypsin-EDTA (Biosera, France) at a 1:3 dilution ratio. Cells from passages three to five were used for the experiments. The culture medium was refreshed every two days [2].

Study groups:

The cultured cells were then randomly divided into six groups as follows:

R1: 660nm laser irradiation at 25mW power and 1J/cm² energy density for 20 seconds

R2: 660nm laser irradiation at 25mW power and 2J/cm² energy density for 40 seconds

IR1: 940nm laser irradiation at 100mW power and 1J/cm² energy density for 8 seconds

IR2: 940nm laser irradiation at 100mW power and 2J/cm² energy density for 16 seconds

C24: No irradiation control group, for assessment at 24 hours

C72: No irradiation control group for assessment at 72 hours

Laser irradiation:

In this study, 660nm and 940nm lasers were irradiated to the cells at a standardized distance of 1cm between the probe tip and culture medium, with the opening of each well in contact with the laser of each well. The irradiations were conducted in standard environmental conditions and under a laminar flow hood (JTLFH130; Jal Tajhiz, Iran). The output power of the laser handpiece was calibrated before use. In this study, 660nm laser (Semiconductor diode; SiroLaser Blue; Dentsply Sirona, Germany) with 25mW power and energy densities of 1 and 2J/cm² for 20 and 40 seconds, respectively, was used for groups R1 and R2. A 940nm laser (InGaAsP semiconductor diode; Epic; Biolase, USA) with 100mW power and energy densities of 1 and 2J/cm² for 8 and 16 seconds, respectively, was used for groups IR1 and IR2. No laser irradiation was applied to the control groups. It should be mentioned that energy density was calculated according to the formula $\frac{T \times P}{A}$, where T indicates time in seconds, P is the power in watts (W) and A is the cross-sectional surface area of the probe in square centimeters (cm²). In the present study, the laser probe had a circular cross-section with a tip diameter of 8mm for the 660nm laser and 10mm for the 940nm laser. After laser irradiation, the culture medium in all wells was replaced with fresh culture medium supplemented with 10% fetal bovine serum (Biosera, France) [13].

Assessment of cell viability and proliferation:

The cells were first seeded in 96 and 24-well plates at a density of 104 cells/well in the 96-well plate, and 7 x 104 cells/well in the 24-well plate. For each laser group, 10 repetitions were included for the MTT assay and 3 repetitions for cell proliferation assessment via cell counting. Three empty wells were placed between the laser-treated wells, and these were filled with water. Assessments were conducted at 24 and 72 hours post-irradiation. The irradiations were performed at 24 hours after seeding the cells in the wells [15].

Assessment of cell viability by the MTT assay:

Tetrazolium salt (Sigma, USA) was dissolved in phosphate-buffered saline (Sigma, USA) at a concentration of 5mg/ml. The solution was sterilized using a 0.2-µm filter and stored at -20°C until use. The MTT assay was performed at 24 and72 hours post-laser irradiation. For the assay, the MTT solution was first diluted 1:10 with Dulbecco's Modified Eagle's Medium (Biosera, France). Next, 10µl of the diluted solution was added to each well. The plates were incubated at 37°C. After 3-4 hours, the wells were emptied upon observing the formation of a purple precipitate under a light microscope (Leica, USA). Then, 100µl of dimethyl sulfoxide (LifeBiolab, Germany) was added to each well to dissolve the formazan crystals. Finally, the optical density (OD) of each well was read at 570nm wavelength by a spectrophotometer (Epoch; Biotek, USA). To calculate the percentage of cell viability, the OD of each

group was divided by the OD of the control group and then multiplied by 100 [16].

Assessment of cell proliferation by the Neubauer chamber and DAPI staining:

At 24 and 72 hours post-laser irradiation, the cells were detached from the bottom of the wells by using 0.5% trypsin/EDTA (Biosera, France). A 20µL aliquot of 0.4% trypan blue solution (Sigma Aldrich, USA) was then mixed with 0.85% phosphate buffered saline for 1 minute. Cells present in 10 µL of the suspension were immediately counted using a Neubauer chamber (Marienfield, Germany) under a light microscope (DM 300; Leica, USA). Cell counting was performed three times for each group. The mean number of cells in the four corner squares of the chamber was used to estimate the number of cells per milliliter of suspension. This value was then used to calculate the total number of cells in each well [17-19].

DAPI staining was performed to qualitatively assess cell density and cell nucleus morphology. Cultured cells were fixed with 70% ethanol (Merck, Germany) at room temperature for 10 minutes, then air-dried for 30 minutes. A 20mg/mL stock solution of DAPI (Sigma Aldrich, USA) was prepared in water, and working solution (2–3µM) was made in phosphate-buffered saline. Each well received 200µL of the 3µM DAPI solution and was incubated on a shaker (FMS2; Finepcr, Korea) for 5 min. Wells were then washed three times with 200µL PBS (5-minute incubations each). Stained nuclei were examined under a fluorescent microscope (IX7; Olympus, Japan) to evaluate nuclear DNA content and relative cell density [20].

Statistical analysis:

Data were analyzed using one-way ANOVA, followed by Tamhane's and Tukey's tests using PASS 11 software at a 0.05 level of significance.

RESULTS

Cell counting:

Fig 1 presents the mean cell count for each group at 24 and 72 hours. The 940nm/2J/cm^2 group exhibited the highest mean cell count, while the 660nm/2J/cm^2 group showed the lowest at both time points of 24 and 72 hours.

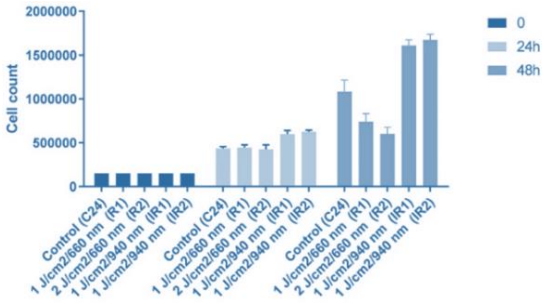

Fig 1. Cell counts of SHED after laser irradiation. The effects of laser irradiation on cell proliferation across different treatment conditions and time points. Error bars represent standard deviation.

Visual assessment of DAPI staining confirmed the Neubauer chamber counts, revealing the highest cell density in the 940nm/2J/cm^2 group and the lowest in the 660nm/2J/cm^2 group at 72 hours, as shown in Fig 2.

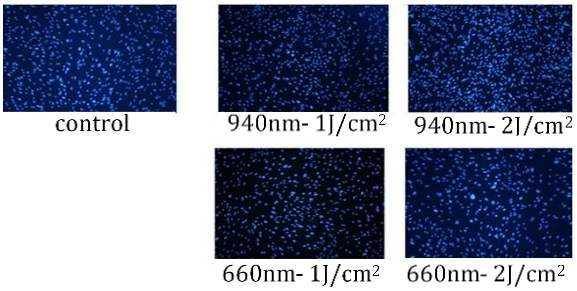

Fig 2. Fluorescent micrographs for qualitative assessment of cell count following DAPI staining

Pairwise comparisons (Table 1) were performed using Tukey's HSD test. Groups with homogenous means were categorized into the same subset. No significant differences were noted within each subset (P>0.999), however, the mean cell count differed significantly between the two subsets at 24 hours (P<0.001).

Subsequently, the groups were divided into three subsets (Table 2), and those with a homogenous means were placed in one subset. No significant difference was noted within each subset (P>0.999), however the mean cell count was significantly different between the two subsets at 72 hours (P<0.001).

Overall, the results indicated that cell counts at 72 hours were significantly higher than those at 24 hours in all groups (P<0.05).

Table 1. Comparison of homogeneous subsets of cell count at 24 hours using Tukey's test

Comparison	Group		P value
Within-group	C24: Control	R1: 1J/cm²/660nm	>0.999
		R2: 2J/cm²/660nm	>0.999
	R1:1J/cm²/660nm	(C24) Control	>0.999
		(R2) 2J/cm²/660nm	>0.999
	R2: 2J/cm²/660nm	(C24) Control	>0.999
		(R1) 1J/cm²/660nm	>0.999
	IR1: 1J/cm²/940nm	(IR2) 1J/cm²/940nm	>0.999
Between-group	C24: Control	(IR1) 1J/cm²/940nm	<0.001*
		(IR2) 2J/cm²/940nm	<0.001*
	R1: 1J/cm²/660nm	(IR1) 1J/cm²/940nm	<0.001*
		(IR2) 2J/cm²/940nm	< 0.001*
	R2: 2J/cm²/660nm	(IR1) 1J/cm²/940nm	<0.001*
		(IR2) 2J/cm²/940nm	<0.001*

* statistically significant

Table 2. Comparison of homogeneous subsets of cell count at 72 hours using Tukey's test

Comparison	Group		P value
Within-group	R1: 1J/cm²/660nm	(R2)660nm- 2J/cm²	>0.999
	C72: Control	-	-
	IR1: 1J/cm²/940nm	(IR2) 2J/cm²/940nm	>0.999
Between-group	C72: Control	(IR1) 1J/cm²/940nm	<0.001*
		(IR2) 2J/cm²/940nm	<0.001*
		(R1) 1J/cm²/660nm	0.0004*
		(R2) 2J/cm²/660nm	<0.001*
	R1: 1J/cm²/660nm	(IR1) 1J/cm²/940nm	<0.001*
		(IR2) 2J/cm²/940nm	<0.001*
		(C72) Control	<0.001*
	R2: 2J/cm²/660nm	(IR1) 1J/cm²/940nm	<0.001*
		(IR2) 2J/cm²/940nm	<0.001*
		(C72)Control	<0.001*
	IR1: 1J/cm²/940nm	(R1) 1J/cm²/660nm	<0.001*
		(R2) 2J/cm²/660nm	<0.001*
		(C72) Control	<0.001*
	IR2: 2J/cm²/940nm	(R1) 1J/cm²/660nm	<0.001*
		(R2) 2J/cm²/660nm	<0.001*
		(C72) Control	<0.001*

* statistically significant; Blank cells indicate groups that did not belong to any homogeneous subset and were significantly different from all other groups ($p < 0.05$).

Cell viability:

Fig 3 shows the mean cell viability, expressed as a percentage, for each group at 24 and 72 hours. At 24 hours, the highest mean cell viability was observed in the 940nm/2J/cm^2 group, while the lowest was recorded in the control group (C24). At 72 hours, the 940nm/1J/cm^2 group exhibited the highest mean viability, whereas the 660nm/2J/cm^2 group showed the lowest.

For pairwise comparisons of the groups at 24 hours using Tukey's test, the study groups were categorized into three subsets, with groups exhibiting homogeneous means placed within the same subset (Table 3). No significant

differences were observed within subsets (P>0.999); however, the mean viability at 24 hours differed significantly among the three subsets (P<0.001).

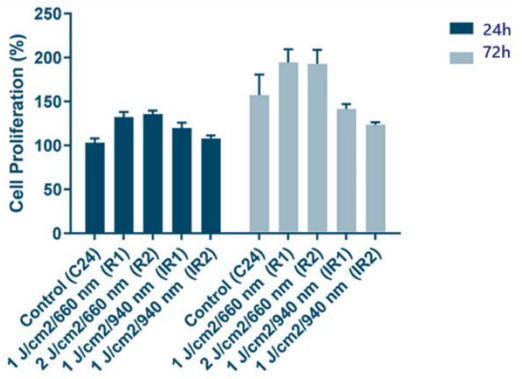

Fig 3. Cell proliferation (%) of SHED after laser irradiation using MTT assay. The graph shows cell proliferation at 24 hours (black bars) and 72 hours (gray bars) for various laser treatments. The control group and groups irradiated with 660nm and 940nm lasers at 1J/cm^2 and 2J/cm^2 energy densities are shown. Error bars represent standard deviation (SD).

For pairwise comparisons of the groups at 72 hours using Tukey's test, the study groups were categorized into four subsets, with groups exhibiting homogeneous means placed within the same subset (Table 4). Within-subset comparisons showed no significant differences (P>0.999); however, the mean viability at 72 hours differed significantly among the four subsets (P<0.001).

At 24 hours, all laser-treated groups showed higher cell viability compared to the control group. By 72 hours, only the 940nm laser groups maintained higher cell viability than the control.

DISCUSSION

This study assessed the effects of 660 and 940nm laser irradiation on the viability and proliferation of SHED.

Previous reports suggest that higher energy density delivered over a shorter exposure time is more effective than lower density with prolonged exposure [21]. In agreement with this, the present study found that cell proliferation was significantly higher in 940 nm laser groups (higher power, shorter exposure time) compared to both the control and 660nm laser groups (lower density, longer exposure time).

Table 3. Comparison of homogeneous subsets of cell viability at 24 hours using Tukey's test

Comparison	Group		P value
Within-group	(C24) Control	(R2) 2J/cm^2 /660nm	>0.999
	(R1) 1J/cm^2/660nm	-	-
	(IR1)940nm- 1J/cm^2	(IR2) 2J/cm^2/940nm	>0.999
Between-group	(C24) Control	(IR1) 1J/cm^2/940nm	0.033*
		(IR2) 2J/cm^2/940nm	<0.001*
		(R1) 1J/cm^2/660nm	<0.001*
	(R2) 660nm- 2J/cm^2	(IR2) 2J/cm^2/940nm	<0.001*
		(R1) 1J/cm^2/660nm	<0.001*
		(IR1) 1J/cm^2/940nm	<0.001*
	660nm- 1J/cm^2 (R1)	(IR2) 2J/cm2/940nm	<0.001*
		(R2) 2J/cm^2/660nm-	<0.001*
		(C24) Control	<0.001*
	(IR1) 940nm- 1J/cm^2	(R1) 1J/cm^2 /660nm	<0.001*
		(R2) 2J/cm^2/660nm	<0.001*
		(C24) Control	<0.001*
	(IR2) 940nm- 2J/cm^2	(R1) 1J/cm^2 /660nm	<0.001*
		(R2) 2J/cm^2/660nm	<0.001
		(C24) Control	<0.001

* statistically significant; Blank cells indicate groups that did not belong to any homogeneous subset and were significantly different from all other groups (p < 0.05).

Table 4. Comparison of homogenous subsets of cell viability at 72 hours using Tukey's test

Comparison	Group		P value
Within-group	(R2) 2J/cm²/660nm	-	-
	(R1) 1J/cm² /660nm	-	-
	(C72) Control	-	-
	(IR2) 2J/cm²/940nm	(IR1) 940nm- 1J/cm2	>0.999
Between-group	(R2) 2J/cm²/660nm	(R1) 660nm- 1J/cm²	<0.001*
		(C72) Control	<0.001*
		(IR2) 940nm-2J/cm²	<0.001*
		(IR1) 940nm- 1J/cm²	<0.001*
	(R1) 1J/cm² /660nm	(R2) 660nm- 2J/cm²	<0.001*
		(IR2) 940nm-2J/cm²	<0.001*
		IR1) 940nm- 1J/cm²	<0.001*
		(C72) Control	0.002*
	Control (C72)	(R2)660nm- 2J/cm²	<0.001*
		(R1)660nm- 1J/cm²	0.002*
		(IR2) 940nm-2J/cm²	0.004*
		(IR1) 940nm- 1J/cm²	0.002*
	(IR2) 2J/cm2/940nm	(R2) 660nm- 2J/cm²	<0.001*
		(R1)660nm- 1J/cm²	<0.001*
		(C72) Control	<0.001*
	(R1) 1J/cm² /660nm	(R2) 660nm- 2J/cm²	<0.001*
		(R1)660nm- 1J/cm²	<0.001*
		(C72) Control	<0.001*

* statistically significant; Blank cells indicate groups that did not belong to any homogeneous subset and were significantly different from all other groups ($p < 0.05$).

Also, the increase in cell proliferation observed at 72 hours compared to 24 hours across all groups highlights the time-dependent nature of proliferation.

Due to the considerable variation in laser parameters reported in the literature, direct comparison of results across studies is not feasible [22]. Nonetheless, it is valuable to highlight the most effective protocols.

Almeida-Junior et al. [23] evaluated the effects of a single and multiple doses of LLLT using 660nm laser on the viability and proliferation of SHED. Their findings showed that, at 72 hours, a single dose of 660nm laser irradiation at 2.5J/cm² energy density with 10mW power for 10 seconds, as well as 7.5J/cm² energy density with 10mW power for 30 seconds significantly enhanced cell proliferation. Additionally, irradiation with a 660nm laser at 2.5J/cm² energy density and 10mW power for 10 seconds resulted in lower cell proliferation at all time points. However, in the present study, irradiation with a 660nm laser at 1J/cm² energy density and 25mW power for 20 seconds resulted in significantly higher cell proliferation at 24 hours compared to the control group, while irradiation at 2J/cm² and 25mW for 40 seconds led to significantly lower proliferation at 72 hours. Additionally, both 660 nm laser groups showed significantly increased cell counts at 72 hours compared to 24 hours. Notably, the 2J/cm² group consistently demonstrated lower proliferation at both time points. These variations may be attributed to differences in the methods used to assess cell proliferation, the composition of the culture medium, and the dosimetry settings for 660nm laser irradiation between studies. Ginani et al. [10] evaluated the effects of LLLT using a diode InGaAlP laser with 660nm wavelength, 30mW power, and 0.5J/cm² (16 seconds) and 1J/cm² (33 seconds) energy density on the

proliferation and viability of SHED at 0, 24, 48, and 72 hours using the MTT assay and Trypan Blue Exclusion (TBE) method and DAPI staining. They reported that an energy density of 1J/cm^2 increased cell proliferation at 48 and 72 hours. No change in cell nuclear morphology was observed, suggesting that cell viability was not adversely affected. They concluded that 660nm laser irradiation at 1J/cm^2 energy density and 30mW power for 33 seconds increased the proliferation of SHED. In contrast, the present findings showed the control group at 72 hours surpassed those of both 660nm laser groups. As energy density is an important parameter for achieving optimal outcomes [2,24], differences in laser dosimetry may explain the discrepancy in results. Additionally, the use of different types of culture media and variations in fetal bovine serum (FBS) concentrations may have contributed to the discrepancies in results. Moura-Netto et al. [25] demonstrated a significant reduction in cell proliferation when a suboptimal FBS concentration (15%) was used. Furthermore, SHED isolated from different individuals may exhibit variable biological behaviors, particularly in the expression of extracellular matrix proteins, which could further account for differences in experimental outcomes. However, unlike the findings of Moura-Netto et al. [25], the present results showed that the use of 10% fetal bovine serum significantly increased cell proliferation in all groups at 24 hours and in 940nm laser groups at 72 hours. This suggests that energy density may partially compensate for suboptimal nutritional conditions, thus promoting SHED proliferation [25]. Marques et al. [2] reported that irradiation with a 660nm laser at 5J/cm^2 energy density and 10mW power for 20 seconds increased SHED viability and proliferation at 24, 48, and 72 hours, while irradiation with 780nm laser at the same energy density and power had negative effects. Furthermore, LLL at 1.2 and 2.5J/cm^2 energy densities was found to impair metabolic activity and proliferation of SHED. They also noted that the red laser at 5J/cm^2 energy density resulted in higher cell viability than infrared laser at 24 hours. In contrast, the present findings showed that red laser irradiation resulted in lower cell proliferation than infrared laser at both 24 and 72 hours. In agreement with our results, de Souza et al. [24] also reported reduced SHED viability following 660nm laser irradiation at 1.2J/cm^2 energy density at 24 hours, as assessed by the MTT assay. Moura-Netto et al. [25] observed increased SHED viability after irradiation with a 660nm laser at 10mW power and 5J/cm^2 energy density for 14 seconds at 48 hours. Consistently, our study found that SHED viability and proliferation at both 24 and 72 hours were significantly higher in the 940nm laser groups compared to the 660nm laser and control groups. Variations in laser irradiation dosimetry, wavelength, and radiation frequency can help explain the differences in results, as Moura-Netto et al. [25] employed two irradiation cycles with a 6-hour interval, whereas the present study used a single-dose irradiation protocol. Also, differences in SHED donors may also account for variations in biological behavior. Moreover, as noted by Moura-Netto et al. [25], the effect of laser irradiation is strongly influenced by the redox state of the cells at the time of irradiation. Specifically, a shift from reduction to oxidation is associated with stimulation, while a more reduced redox state tends to inhibit proliferation. When the initial redox potential is optimal or near-optimal, the cellular response is weak or absent. However, if the redox state deviates from this optimal range, the cellular response is enhanced. Cells with lower than normal pH levels are more sensitive to light and LLLT compared to cells with optimal or near-optimal pH [21,25]. This factor may also affect the experimental outcomes. In a study by Soares et al. [26], a 660nm diode laser with 30mW power was applied using 1 and 0.5J/cm^2 energy densities, delivered in two sessions lasting 33 and 16 seconds, respectively. Their results demonstrated that the group treated with 1J/cm^2 energy density exhibited significantly higher cell proliferation at 48 and 72 hours compared to

both the 0.5J/cm^2 group and control groups. Their findings were consistent with the present results at 24 hours, but differed at 72 hours, which may be attributed to variations in irradiation frequency, culture medium type, and irradiation duration. According to Huang et al. [27], laser therapy elicits a dose-dependent biological response in cells, with each successive dose potentially producing a cumulative effect. This was supported by Soares et al. [26], who demonstrated that infrared laser irradiation at an energy density of 1J/cm^2 positively influenced the proliferation of human dental pulp stem cells (DPSCs). Their results align with the present study's findings and may help explain observed differences across studies.

Only a limited number of studies have assessed the effects of LLLT on SHED, with most focusing on the red laser [2,10,23-25]. However, beyond dosage, the efficacy of LLLT also depends on the extent of light absorption by the target tissue [2,28]. Red lasers are typically employed for superficial treatments, with low doses ranging from 0.01 to 10J/cm^2, which are sufficient. In contrast, to reach deeper structures, infrared lasers are more appropriate and require higher doses, typically between 10-50J/cm^2 [28]. The ideal wavelength depends on the properties of the target light receptor molecules [2]. Maximum tissue penetration occurs within the "optical window," a range in which tissue chromophores exhibit minimal absorption. Within this window, red and infrared light can reach intracellular targets such as mitochondrial cytochromes (red laser) or cell membranes (infrared laser). In contrast, wavelengths below 600nm and above 900nm are strongly absorbed by major tissue chromophores, limiting their penetration and effectiveness [2,28]. Although the photochemical and photophysical properties of lasers vary with wavelengths, and red and infrared lasers exert different effects, both types are capable of modulating cellular activity. Thus, the biological response following cell irradiation is attributed to photochemical and photophysical changes resulting from absorption of non-ionizing radiation. In the visible light range, molecules absorb photons, causing electron transitions to higher energy orbits; energy is then released as the electrons return to their ground state. In contrast, the absorption of infrared light induces molecular rotations and vibrations [2].

Given the wide range of laser parameters and methodologies used in low-level laser therapy (LLLT), the foundation for understanding the photobiomodulation effects of lasers on cells lies in the reproducibility of in vitro results to ensure their accuracy and reliability [29].

The primary strength of this study was the evaluation of the effect of a 940nm laser on the viability and proliferation of SHED, which has not been previously investigated. The observed optimal effects at both 24 and 72 hours may contribute to the standardization of laser parameters to enhance efficacy. These findings also pave the way for future studies to explore additional factors influencing the viability and proliferation of SHED.

This study had some limitations; the most notable being the lack of primary response parameters for the comparison of the effects of different LLL wavelengths on SHED. Thus, further studies are required to clarify the impact of LLL parameters on the viability and proliferation of SHED.

Future studies are needed to compare the effects of different LLL parameters, as well as multiple doses versus a single dose, on the viability and proliferation of SHED. Since Trypan Blue protein can interfere with fluorescence, flow cytometry-based assessment may serve as a faster and more reliable alternative method to the conventional TBE method using the Neubauer chamber for evaluating cell viability and counting. Moreover, polymerase chain reaction and Sulforhodamine B staining can be employed in future research to assess cell viability and proliferation following laser irradiation.

CONCLUSION

Based on our current study, low-level laser therapy, particularly at a wavelength of 940nm, promotes the viability and proliferation of stem cells, suggesting that this therapy is a promising tool in regenerative medicine and tissue engineering.

CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES

1. Mahla RS. Stem Cells Applications in Regenerative Medicine and Disease Therapeutics. Int J Cell Biol. 2016;2016:6940283.
2. Marques NP, Lopes CS, Marques NCT, Cosme-Silva L, Oliveira TM, Duque C, et al. A preliminary comparison between the effects of red and infrared laser irradiation on viability and proliferation of SHED. Lasers Med Sci. 2019 Apr;34(3):465-471.
3. Bakhtiar H, Mazidi S A, Mohammadi Asl S, Ellini MR, Moshiri A, Nekoofar MH, et al. The role of stem cell therapy in regeneration of dentine-pulp complex: a systematic review. Prog Biomater. 2018 Dec;7(4):249-268.
4. Tocci A, Forte L. Mesenchymal stem cell: use and perspectives. Hematol J. 2003;4(2):92-6.
5. Martinez Saez D, Sasaki RT, Neves AD, da Silva MC. Stem Cells from Human Exfoliated Deciduous Teeth: A Growing Literature. Cells Tissues Organs. 2016;202(5-6):269-280.
6. Sanz AR, Carrión FS, Chaparro AP. Mesenchymal stem cells from the oral cavity and their potential value in tissue engineering. Periodontol 2000. 2015 Feb;67(1):251-67.
7. Borzabadi-Farahani A. Effect of low-level laser irradiation on proliferation of human dental mesenchymal stem cells; a systemic review. J Photochem Photobiol B. 2016 Sep;162:577-582.
8. Xie F, He J, Chen Y, Hu Z, Qin M, Hui T. Multi-lineage differentiation and clinical application of stem cells from exfoliated deciduous teeth. Hum Cell. 2020 Apr;33(2):295-302.
9. Rosa V, Dubey N, Islam I, Min KS, Nör JE. Pluripotency of Stem Cells from Human Exfoliated Deciduous Teeth for Tissue Engineering. Stem Cells Int. 2016;2016:5957806..
10. Ginani F, Soares DM, de Oliveira Rocha HA, de Souza LB, Barboza CAG. Low-level laser irradiation induces in vitro proliferation of stem cells from human exfoliated deciduous teeth. Lasers Med Sci. 2018 Jan;33(1):95-102.
11. Zaccara IM, Ginani F, Mota-Filho HG, Henriques ÁC, Barboza CA. Effect of low-level laser irradiation on proliferation and viability of human dental pulp stem cells. Lasers Med Sci. 2015 Dec;30(9):2259-64.
12. Marques NCT, Neto NL, Prado MTO, Vitor LLR, Oliveira RC, Sakai VT, et al. Effects of PBM in different energy densities and irradiance on maintaining cell viability and proliferation of pulp fibroblasts from human primary teeth. Lasers Med Sci. 2017 Sep;32(7):1621-1628.
13. Fernandes AP, Junqueira Mde A, Marques NC, Machado MA, Santos CF, Oliveira TM, et al. Effects of low-level laser therapy on stem cells from human exfoliated deciduous teeth. J Appl Oral Sci. 2016 Jul-Aug;24(4):332-7
14. Navaei-Nigjeh M, Moloudizargari M, Baeeri M, Gholami M, Lotfibakhshaiesh N, Soleimani M et al. Reduction of marginal mass required for successful islet transplantation in a diabetic rat model using adipose tissue-derived mesenchymal stromal cells. Cytotherapy. 2018 Sep;20(9):1124-1142.
15. Sylvester PW. Optimization of the tetrazolium dye (MTT) colorimetric assay for cellular growth and viability. Methods Mol Biol. 2011;716:157-68.
16. Feoktistova M, Geserick P, Leverkus M. Crystal Violet Assay for Determining Viability of Cultured Cells. Cold Spring Harb Protoc. 2016 Apr 1;2016(4):pdb.prot087379.
17. Felice DL, Sun J, Liu RH. A modified methylene blue assay for accurate cell counting. Journal of Functional Foods. 2009 Jan 1;1(1):109-18.
18. Avelar-Freitas BA, Almeida VG, Pinto MC, Mourão FA, Massensini AR, Martins-Filho OA, et al. Trypan blue exclusion assay by flow cytometry. Braz J Med Biol Res. 2014 Apr;47(4):307-15.
19. Martin-Piedra MA, Garzon I, Oliveira AC, Alfonso-Rodriguez CA, Carriel V, Scionti G, et al. Cell viability and proliferation capability of long-term human dental pulp stem cell cultures. Cytotherapy. 2014 Feb;16(2):266-77.
20. Ligasová A, Koberna K. Quantification of fixed adherent cells using a strong enhancer of the fluorescence of DNA dyes. Sci Rep. 2019 Jun 18;9(1):8701.
21. Eduardo Fde P, Bueno DF, de Freitas PM, Marques MM, Passos-Bueno MR, Eduardo Cde P, et al. Stem cell proliferation under low intensity laser irradiation: a preliminary study. Lasers Surg Med. 2008 Aug;40(6):433-8.
22. Paschalidou M, Athanasiadou E, Arapostathis K, Kotsanos N, Koidis PT, Bakopoulou A, et al. Biological effects of low-level laser irradiation (LLLI) on stem cells from human exfoliated deciduous teeth (SHED). Clin Oral Investig. 2020 Jan;24(1):167-180.
23. Almeida-Junior LA, Marques NCT, Prado MTO, Oliveira TM, Sakai VT. Effect of single and multiple doses of low-level laser therapy on viability and proliferation of stem cells from human exfoliated deciduous teeth (SHED). Lasers Med Sci. 2019 Dec;34(9):1917-1924.
24. Souza L, Rinco U, Aguiar D, de Almeida

Junior L, Silva L, Oliveira T, et al. Effect of low-level laser therapy on viability and proliferation of stem cells from exfoliated deciduous teeth under different nutritional conditions. Laser Phys. 2018 Jan 17;28(2):025603.
25. Moura-Netto C, Ferreira LS, Maranduba CM, Mello-Moura ACV, Marques MM. Low-intensity laser phototherapy enhances the proliferation of dental pulp stem cells under nutritional deficiency. Braz Oral Res. 2016 May 31;30(1):S1806-83242016000100265.
26. Soares DM, Ginani F, Henriques ÁG, Barboza CA. Effects of laser therapy on the proliferation of human periodontal ligament stem cells. Lasers Med Sci. 2015 Apr;30(3):1171-4.
27. Huang YY, Chen AC, Carroll JD, Hamblin MR. Biphasic dose response in low level light therapy. Dose Response. 2009 Sep 1;7(4):358-83.
28. Pandeshwar P, Roa MD, Das R, Shastry SP, Kaul R, Srinivasreddy MB. Photobiomodulation in oral medicine: a review. J Investig Clin Dent. 2016 May;7(2):114-26.
29. Tunér J, Jenkins PA. Parameter Reproducibility in Photobiomodulation. Photomed Laser Surg. 2016 Mar;34(3):91-2.