

How to Cite:

Yavagal, C. M., Vikaraman, K. ., Yavagal, P. C., Borkar, T., Chaurasia, P., & Kalmani, B. R. (2022). Photobiomodulatory effects of multi wavelength diode lasers on the proliferation and differentiation of human dental pulp stem cells: An ex-vivo study. *International Journal of Health Sciences*, 6(S1), 2268–2279. <https://doi.org/10.53730/ijhs.v6nS1.5164>

Photobiomodulatory Effects of Multi Wavelength Diode Lasers on the Proliferation and Differentiation of Human Dental Pulp Stem Cells: An Ex-Vivo Study

Chandrashekar M Yavagal

Professor and Head, Department of Pediatric Dentistry, Maratha Mandal's Nathajirao G. Halgekar Institute of Dental Sciences & Research Centre, Belgaum, Karnataka, India - 590019

KS Vikaraman

Post graduate student, Department of Pediatric Dentistry, Maratha Mandal's Nathajirao G. Halgekar Institute of Dental Sciences & Research Centre, Belgaum, Karnataka – 590019

Puja C Yavagal

Professor, Department of Public Health Dentistry, Bapuji Dental College and Hospital, Davangere, Karnataka, India-577004

Tanay Borkar

Research Fellow, Asian Academy of Laser Therapy, Kochi, India -682024

Pinki Chaurasia

Research Fellow, Asian Academy of Laser Therapy, Kochi, India-682024

Basavanth Reddy Kalmani

Research Fellow, Asian Academy of Laser Therapy, Kochi, India-682024

Abstract---Photobiomodulation is currently trending as a potential stimulator of several cell lines including human dental pulp stem cells (HDPSc) proliferation and differentiation. This study evaluated the Photo-biomodulatory effects of different wavelength diode lasers on HDPSc proliferation and differentiation. Dental pulp stem cells were recovered from human mature premolar teeth extracted for orthodontic reasons, isolated and cultured followed by irradiation with different wavelength laser diodes (660 nm, 450nm and 810nm, 100 mW, all standardized to continuous mode, for 3 minutes at a distance of 1cm from the cells). Cell proliferation was analyzed at 1, 3, and 5 days by flow cytometry. Cell differentiation was analyzed by Alizarin

Red Staining at days 7, 14 and 21. Cell proliferation and differentiation were compared with a negative control where cells were maintained in a culture medium without any irradiation and a positive control where pulp cells were cultured in a growth induction medium - Mineral trioxide aggregate (MTA). One -Way and Repeated measures ANOVA followed by Tukey's HSD post Hoc test were used for statistical analysis. Proliferating cells were significantly higher in the positive control and Infra-red-PBM groups compared to other groups. (Day 3: $p=0.002$, Day 5: $p=0.00$). Differentiating cells were highest in the positive control and Blue Diode PBM group compared to other groups. (Day 14: $F=9.69$, $df=4$, $p=0.00$; Day 21: $F=19.14$, $df=4$, $p=0.00$). Photobiomodulation clearly showed to improve the HDPSc proliferation as well as their differentiation.

Keywords---Photobiomodulation, LLLT, Stem cells, Dental pulp, Proliferation, Differentiation.

Introduction

Dental pulp, which is a specialized connective tissue, maintains dentin homeostasis, sensation, nourishment, and defense. It has the ability to generate reparative dentin under clinically relevant insults such as trauma or infection. Dental pulp cell differentiation is clinically important as they have the ability to develop into odontoblast-like cells, which are hypothesized to be responsible for secreting the dentin matrix and producing reparative dentin thus allowing therapeutic interventions that maintain the vitality of the tooth. Dental Pulp Stem Cells (DPSCs) have mesenchymal stem cell like characteristics, such as the ability to self-renew and differentiate into several lineages. They have the unique ability to differentiate into not just normal mesodermal cell lineages such as osteogenic, chondrogenic, and adipogenic lineages, but also ectodermal and endodermal cell lineages (Yamada et al.,2019). They are seen to proliferate at a higher rate than bone marrow derived mesenchymal stem cells (Nakamura et al.,2009). DPSCs were shown in vivo to be capable of reconstituting functioning dentin/pulp complexes as well as other tissues such as bone, cementum, blood vessels, and neural tissues (Yamada et al.,2019). They are regarded to be potential stem cell sources for clinical usage because of their ease of access, isolation by noninvasive routine clinical procedures, low ethical problems, and high proliferation capacity. Recently photobiomodulation is being tested as a potential stimulating modality for the proliferation and differentiation of dental pulp stem cells. (Malthiery et al.,2021).

Photobiomodulation (PBM), originally known as low-level light therapy (LLLT) uses low-power irradiation from lasers or light-emitting diode (LED) devices to irradiate human tissues to either relieve pain or stimulate cellular healing. Photostimulatory and photo-biomodulatory effects of PBM on cell proliferation, metabolism, regeneration, and anti-inflammatory response have been well documented (Malthiery et al.,2021). PBM is also shown to increase stem cells production in culture media or to cause specific differentiation of cell types (Malthiery et al.,2021). PBM effects involve intracellular photoreceptors (such as

cytochrome C oxidase, porphyrin, or flavoproteins) inducing signaling cascades (redox pathways and transcription factors) at the cellular level (Malthiery et al.,2021). A systematic review conducted by Marques MM et al., 2016 summarized no deleterious effects of PBM on dental derived mesenchymal stem cells. The study pointed towards scarce number of publications in this area underlining the potential importance of PBM therapy in improving dental derived stem cell viability and proliferation. On adipose-derived stem cells, shorter wavelengths (420 nm and 540 nm) have been found to be more effective in stimulating osteoblastic differentiation compared with 660 nm and 810 nm (Wang et al.,2016). Majority of studies have tested red or infrared light irradiation and a few have tested green and blue light irradiation on dental stem cells proliferation and differentiation with inconsistent results (Marques MM et al.,2016) This research equipoise prompted to plan an ex-vivo study to assess and compare the photo-biomodulatory effects of different wavelength diode lasers on dental pulp stem cells proliferation and differentiation. Study tested the null hypothesis that there was no difference in the photo-biomodulatory effects of different wavelength diode laser irradiation on dental pulp stem cells proliferation and differentiation.

Methodology

An ex-vivo study was planned. Ethical clearance was obtained from the institutional review board of the research Centre where the study was conducted. All the laboratory procedures were carried out with strict aseptic precautions and wavelength specific eye goggles were worn the investigators during use of lasers.

Isolation and culture of human dental pulp stem cells

Dental pulp stem cells (DPSCs) were recovered from human mature premolar teeth extracted for orthodontic reasons, from healthy children. Written informed consent was obtained from the parents of children for collection of extracted teeth specimens. The teeth samples were longitudinally cleaved after cleaning and disinfecting the surface with 70% ethanol. A longitudinal furrow was made using a diamond disc and sectioned using a chisel. Pulp tissue was gently separated and placed in Dulbecco's modified eagle's medium (DMEM) and transported to the laboratory. Pulp tissue was then washed with phosphate-buffered solution (PBS) for 3 times (1 minute for each). The pulp was cut into 0.4X0.5X0.5mm tissue sections using sterilized eye scissors placed into a 1.5ml tube. Enzyme digestion was carried out as per the procedure followed by Kukreja et al.,2021. Minced pulp tissues were digested in a solution of 3 mg/ml collagenase type I and 4 mg/ml dispase for 30–60min at 37°C. Cell suspensions were obtained by passing the digested tissues through a 70-µm cell strainer. Each cell suspensions (1×10^6 cells/flask) were seeded in T 25 tissue flasks containing, DMEM supplemented with 10% fetal bovine serum (FBS), 1% antibiotic and antimycotic, 10^{-8} mol/l dexamethasone, 0.05%g/l L-Ascorbic Acid and 2.16g/l glycerol 2-phosphate disodium salt hydrate in a humidified atmosphere with 5% CO₂ at 37°C and the medium was changed every 3 days. Cells were grown to confluence and continuously passed at a 1:3 ratio when confluent. Cells were counted using hemocytometer and base line number was calculated.

Preparation of Mineral Trioxide Aggregate (MTA) specimens (for positive control)

White MTA powder (0.2g) was mixed with distilled water in a liquid /powder ratio 0.3 ml/g to make the cement. It was allowed to set at 37°C at 100% relative humidity for 24 hours. The human dental pulp cells (HDPCs) were then seeded in 24 well plates containing 2ml of DMEM solution and allowed to attach overnight. The set MTA was placed on the bottom of 6.5 mm diameter trans well inserts which fitted into the wells of a 24 well, cell culture plate. The trans-wells contained permeable membranes (0.4micron pore size) and were used to prevent direct physical interaction of MTA with pulp cells.

Cell proliferation analysis

Cell Proliferation analysis was done at 1st, 3rd and 5th day after photobiomodulation. The cells were seeded in a 96-well flat-bottom micro plate and maintained at 37°C in 95% humidity and 5% CO₂ for overnight. Five different groups were established. The laser irradiation was done as per the protocol. The cells were incubated for one, three and five days respectively. The wells were washed twice with PBS and 20 µL of the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) and staining solution was added to each well and plate was incubated at 37°C. After 4hrs, 100 µL of DMSO was added to each well to dissolve the formazan crystals, and the optical density was recorded at 570 nm using a microtiter plate reader according to a protocol followed by Daniela et al.,2018. Cell proliferation (%) was calculated using following formula. Cell proliferation (%) = Mean OD of test compound/Mean OD of negative control x 100 which was expressed in terms of surviving cells percentage. Analysis was done using GraphPad Prism version 5.1 for Windows, San Diego, California USA.

Cell differentiation analysis

Cell differentiation assay was carried out by Alazarin Red Staining method at 7th,14th and 21st day after laser irradiation. The cells were seeded at a density of approximately 1×10⁵cells/well in 24-well flat-bottom micro plate and maintained at 37°C in 95% humidity and 5% CO₂ overnight. Five interventional groups were established and cells were treated with different types of laser irradiation. The cells were incubated for another 7, 14 and 21 days. After the incubation period the media was removed and cells were washed and fixed with 95% ethanol for 15 minutes at 4°C. The cells were then stained with 2% Alizarin Red S (pH 4.1- 4.3) for 15 minutes. The red stain was solubilized with 300µl of 33% glacial acetic acid solution and subjected to spectrophotometric evaluation according to the protocol of Gregory et al 2004, using a microplate reader to measure the absorbance at 415 nm. The same protocol was repeated three times independently, giving three different readings.

Interventional Groups:

- Negative Control: HDPCs maintained in the culture medium throughout the experiment period (DMEM with 10 % FBS, 1 % penicillin- streptomycin).

- Positive Control (MTA Group): HDPCs maintained in the cell culture medium throughout the experiment period supplemented with growth induction medium (Mineral Trioxide Aggregate)
- Blue light (BL) Group: HDPCs maintained in the cell culture medium throughout the experiment which was exposed to blue laser light. (Instrument- Novolase, Novolase technologies, India; wavelength: 450nm, power-100mw, Irradiation distance from the culture plate-1cm; duration: 3 minutes) Figure 1.
- Red Laser light (RL) Group: HDPCs maintained in the culture medium throughout the experiment which was exposed to red laser light. (Instrument- Novolase, Novolase technologies, India; wavelength: 660nm, power-100mw, Irradiation distance from the culture plate-1cm; duration: 3 minutes) Figure 2.
- Infrared Light (IRL) Group: HDPCs maintained in the culture medium throughout the experiment which is exposed to infrared light. (Instrument- Novolase, Novolase technologies, India; wavelength: 810nm, power-100 mw, Irradiation distance from the culture plate-1cm; duration: 3 minutes) Figure 3.

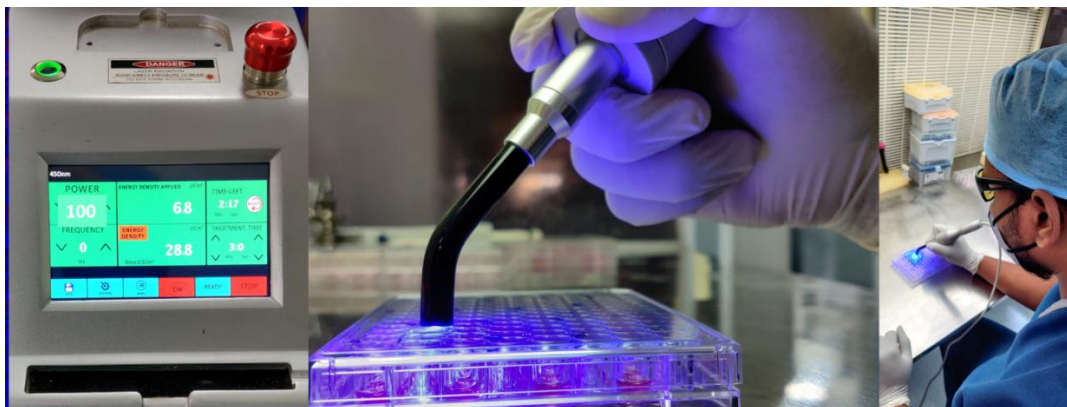


Figure 1. Blue laser (450nm) irradiation of Human dental Pulp stem cells (HDPCs)



Figure 2. Red (660 nm) laser irradiation of Human dental Pulp stem cells (HDPCs)



Figure 3. Infra Red (810nm) laser irradiation of Human dental Pulp stem cells (HDPSCs)

Statistical analysis

IBM SPSS Statistics for Windows, version 21 (IBM Corp., Armonk, N.Y., USA) was used for statistical analysis. Level of significance was set at $p < 0.05$. Descriptive statistics were expressed in terms of mean number of surviving cells post intervention in all the groups. Shapiro Wilk's test denoted normal distribution of data hence, parametric tests were employed for data analysis. One -Way and Repeated measures ANOVA followed by Tukey's HSD post Hoc test were used to determine inter and intragroup significant differences respectively. Effect size was expressed as partial eta square value.

Results

Results depicted by Table 1 indicate that, there was increase in the mean number of proliferating cells at day 5 compared to day 1 in all the groups. However, this was statistically significant in positive control group (MTA group) ($p = 0.03$, $\eta_p^2 = 0.99$). Between group comparisons indicated that at day 3 and 5, mean number of surviving cells were significantly higher in PC group and IRL groups compared to other groups. (Day 3: $p = 0.002$, $\eta_p^2 = 0.79$, Day 5: $p = 0.00$, $\eta_p^2 = 0.94$). These results suggest that the infrared photobiomodulation was as effective as MTA in promoting dental pulp cell proliferation and showed superior cell proliferation compared to other groups.

Table 2 denotes that there was significant increase in number of differentiating cells in blue Laser (BL) group, Red Laser group (RL) and Positive control group (PC) on day 21 compared to day 7. ($p \leq 0.05$). Between group comparisons revealed that at day 14 and 21, mean number of differentiating cells were highest in PC group followed by BL group compared to other groups. (Day 14: $F = 9.69$, $df = 4$, $p = 0.00$, $\eta_p^2 = 0.72$; Day 21: $F = 19.14$, $df = 4$, $p = 0.00$, $\eta_p^2 = 0.83$) (Figure 4)

Table 1
Intra and Intergroup comparison of mean number of surviving (proliferating) dental pulp cells

Groups	Mean proliferating cells %			
	Day1	Day3	Day5	Repeated measures Anova Value
Negative Control	100±1.71	99.99±7.07 ^{AB}	100±1.08 ^{GHI}	F=0.0, df=2, P=1, $\eta_p^2=0$
Blue Light PBM	101.72±2.07	101.20±3.16 ^{CD}	106.29±1.47 ^{GJK}	F=1.46, df=2, P=0.5, $\eta_p^2=0.7$
Red Light PBM	101.29±1.70	101.65±0.94 ^{EF}	103.96±2.25 ^{LM}	F=0.72, df=2, P=0.63, $\eta_p^2=0.5$
Infrared PBM	103.03±1.01	111.66±1.16 ^{ACE}	115.93±3.74 ^{HJL}	F=15.18, df=2, P=0.17, $\eta_p^2=0.96$
Positive Control (MTA)	104.03±1.0 ^a	114.53±2.54 ^{BDF}	118.71±0.52 ^{aIKM}	F=363.80, df=2, *P=0.03, $\eta_p^2=0.99$
One -way Anova	F=3.01, df=4, P=0.07, $\eta_p^2=0.54$	F=9.95, df=4, *P=0.002, $\eta_p^2=0.79$	F=42.40, df=4, *P=0.00, $\eta_p^2=0.94$	
F=Anova value, df-degrees of freedom=probability value, η_p^2 = partial eta square (effect size) *Statistically significant at P<0.05, Same Capital letters superscript indicates significant difference between groups, Same small letters superscript indicates significant difference within groups				

Table 2
Intra and Intergroup comparison of mean number of differentiating dental pulp stem cells

Groups	Mean differentiating cells %			
	Day7	Day14	Day21	Repeated measures Anova Value
Negative Control	100±2.11	100±2.37 ^{AB}	100.01±5.16 ^{EF}	F=0.0, df=2, P=1.00, $\eta_p^2=0$
Blue Light PBM	104.56±3.03 ^a	111.69±1.33 ^{ba}	120.08±3.62 ^{abEG}	F=19.15, df=2, *P=0.05, $\eta_p^2=0.9$
Red Light PBM	101.54±4.55 ^c	105.88±2.08 ^c	109.77±5.23 ^{ch}	F=18.63, df=2, *P=0.05, $\eta_p^2=0.94$
Infrared PBM	99.73±0.44	103.65±7.80 ^D	105.14±6.88 ^{GI}	F=1.30, df=2, P=0.43, $\eta_p^2=0.5$
Positive Control (MTA)	104.23±2.97 ^d	115.51±2.73 ^{eBCD}	126.31±2.65 ^{deFHI}	F=63.36, df=2, *P=0.01, $\eta_p^2=0.98$
One -way Anova	F=2.41, df=4, P=0.09, $\eta_p^2=0.39$	F=9.69, df=4, *P=0.00, $\eta_p^2=0.72$	F=19.14, df=4, *P=0.00, $\eta_p^2=0.83$	
F=Anova value, df-degrees of freedom=probability value, η_p^2 = partial eta square (effect size) *Statistically significant at P<0.05, Same Capital letters superscript indicates significant difference between groups, Same small letters superscript indicates significant difference within groups				

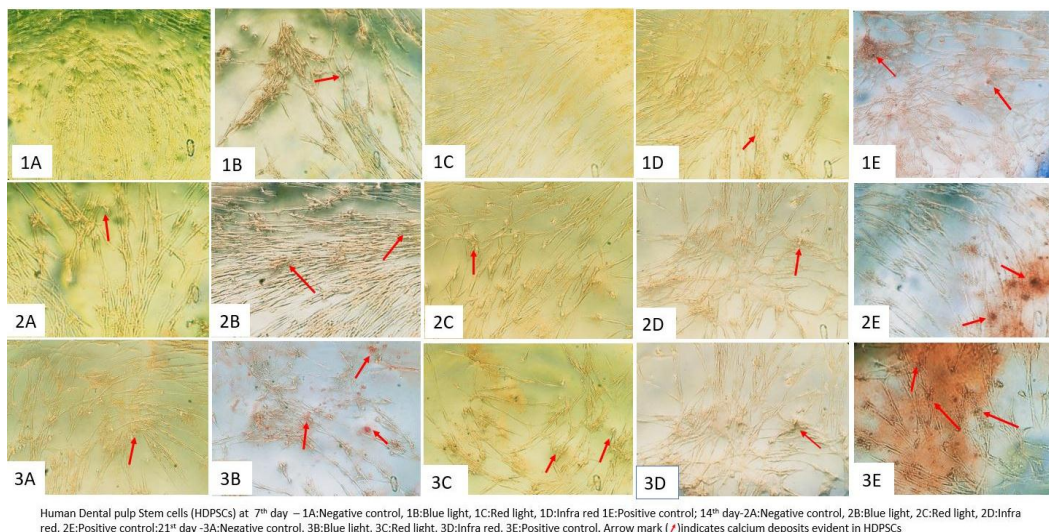


Figure 4. Microscopic Views of Alizarin Red S staining of Human dental Pulp stem cells (HDPCs)

Discussion

The study assessed and compared the photo-biomodulatory effects of different wavelengths of diode lasers on human dental pulp stem cell proliferation and differentiation and compared the effects with mineral trioxide aggregate as a positive control. The results indicated that the photobiomodulation with Infrared light resulted in significant pulp cell proliferative effects compared to photobiomodulation with other light sources. The efficacy of infrared light was comparable with the cell proliferatory effect of mineral trioxide aggregate. However, cell differentiating effect of photobiomodulation was better with blue light compared to other groups. Our results are in accordance with previous studies where, red or infrared light irradiation have shown profound effect on improving dental stem cells proliferation and differentiation (Marques et al.,2016). Few studies have pointed out the positive effect of red laser light (660nm) on dental pulp cell proliferation and differentiation (Ferriera et al.,2019,Garrido et al.,2019) However, study by Pereira et al.,20112, demonstrated no effect of laser PBM with 660 nm on dental pulp stem cell proliferation and differentiation. Using 810-850 nm infrared diodes, few studies demonstrated increased dental pulp stem cell proliferation and differentiation (Tabatabaei et al.,2015), (Kim et al.,2015), (Sivakumar et al.,2019). A study done by Malthirey et al.,2021, showed positive effect of green laser (532nm) on HDPCs proliferation and differentiation. On literature search authors could not find any study testing the effect of blue light on HDPCs proliferation and differentiation. However, study done by Yang et al.,2020, tested low-energy blue light irradiation on the osteogenic differentiation of stem cells from the apical papilla (SCAPs) of human teeth which showed improved osteogenic differentiation of the SCAPs. At 4 J/cm², blue light up regulated the expression levels of the osteogenic/dentinogenic genes ALP, dentin sialo phosphoprotein (DSPP), dentin matrix protein-1 (DMP-1), and osteocalcin (OCN) in SCAPs.

Photobiomodulation induces reactive oxygen species (ROS) in a dose-dependent manner, which, in turn, activates latent TGF- β 1 (LTGF- β 1) via a specific methionine residue (at position 253 on LAP). Further, this laser activated TGF- β 1 is capable of inducing proliferation and differentiation of human dental stem cells into odontoblast like cells (Arnayet al., 2007). The effects of PBM therapy on ddMSCs (dental derived mesenchymal stem cells) seems to follow the Arndt-Schulz law which indicates that, when the lowest (0.05 J/cm²) and the highest (42 J/cm²) energy densities are applied, no effects are observed. Therapeutic window was evidenced between 1–4 J/cm² (Marques et al., 2016).

Red light and infrared light are commonly used for stimulating HDPCs in various studies as they have shown positive effect on cell proliferation and differentiation. 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 leading to increase in intracellular matrix metalloproteinase levels and reduced ROS levels (Wang et al., 2016). Red wavelengths can penetrate 0.5–1 mm and Near Infra Red wavelengths can penetrate 2 mm before losing 37% of their intensity. Therefore, deeper tissues such as bone have shown better response with near-infrared lasers. When determining the effect of irradiation, the number of mitochondria in a cell is an important factor to consider as they tend to be the initial site of light absorption. Cytochrome c oxidase (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 (Zein et al., 2018). Mineral trioxide aggregate is an established inducer of dental pulp cell proliferation and differentiation hence was chosen for the positive control group. MTA-treated DPSCs, demonstrated higher ALP activity and formed more mineralized nodules than the untreated group in a study done by Wang et al. The odonto/osteoblastic markers (Alp, Runx2/RUNX2, Osx/OSX, Ocn/OCN, and Dspp/DSP, respectively) in MTA-treated DPSCs were significantly upregulated. Mineral trioxide aggregate enhanced the odonto/osteogenic capacity of DPSCs from inflammatory sites via activating the NF- κ B pathway. A study done by Zhu et al., 2019, showed that, accelerated-set MTA (2.5% disodium hydrogen phosphate and 5% calcium chloride) promoted better differentiation in DPSC niches (human dental pulp cell niches) than conventional MTA (Zhu et al., 2019), (Kulkarni et al., 2020).

The results of the present study highlight that photobiomodulation may improve human derived dental pulp cell proliferation and differentiation. These results can be imported to tissue engineering along with stem cells and growth factors to improve tissue regeneration and healing. The reporting of laser parameters in the current study follow the WALT guidelines which allows for replication of the suggested photobiomodulation methodology. However, further translational research needs to be conducted in this area to elucidate the potential and legitimacy of photobiomodulation therapy as an effective tool for dental pulp cell regeneration. Given the broad range of roles that ROS and TGF- β can mediate in vivo and the popularity of laser devices in current clinical settings, the insights from this study could also promote clinical translation of photobiomodulation to modulate pain, inflammation, or immune responses, and promote tissue

regeneration of dentin-pulp complex and associated tissues. The proliferation of dental pulp cells, is a time-consuming process, and the development of methods that increase the number of available cells within a short period of time is fundamental. In this context, photobiomodulation therapy can serve as an excellent inducer of dental pulp cell proliferation.

Conclusion

Photobiomodulation improved dental pulp stem cells proliferation and differentiation. Infrared laser irradiation resulted in significant pulp cell proliferative effects compared to other wavelengths. The efficacy of infrared laser was even comparable with the cell proliferative effect of mineral trioxide aggregate. However, cell differentiating effect of photobiomodulation was better with blue laser compared to other groups and was again comparable to MTA group.

Abbreviations

HDPSc- human dental pulp stem cells, DPSc- Dental pulp stem cells, PBM- Photobiomodulation, MTA-mineral trioxide aggregate, ANOVA- Analysis of Variance, LLLT- Low level laser therapy, LED- Light emitting diode, DMEM - Dulbecco's modified eagle's medium, PBS- phosphate-buffered solution, MTT-3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide-Optical density, SCAPS - stem cells from the apical papilla of teeth, DSPP - dentin sialo phosphoprotein, DMP- dentin matrix protein, OCN- osteocalcin, ddMSC - dental derived mesenchymal stem cells, ROS- reactive oxygen species, WALT- World Association for Laser Therapy.

Acknowledgments

Authors acknowledge the support of Dr. Kishore Bhat, Director and Mr. Vijay Kumbar, Research Officer, at Central Research Laboratory, Maratha Mandal's Nathajirao G. Halgekar Institute of Dental Sciences & Research Centre, Belgaum, India towards microbiological analysis.

References

- Arany PR, Nayak RS, Hallikerimath S, Limaye AM, Kale AD, Kondaiah P. Activation of latent TGF-beta1 by low power laser in vitro correlates with increased TGF-beta1 levels in laser-enhanced oral wound healing. *Wound Repair Regen*, 2007;15:866-874
- Daniela Ferreira Araújo B, Luciana Oliveira P, Izabel Cristina Rodrigues da S, Ricardo Bentes A, Ana Cristina Barreto B. Culture of human dental pulp cells at variable times post-tooth extraction. *Braz Oral Res*, 2018 Feb 1;32:e003.
- Ferreira LS, Diniz IMA, Maranduba CMS, Miyagi SPH, Rodrigues MFSD, Moura-Netto C, Marques MM. Short-term evaluation of photobiomodulation therapy on the proliferation and undifferentiated status of dental pulp stem cells. *Lasers Med Sci*, 2019 Jun;34(4):659-666

- Garrido PR, Pedroni ACF, Cury DP, Moreira MS, Rosin F, Sarra G, Marques MM. Effects of photobiomodulation therapy on the extracellular matrix of human dental pulp cell sheets. *J Photochem Photobiol B*. 2019 May;194:149-157.
- Gregory CA, Gunn WG, Peister A, Prockop DJ. An Alizarin red-based assay of mineralization by adherent cells in culture: comparison with cetylpyridinium chloride extraction. *Anal Biochem*, 2004 Jun 1;329(1):77-84.
- Kim HB, Baik KY, Choung PH, Chung JH. Pulse frequency dependency of photobiomodulation on the bioenergetic functions of human dental pulp stem cells. *Sci Rep*, 2017 Nov 21;7(1):15927
- Kukreja BJ, Bhat KG, Kukreja P, Kumber VM, Balakrishnan R, Govila V. Isolation and immunohistochemical characterization of periodontal ligament stem cells: A preliminary study. *Journal of Indian Society of Periodontology*, 2021 Jul;25(4):295.
- Kulkarni S, Meer M, George R. The effect of photobiomodulation on human dental pulp-derived stem cells: systematic review. *Lasers Med Sci*. 2020 Dec;35(9):1889-1897. doi: 10.1007/s10103-020-03071-6. Epub 2020 Jun 22. PMID: 32572661.
- Malthiery E, Chouaib B, Hernandez-Lopez AM, Martin M, Gergely C, Torres JH, Cuisinier FJ, Collart-Dutilleul PY. Effects of green light photobiomodulation on Dental Pulp Stem Cells: enhanced proliferation and improved wound healing by cytoskeleton reorganization and cell softening. *Lasers Med Sci*, 2021 Mar;36(2):437-445.
- Marques MM, Diniz IM, de Cara SP, Pedroni AC, Abe GL, D'Almeida-Couto RS, Lima PL, Tedesco TK, Moreira MS. Photobiomodulation of Dental Derived Mesenchymal Stem Cells: A Systematic Review. *Photomed Laser Surg*, 2016 Nov;34(11):500-508.
- Nakamura S., Yamada Y., Katagiri W., Sugito T., Ito K., Ueda M. Stem cell proliferation pathways comparison between human exfoliated deciduous teeth and dental pulp stem cells by gene expression profile from promising dental pulp. *J. Endod.* 2009;35:1536-1542.
- Pereira LO, Longo JPF, Azevedo RB. Laser irradiation did not increase the proliferation or the differentiation of stem cells from normal and inflamed dental pulp. *Arch Oral Biol*, 2012;57:1079-1085.
- Shivakumar TT, Muruppel AM, Joseph AP, Reshmi JA, Ramachandran R, Nair PD et al (2019) Photobiomodulatory effect delivered by low-level laser on dental pulp stem cell differentiation for osteogenic lineage. *Laser Dent Sci*, 2019; 3:175
- Tabatabaei FS, Torshabi M, Nasab MM, Khosraviani K, Khojasteh A. Effect of low-level diode laser on proliferation and osteogenic differentiation of dental pulp stem cells. *Laser Phys*, 2015 Jul 29;25(9):095602.
- Wang Y, Huang Y-Y, Wang Y et al. Photobiomodulation (blue and green light) encourages osteoblastic-differentiation of human adipose-derived stem cells: role of intracellular calcium and light-gated ion channels. *Sci Rep*, 2016; 6:33719.
- Wang Y, Huang YY, Wang Y, Lyu P, Hamblin MR. Red (660 nm) or near-infrared (810 nm) photobiomodulation stimulates, while blue (415 nm), green (540 nm) light inhibits proliferation in human adipose-derived stem cells. *Sci Reps*, 2017 Aug 10;7(1):1-0.

- Wang Y, Yan M, Fan Z, Ma L, Yu Y, Yu J. Mineral trioxide aggregate enhances the odonto/osteogenic capacity of stem cells from inflammatory dental pulps via NF- κ B pathway. *Oral Dis*, 2014 Oct;20(7):650-8.
- Yamada Y, Nakamura-Yamada S, Kusano K, Baba S. Clinical Potential and Current Progress of Dental Pulp Stem Cells for Various Systemic Diseases in Regenerative Medicine: A Concise Review. *Int J Mol Sci*, 2019 Mar 6;20(5):1132.
- Yang Y, Zhu T, Wu Y, Shu C, Chen Q, Yang J, Luo X, Wang Y. Irradiation with blue light-emitting diode enhances osteogenic differentiation of stem cells from the apical papilla. *Lasers Med Sci*, 2020 Dec;35(9):1981-1988.
- Zein R, Selting W, Hamblin MR (2018) Review of light parameters and photobiomodulation efficacy: dive into complexity. *J Biomed Opt*, 2018, 23(12):120901
- Zhu T, Wu Y, Zhou X, Yang Y, Wang Y. Irradiation by blue light-emitting diode enhances osteogenic differentiation in gingival mesenchymal stem cells in vitro. *Lasers Med Sci*. 2019 Sep;34(7):1473-1481.