ORIGINAL RESEARCH

# Effect of Low-level Laser Therapy on the Viability and Proliferation of Gingival Mesenchymal Stem Cells: An *Ex Vivo* Pilot Study

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## Abstract

**Aim:** The present *ex vivo* study was designed to assess the *in vitro* effects of low-level laser therapy (LLLT) on the viability and proliferation of gingival mesenchymal stem cells (GMSCs).

**Materials and methods:** Gingival explants were obtained from the gingival collar of teeth advised for minor gingival surgical procedures. Following processing, the GMSCs were irradiated with a 660 nm diode laser according to the following groups. Group I—1 J/cm$^2$, 25 mW, and 40 seconds; group II—2 J/cm$^2$, 50 mW, and 10 seconds; and group III—no irradiation (control group). 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay was done to assess the rate of cell proliferation by measuring the absorbance values using a spectrometer. The mean values of absorbance in each of the three groups were considered for statistical analysis separately at 12 and 24 hours, respectively.

**Results:** The absorbance values of tetrazolium reduction were directly proportional to the rate of cell proliferation. Both groups I and II showed statistically significant differences in the absorbance rates from 12 to 24 hours after irradiation. Both at 12 and 24 hours after irradiation, group I exhibited a greater absorbance value compared to group II and this difference was statistically significant ($p < 0.05$).

**Conclusion:** Low-level laser therapy (LLLT) using 660 nm diode laser with different energies showed a positive effect on *in vitro* proliferation of GMSCs. The rate of proliferation was comparatively more significant at 12 hours and lower energy.

**Clinical significance:** This study provides a basis for the probable application of LLLT as a tool in tissue engineering using GMSCs.

**Keywords:** Biostimulation, Gingival mesenchymal stem cells, Low-level laser therapy.

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## Introduction

The goal of periodontal therapy is the regeneration of lost tissues to their original form, architecture and function. This demands an abundance of regenerative cells, a conducive environment, and appropriate signals. The contribution of MSCs in the field of regenerative medicine and therapeutics is invaluable. MSCs have been identified to possess the potential for plastic adherence, self-renewing ability, and trilineage differentiation.[1,2] They also possess inherent regenerative properties and maintain tissue homeostasis, apart from their immunomodulatory effects. They have also shown promise in the management of inflammatory disorders, autoimmune diseases, malignancies, and wounds, among others.[3–7]

Among the various sources of MSCs, GMSCs have been the most sought-after since their discovery in 2009. They are readily accessible, do not require invasive procurement procedures, nontumorigenic, and are expandable in nature. They have been proven successful in treating skin disorders, allergic disorders, inflammatory and autoimmune disorders, and aid in wound healing.[8–17]

When harvested from the bone marrow, MSCs make up 1-minute fraction of nucleated cells and account for approximately 0.001–0.01% of all cells in each aspirate, depending on the technique. However, the therapeutic application of MSCs often requires a larger number of cells, which necessitates *ex vivo* expansion postharvest. As mammalian cells require 18–24 hours to double in cultures, there is always a risk of contamination.

Low-level laser therapy (LLLT) is a type of noninvasive, nonthermal therapy based on nonionizing light sources, including lasers, light-emitting diode, and broadband light, in the visible and infrared spectrum (600–1070 nm) that has attracted special attention and its applications in dentistry and medicine is continuously growing. This therapy involves the use of low-level laser light to achieve hemostasis, eliminate periodontal infections, improve tissue development, regeneration, reduce pain, and promote wound healing.[18] LLLT has been shown to enhance the proliferation of fibroblasts, endothelial cells, skeletal cells, keratinocytes, myoblasts, MSCs, and cardiac stem cells.[19,20] Study by Bai et al. has shown that LLLT demonstrated osteogenesis and angiogenesis by enhanced vascularization in mouse bone marrow mesenchymal stem cells.[21]

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**Conflict of interest:** None

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Their effect on GMSCs has not been elucidated to date. The present *ex vivo* study was designed to assess the *in vitro* effects of LLLT on the viability and proliferation of GMSCs.

## Material and Methods

This exploratory, observational study was carried out at a tertiary dental hospital in South India.

### Patient Selection

Patients, aged between 18 and 55 years, who were advised minor periodontal surgical procedures (gingivoplasty, gingivectomy, and crown lengthening) or tooth extraction at the outpatient department of a tertiary dental hospital in South India, were recruited for the study after obtaining informed consent. The study protocol was approved by the Institute Ethics Committee (JSSDCH/PGS/Ethical/2015-16).

### Inclusion and Exclusion Criteria

Patients aged between 18 and 55 were selected for the study.

Smokers, lactating and pregnant women, and individuals with systemic diseases were excluded from the study.

### Sample Size

A total of five patients were selected from the outpatient department of periodontology. Two explants were lost during the process and finally, three samples were subjected to the experiment.

### Isolation and Culture of Gingival Tissue

Gingival explants were obtained from the gingival collar of teeth advised for extraction/gingivectomy/gingivoplasty/crown lengthening procedures using the No. 15 Bard Parker blade. The excised gingival tissue was washed in phosphate buffered solution (PBS) and Gibco. Later teased and digested overnight with 0.5 mg/mL collagenase blend type H (Sigma-Aldrich) at 37°C in an incubator. The digested tissue was washed with PBS and plated in Knockout Dulbecco's Modified Eagles Medium (KO-DMEM, Gibco, catalog no. 10829-18) supplemented with 10% fetal bovine serum (FBS) (Australian FBS, hiMedia, catalog no. RM9951), Glutamax (Gibco, catalog no. 35050-061) and antibiotics namely, 100 U/mL—1% penicillin, 100 µg/mL—1% streptomycin, and 1% amphotericin. After 24 hours, the floating debris was removed and the adherent cells were allowed to grow till confluence. They were passaged further with 0.25% trypsin-ethylenediaminetetraacetic acid and plated in a complete medium containing KO-DMEM, FBS 10%, Glutamax—1%, and antibiotic-antimycotic—1%. The flasks were incubated at 37°C in 5% carbon dioxide. The cells were allowed to grow for 3–4 days. They reached confluence; they were checked using phase contrast inverted microscopy. The basic medium was changed on a regular basis 3 times in 1 week.

### Immunophenotype Characterization of Gingival Cells by Flow Cytometry

Flow cytometry was used to determine the immunophenotypic characteristics of GMSCs. Around $0.5 \times 10^6$ gingival cells were obtained from the third or fourth passage and incubated with specific monoclonal antibodies. (Fig. 1). The primary antibodies used were:

- A cluster of differentiation (CD) 73—catalog no. 550257.
- CD105—catalog no. 561443.

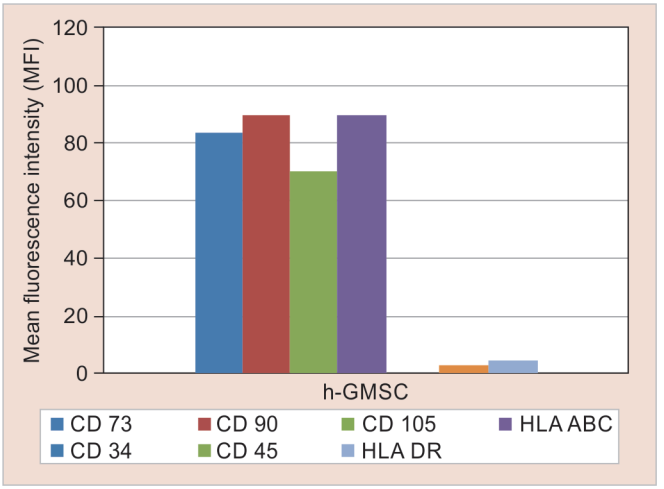



**Fig. 1:** Graphical Representation of expression of stem cell markers of GMSCs isolated and cultivated

- CD90—catalog no. 555596.
- CD45—catalog no. 555482.
- CD34—catalog no. 550761.
- Human leukocyte antigen (HLA) D catalog no. 347363.
- HLA ABC catalog no. 555552 from BD Pharmingen to maintain a temperature of 4°C.

Fluorescein isothiocyanate (FITC), peridinin chlorophyll protein, or phycoerythrin (PE), in 250 µL of flow cytometry staining buffer, which contained sodium azide was conjugated for a duration of 30 minutes at room temperature. Around 4 mL PBS was added to dilute the cells and then centrifuged. Resuspended with 600 µL of PBS containing 2% formaldehyde. Later it was analyzed using cell quest prosoftware with a flow cytometer. Immunoglobulin G (IgG) 1, FITC, and IgG1 PE monoclonal antibodies were the isotypes used.

### Cryopreservation

Media was removed from culture flasks and the cell monolayer was washed with PBS, trypsinized, and then centrifuged at 1200 rpm for 5 minutes. The cell pellet thus obtained was gently tapped to loosen it, after which 1 mL of cryopreservation medium (90% FBS + 10% DMSO) was added to the pellet and gently pipetted to get a single-cell suspension. The suspension was then transferred to cryovials and kept in a cryocooler at –80 for 1 day, after which vials were transferred to liquid nitrogen canisters for long-term storage. The tissue explant was stored in complete media supplemented with antibiotic and antimycotic and transported on ice packs to the experimental lab.

### Thawing

Cryovial was removed from the liquid nitrogen canister and immersed in a 37°C water bath for it to thaw. When a small chunk of ice was remaining, the vial was removed from the water bath, thoroughly cleaned with 70% ethanol, and then taken inside the hood, after which the vial was opened, and the contents were transferred to a conical falcon containing prewarmed media. The falcon was then centrifuged to pellet the cells. The pellet was then gently tapped to loosen it, resuspended in 1 mL media, and transferred to a flask containing an appropriate amount of media.

### Process of Irradiation

The cells were seeded in the plates with α MEM + 10% FBS (modified eagles medium, Gibco catalog no. 10829-18, Australian FBS, hiMedia,

catalog no. RM 9951) and were allowed to attach for 24 hours. The plates were covered with black cardboard with holes created only for the test wells. Prior to irradiation, the medium was changed to fresh 1% FBS. Following this, the required plates were irradiated with a 660 nm diode laser following block randomization and group categorization as follows according to the LLLT energy density.

- Group I: 1 J/cm$^2$, 25 mW, and 40 seconds.
- Group II: 2 J/cm$^2$, 50 mW, and 10 seconds.
- Group III: No irradiation (control group).

The probe tip was kept perpendicular and 1 mm away from the well to be covering a spot of 0.07 cm$^2$.

### MTT Assay

The 3-(4,5-dimethylthiazol-2-yl)—2,5-diphenyl-2H-tetrazolium bromide (MTT) assay was done to assess the rate of cell proliferation by measuring the absorbance values of tetrazolium reduction to a purple-colored formazan dye using a spectrometer.

The mean values of absorbance in each of the three groups were considered for statistical analysis separately at 12 hours and 24 hours, respectively.

### Statistical Analysis

Statistical analysis was performed using Statistical Package for the Social Sciences 23 statistical software. The cell proliferation data were analyzed by two-way analysis of variance (ANOVA), followed by Tukey's test. Differences were considered significant at $p < 0.05$. Data were expressed as mean value and standard deviation.

## Results

The absorbance values of tetrazolium reduction with MTT assay were directly proportional to the rate of cell proliferation.

At 12 hours, the tests of between-subjects effects revealed that there was a significant difference between groups I, II, and III. The mean values in groups I, II, and III at 12 hours were 1.364 ± 0.274, 1.251 ± 0.112, and 1.104 ± 0.061, respectively. On pairwise intergroup comparison and *post hoc* correction at 12 hours, there was a statistically significant difference in mean values, with group I showing the highest value (1.364 ± 0.274) and group III showing the least value (1.104 ± 0.061) with a *p*-value of 0.000.

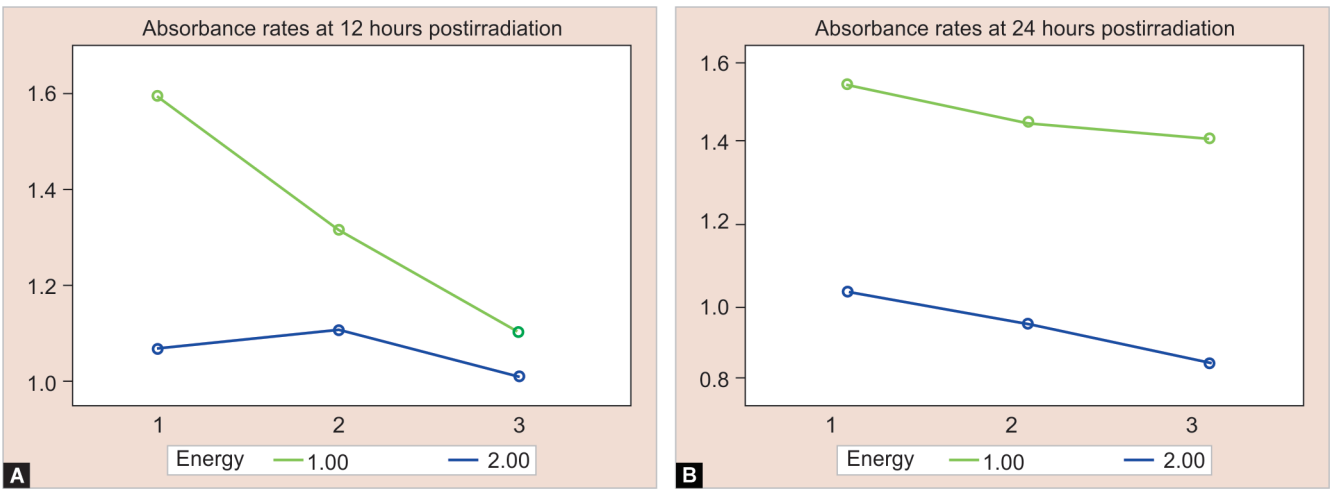
The mean values in groups I, II, and III at 24 hours were, respectively, 1.272 ± 0.256, 1.193 ± 0.248, and 1.132 ± 0.265. On pairwise intergroup comparison and *post hoc* correction at 24 hours, there were statistically significant differences in mean values, with group I showing the highest value, and group III showing the least value.

Both groups I and II showed statistically significant differences in the absorbance rates from 12 to 24 hours after irradiation. Both at 12 and 24 hours after irradiation, a group I exhibited greater absorbance value compared to group II, and this difference was statistically significant ($p < 0.05$) (Fig. 2). One-way ANOVA showed a statistically significant difference in both intragroup and intergroup comparison at 12 hours. However, at 24 hours, there was no statistically significant difference both in intragroup and intergroup comparison. (Tables 1, 2, and 3)

## Discussion

For decades LLLT has been used for its bio-stimulative property. However, there is a continuous search for the best laser protocol to enhance its biostimulatory effect regarding low-energy irradiation. The laser energy is utilized by the mitochondrial respiratory chain to produce adenosine triphosphate, thereby increasing deoxyribonucleic acid activity, and the formation of ribonucleic acid and proteins.

Very few studies have assessed the effects of LLLT on dental stem cells and none have been done on GMSCs. GMSCs have been proven to be effective in the treatment of skin disorders, autoimmune and inflammatory disorders, periodontal diseases, and nerve regeneration.[22–25] GMSCs exposure to LLLT may also enhance its biological properties. Hence this pilot study was conducted to explore the beneficial effects of LLLT on the proliferation of GMSCs. An extensive literature search revealed that this study is the first of its kind to evaluate the effect of LLLT on the proliferation of GMSCs and compare two energy densities at 12 and 24 hours. The application of both energy densities of LLLT on GMSCs has been shown to increase their proliferation rates both at 12 and 24 hours, the intergroup difference being statistically significant in comparison to the control group at each time point. These findings were in accordance with the results of the studies that have revealed increased proliferation of stem cells from human exfoliated deciduous teeth, human periodontal ligament stem cells, and dental pulp stem cells with the use of a 660 nm laser in a wide range of energies.[26–31] laser wavelengths of red or near-infrared (600–1200 nm) are beneficial for biological effects and energy fluences of 0.05–10 J/cm$^2$ to induce




**Figs 2A and B:** Absorbance rates at 12 and 24 hours postirradiation

**Table 1:** Two-way ANOVA for energy levels and laser groups at 12 hours. Tests of between-subjects effects

| *Source* | *Type III Sum of squares* | *Degree of freedom* | *Mean square* | *F* | *Significance* | *Partial eta squared* |
|---|---|---|---|---|---|---|
| Corrected model | 0.742[a] | 5 | 0.148 | 67.042 | 0.000* | 0.949 |
| Intercept | 33.188 | 1 | 33.188 | 14994.739 | 0.000* | 0.999 |
| laser | 0.286 | 2 | 0.143 | 64.591 | 0.000* | 0.878 |
| energy | 0.373 | 1 | 0.373 | 168.328 | 0.000* | 0.903 |
| laser*energy | 0.167 | 2 | 0.083 | 37.709 | 0.000* | 0.807 |
| Error | 0.040 | 18 | 0.002 | | | |
| Total | 35.669 | 24 | | | | |
| Corrected total | 0.782 | 23 | | | | |

R squared = 0.949 (adjusted R squared = 0.935). There is a significant difference between group I and II as well as low, high laser and control group at 12 hours; *$p$ = 0.000 is very highly significant

**Table 2:** Mean and standard deviation for lasers at 12 hours

| | | | *95% confidence interval* | |
|---|---|---|---|---|
| *Laser* | *Mean* | *Standard error* | *Lower bound* | *Upper bound* |
| 1.00 | 1.364 | 0.019 | 1.323 | 1.404 |
| 2.00 | 1.251 | 0.019 | 1.211 | 1.292 |
| 3.00 | 1.104 | 0.014 | 1.075 | 1.133 |

1, low laser; 2, high laser; 3, control

**Table 3:** Univariate test for laser at 12 hours

| | *Sum of squares* | *Degree of freedom* | *Mean square* | *F* | *Significance* | *Partial eta squared* |
|---|---|---|---|---|---|---|
| Contrast | 0.286 | 2 | 0.143 | 64.591 | 0.000* | 0.878 |
| Error | 0.040 | 18 | 0.002 | | | |

The *F* tests the effect of laser. This test is based on the linearly independent pairwise comparisons among the estimated marginal means. There is a very high significant difference between lasers and the control group at 12 hours. *$p$ = 0.000

cell proliferation, contrary to energies greater than 10 J/cm$^2$ being antiproliferative.[32] This study is in accordance with the above study as 660 nm wavelength was used and proved to be beneficial for the proliferation of GMSCs. Studies have demonstrated that energy densities ranging from 0.5–4 J/cm$^2$ have been more effective in stimulating cellular growth.[33] The results of our study demonstrated that the lower energy density showed better results in comparison to the higher energy density, which was more significant at 12 hours. This revelation could speculate the criticality of choosing appropriate energy densities to provide the optimum desired effect and the sensitivity of MSCs to biomodulation by LLLT. Research has shown an added benefit in cellular proliferation with a 2nd-time application.[29,34] Our study showed a significant biostimulatory effect at 12 hours for both groups; however, the same was not maintained at 24 hours, which could be indicative of endorsing the need for the consequent application to maintain the biostimulatory effects. Further, the irradiation at a lower energy density seemed to produce a greater biostimulatory effect, the difference being statistically significant. This is in accordance with the study of Fernandes et al., who reported that the results of MTT assay in their study showed the group with the lowest dose of 1.2 J/cm$^2$ demonstrated more favorable results on cell viability as much as 6–24 hours after irradiation compared with the others groups.[35] However, the researchers also used a crystal violet assay to assess the cell viability, which could be one of the limitations of our study. As ours is a novel pilot study, a smaller sample size and assessment with the MTT test alone were undertaken to provide a basis for future expansive research with financial support. However, stem cell research with periodontal ligament cells has been done with a smaller sample size.[35] In summary, our study substantiated that LLLT with different energy densities has a positive influence on *in vitro* viability and proliferation rates of GMSCs and may be a useful tool for tissue engineering. It did provide a basis for future researchers on GMSCs treatment with LLLT. However, further studies are needed to standardize the laser parameters to improve the yield of GMSCs cells in culture and to understand and explore the possibility of sustained effects of biostimulation on GMSCs, which would open a surfeit of therapeutic regenerative opportunities. Innovative therapies with the added benefit of LLLT and stem cell regeneration can provide a plethora of treatment modalities for human diseases naturally. The combination of light-based biomodulation of regenerative cells and their therapeutic potential can find their future applications in photo diagnosis, phototherapy, and mesenchymal stem cell-based regenerative therapies.

## Conclusion

Low-level laser therapy (LLLT) using 660 nm diode laser with different energies showed a positive effect on *in vitro* proliferation of GMSCs. The rate of proliferation was comparatively more significant at 12 hours and low-energy. This study provides a basis on which further work on repetitive doses of irradiation can be investigated to exploit its benefits. LLLT can be a beneficial tool in tissue engineering using GMSCs.

## References


1. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8(4):315–317. DOI: 10.1080/14653240600855905
2. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284(5411):143–147. DOI: 10.1126/science.284.5411.143
3. Soler R, Orozco L, Munar A, et al. Final results of a phase I-II trial using ex vivo expanded autologous Mesenchymal stromal cells for the treatment of osteoarthritis of the knee confirming safety and suggesting cartilage regeneration. Knee 2016;23(4):647–654. DOI: 10.1016/j.knee.2015.08.013Z
4. Lin F, Ichim TE, Pingle S, et al. Mesenchymal stem cells as living anti-inflammatory therapy for COVID-19 related acute respiratory distress syndrome. World J Stem Cells 2020;12(10):1067–1079. DOI: 10.4252/wjsc.v12.i10.1067
5. Le Blanc K, Frassoni F, Ball L, et al. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. Lancet 2008;371(9624):1579–1586. DOI: 10.1016/S0140-6736(08)60690-X
6. Jung Y, Kim JK, Shiozawa Y, et al. Recruitment of mesenchymal stem cells into prostate tumours promotes metastasis. Nat Commun 2013;4:1795. DOI: 10.1038/ncomms2766
7. Golchin A, Farahany TZ, Khojasteh A, et al. The clinical trials of mesenchymal stem cell therapy in skin diseases: an update and concise review. Curr Stem Cell Res Ther 2019;14(1):22–33. DOI: 10.2174/1574888X13666180913123424
8. Yañez RM, Lamana ML, Garcia-Castro J, et al. In vitro and in vivo immunomodulatory effects of mesenchymal stem cells from adipose tissue. Blood 2005;106(11):3098. DOI: 10.1182/blood.V106.11.3098.3098
9. Zhang QZ, Su WR, Shi SH, et al. Human gingiva-derived mesenchymal stem cells elicit polarization of m2 macrophages and enhance cutaneous wound healing. Stem Cells 2010;28(10):1856–1868. DOI: 10.1002/stem.503
10. Li P, Zhao Y, Ge L. Therapeutic effects of human gingiva-derived mesenchymal stromal cells on murine contact hypersensitivity via prostaglandin E2-EP3 signaling. Stem Cell Res Ther 2016;7(1):103. DOI: 10.1186/s13287-016-0361-9
11. Shi Q, Qian Z, Liu D, et al. GMSC-derived exosomes combined with a chitosan/silk hydrogel sponge accelerates wound healing in a diabetic rat skin defect model. Front Physiol 2017;8:904. DOI: 10.3389/fphys.2017.00904
12. Linard C, Tissedre F, Busson E, et al. Therapeutic potential of gingival fibroblasts for cutaneous radiation syndrome: comparison to bone marrow-mesenchymal stem cell grafts. Stem Cells Dev 2015;24(10):1182–1193. DOI: 10.1089/scd.2014.0486
13. Lu Y, Xu Y, Zhang S, et al. Human gingiva-derived mesenchymal stem cells alleviate inflammatory bowel disease via IL-10 signalling-dependent modulation of immune cells. Scand J Immunol 2019;90(3):e12751. DOI: 10.1111/sji.12751
14. Chen M, Su W, Lin X, et al. Adoptive transfer of human gingiva-derived mesenchymal stem cells ameliorates collagen-induced arthritis via suppression of Th1 and Th17 cells and enhancement of regulatory T cell differentiation. Arthritis Rheum 2013;65(5):1181–1193. DOI: 10.1002/art.37894
15. Gu Y, Shi S. Transplantation of gingiva-derived mesenchymal stem cells ameliorates collagen-induced arthritis. Arthritis Res Ther 2016;18(1):262. DOI: 10.1186/s13075-016-1160-5
16. Zhang Q, Shi S, Liu Y, et al. Mesenchymal stem cells derived from human gingiva are capable of immunomodulatory functions and ameliorate inflammation-related tissue destruction in experimental colitis. J Immunol 2009;183(12):7787–7798. DOI: 10.4049/jimmunol.0902318
17. Ni X, Xia Y, Zhou S, et al. Reduction in murine acute GVHD severity by human gingival tissue-derived mesenchymal stem cells via the CD39 pathways. Cell Death Dis 2019;10(1):13. DOI: 10.1038/s41419-018-1273-7
18. Zhang W, Zhou L, Dang J, et al. Human gingiva-derived mesenchymal stem cells ameliorate streptozoticin-induced T1DM in mice via suppression of T effector cells and up-regulating treg subsets. Sci Rep 2017;7(1):15249. DOI: 10.1038/s41598-017-14979-5
19. Anders JJ, Lanzafame RJ, Arany PR. Low-level light/laser therapy versus photobiomodulation therapy. Photomed Laser Surg 2015;33(4):183–184. DOI: 10.1089/pho.2015.9848
20. Barboza CA, Ginani F, Soares DM, et al. Low-level laser irradiation induces in vitro proliferation of mesenchymal stem cells. Einstein (Sao Paulo) 2014;12(1):75–81. DOI: 10.1590/S1679-45082014AO2824
21. Bai J, Li L, Kou N, et al. Low-level laser therapy promotes bone regeneration by coupling angiogenesis and osteogenesis. Stem Cell Res Ther 2021;12(432):1–18. DOI: 10.1186/s13287-021-02493-5
22. Shi Y, Wang Y, Li Q, et al. Immunoregulatory mechanisms of mesenchymal stem and stromal cells in inflammatory diseases. Nat Rev Nephrol 2018;14(8):493–507. DOI: 10.1038/s41581-018-0023-5
23. Fawzy El-Sayed KM, Mekhemar MK, Beck-Broichsitter BE, et al. Periodontal regeneration employing gingival margin-derived stem/progenitor cells in conjunction with IL-1ra-hydrogel synthetic extracellular matrix. J Clin Periodontol 2015;42(5):448–457. DOI: 10.1111/jcpe.12401
24. Zhang Q, Nguyen PD, Shi S, et al. Neural crest stem-like cells non-genetically induced from human gingiva-derived mesenchymal stem cells promote facial nerve regeneration in rats. Mol Neurobiol 2018;55(8):6965–6983. DOI: 10.1007/s12035-018-0913-3
25. Feng J, Li X, Zhu S, et al. Photobiomodulation with 808-nm diode laser enhances gingival wound healing by promoting migration of human gingival mesenchymal stem cells via ROS/JNK/NF-κB/MMP-1 pathway. Lasers Med Sci 2020;35(8):1831–1839. DOI: 10.1007/s10103-020-03040-z
26. Wang L, Liu C, Wu F. Low-level laser irradiation enhances the proliferation and osteogenic differentiation of PDLSCs via BMP signaling. Lasers Med Sci 2022;37(2):941–948. DOI: 10.1007/s10103-021-03338-6
27. Wu JY, Chen CH, Yeh LY, et al. Low-power laser irradiation promotes the proliferation and osteogenic differentiation of human periodontal ligament cells via cyclic adenosine monophosphate. Int J Oral Sci 2013;5(2):85–91. DOI: 10.1038/ijos.2013.38
28. Soares DM, Ginani F, Henriques ÁG, et al. Effects of laser therapy on the proliferation of human periodontal ligament stem cells. Lasers Med Sci. 2015;30(3):1171–1174. DOI: 10.1007/s10103-013-1436-9
29. Ginani F, Soares DM, de Oliveira Rocha HA, et al. Low- level laser irradiation induces in vitro proliferation of stem cells from human exfoliated deciduous teeth. Lasers Med Sci 2018;33:95–102. DOI: 10.1007/s10103-017-2355-y
30. Zaccara IM, Ginani F, Mota-Filho HG, et al. Effect of low-level laser irradiation on proliferation and viability of human dental pulp stem cells. Lasers Med Sci 2015;30(9):2259–2264. DOI: 10.1007/s10103-015-1803-9
31. AlGhamdi KM, Kumar A, Moussa NA. Low-level laser therapy: a useful technique for enhancing the proliferation of various cultured cells. Lasers Med Sci 2012;27(1):237–249. DOI: 10.1007/s10103-011-0885-2
32. Pereira AN, Eduardo Cde P, Matson E, et al. Effect of low-power laser irradiation on cell growth and procollagen synthesis of cultured fibroblasts. Lasers Surg Med 2002;31(4):263–267. DOI: 10.1002/lsm.10107
33. Almeida-Junior LA, Marques NCT, Prado MTO, et al. 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;34(9):1917–1924. DOI: 10.1007/s10103-019-02836-y
34. Fernandes AP, Junqueira Mde A, Marques NC, et al. Effects of low-level laser therapy on stem cells from human exfoliated deciduous teeth. J Appl Oral Sci 2016;24(4):332–337. DOI: 10.1590/1678-775720150275
35. Kato H, Taguchi Y, Tominaga K, et al. High glucose concentrations suppress the proliferation of human periodontal ligament stem cells and their differentiation into osteoblasts. J Periodontol 2016;87(4):e44–e51. DOI: 10.1902/jop.2015.150474