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Exploring the Effects of Low-Level Laser Therapy on the Cytocompatibility and Osteo/Odontogenic Potential of Gingival-Derived Mesenchymal Stem Cells: Preliminary Report

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Abstract: Numerous tissue engineering uses for gingival-derived mesenchymal stem cells (GMSCs) have been demonstrated. Recently, low-level laser therapy (LLLT) has been projected as a factor that can improve MSCs' regeneration capacity. Therefore, the aim of this research was to examine the impact of LLLT at 1.5 J/cm^2 and 3 J/cm^2 on the viability and osteo/odontogenic potential of GMSCs. An MTT assay was performed to detect viability. Osteo/odontogenic differentiation was evaluated using Alizarin Red S staining and qRT-PCR for the evaluation of the RUNX2, OC, DMP1, and DSPP genes. A two-way ANOVA with Tukey's post hoc test was used to determine the statistical significance between groups. The results revealed that LLLT of both energy densities had no cytotoxic effect on GMSC viability. LLLT of 1.5 J/cm^2 demonstrated better viability than the higher energy density (3 J/cm^2). Furthermore, the osteo/odontogenic differentiation potential was promoted following LLLT radiation, where both groups exhibited mineralized nodule formation, with the low-energy laser having a significantly higher Alizarin Red S stain level. A qRT-PCR analysis revealed higher expression levels of osteogenic and odontogenic markers in the LLLT groups compared to the control group. In conclusion, this study showed the potential application of LLLT as a non-toxic and effective strategy to enhance the regenerative capacity of GMSCs for tissue engineering and clinical treatments in the oral and craniofacial fields.

Keywords: dentistry; tissue engineering; gingival-derived mesenchymal stem cells; periodontology; low-level laser therapy; osteogenic differentiation; odontogenic differentiation

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1. Introduction

Tissue engineering is a modern multidisciplinary field with the ultimate objective of healing and rebuilding damaged tissues [1]. Mesenchymal stem cells (MSCs) are critical components in the process of tissue engineering owing to their distinct characteristics [2,3]. MSCs from oral sources have been proven to contain the necessary attributes for tissue regeneration utilization [4–9]. Dental MSCs are a unique set of MSCs that can exceed the properties of bone marrow MSCs (BMMSCs), which are regarded as the predominant kind of MSCs exploited in tissue engineering [10–12]. This is because of their multipotency traits, accessibility, and high proliferation rates.

A unique type of MSC of oral origin is isolated from the gingiva, known as gingival-derived MSCs (GMSCs). This type of MSC has yet to receive much attention from researchers. Gingival tissue collected through non-invasive dental procedures, such as crown lengthening and gingival biopsies, is a rich source of GMSCs. Because of this, they have the

potential to be a very useful source of MSCs for the tissue engineering of the craniofacial and oral regions [13,14].

GMSCs share the properties of other MSCs; they have a self-renewal capacity, multi-differentiation potential, and acquire cell-surface-specific proteins such as CD73, CD105, and CD90, which characterize MSCs [15,16]. It is feasible to promote GMSCs into differentiating into numerous cell types, such as osteoblasts, chondrocytes, adipocytes, and fibroblasts [17,18]. In addition, it has been proven that they can differentiate into odontoblasts, cells that form dentin [19]. The high proliferative capacity of GMSCs has been reported in many studies. It has been demonstrated that it may surpass the capacity of BMMSCs [20]. At the same time, GMSCs have no tumorigenicity after a long culture period and long-term inoculation in animal models [21]. Moreover, GMSCs have the potential, under specific induction conditions, to differentiate into ectodermal and endodermal cell types [22,23]. In addition to this, it has been demonstrated that GMSCs also possess anti-inflammatory and immunomodulatory capabilities, which position these cells as a promising source of MSCs for clinical applications involving the treatment of many diseases [24,25].

The advantageous and promising characteristics of GMSCs have made researchers devote a lot of time and effort to uncover the potential uses of MSCs in tissue engineering. GMSCs have been used to treat skin disorders and autoimmune diseases and promote nerve regeneration [26–28]. Furthermore, GMSCs have been utilized to treat oral and craniofacial disorders. Sun et al. [29] have demonstrated the ability of GMSCs to treat periodontal inflammation and promote periodontal regeneration. Moreover, the therapeutic uses of GMSCs in the craniofacial region include the treatment of peri-implantitis, maxillofacial bone defects, and oral mucositis and the promotion of facial nerve regeneration and salivary gland regeneration [22,28,30–32].

The potential for regeneration of GMSCs can be inhibited by factors such as aging and illness. With age, the proliferative capacity and multipotency of the MSCs decline, compromising their regenerative ability. The therapeutic potential of MSCs may also be reduced due to decreases in the number of MSCs caused by underlying conditions [33]. As with other stem cells, GMSCs have a finite life span and are subject to cellular senescence, which inhibits their ability to proliferate and differentiate. Because of their advancing age, they may not be as useful for tissue regeneration in the long run. Also, compared to BM-MSCs and PDLSCs, GMSCs have been shown to have a lower osteogenic potential [34].

Several methods, such as the addition of cytokines, growth factors, and bioactive substances, have been implemented to enhance MSCs' regeneration potential. The employment of a laser with a wavelength between 630 and 980 nm, as in low-level laser therapy (LLLT), is one such strategy. Due to the lack of heat and tissue damage caused by these lasers, LLLT is often called a "cold laser" [35]. These red and near-infrared electromagnetic waves have been shown to promote the proliferative capacity of MSCs by stimulating the action of growth factors and enzymes and the synthesis of ATP within the cells [36,37].

The therapeutic effect of LLLT is related to many parameters. The wavelength, power and energy densities, type of laser used, pulse structure, duration, and repetition of irradiation are all factors that determine the effectiveness of LLLT for bio-stimulation [36].

Because of its distinct properties, LLLT has proven to be a useful technique for regenerating damaged tissues. Tissue repair, accelerated wound healing, and inflammatory modulation are all areas where LLLT has been put to use [38]. By stimulating many signaling pathways involved in osteogenic differentiation, LLLT also expedites bone healing [39,40]. Oral diseases such as mucositis, TMJ inflammation, aphthous stomatitis, and dentinal hypersensitivity have also been treated with LLLT [41–43].

Because LLLT has been shown to be a successful treatment method for a wide variety of disorders, it was used to biologically stimulate MSCs. LLLT has been shown to have a stimulatory effect on DPSCs, SHED [44], SCAP [45], and PDLSCs [46], promoting their proliferation, viability, and differentiation.

The bio-stimulatory effect of LLLT is related to the cellular absorption of LLLT, which takes place in the mitochondrial respiratory chain, and results in an increase in ATP levels, the upregulation of growth factors, and the inhibition of apoptosis. In addition, the laser's anti-inflammatory and anti-edematous actions are caused by an increase in the body's microcirculation, which speeds up blood flow to the affected area.

On the other hand, not a lot is known about how LLLT affects GMSCs. In light of this, the purpose of this research was to investigate the effect of LLLT on the osteogenic and proliferative capabilities of GMSCs, with the end goal of enhancing the regenerative potential of GMSCs as a conveniently available and promising form of MSCs. We hypothesized that LLLT with a low energy density (1.5 J/cm^2) would promote viability and induce osteo/odontogenic differentiation more effectively than high-level laser therapy (HLT) with a high energy density (3 J/cm^2).

2. Materials and Methods

2.1. GMSC Isolation and Culture

After receiving written consent, gingival tissues were collected from healthy patients aged 20–40 who had undergone crown-lengthening treatments. Gingival tissues were collected from 5 patients, and a pool of cells was obtained to provide a yield of stem cells large enough to conduct the whole experiment with the repetition of each experiment three times and to provide a biological duplicate excluding the "patient source" variable [47,48].

We used collagenase type I (Sigma, St. Louis, MO, USA) and dispase type II (Sigma, St. Louis, MO, USA) on the collected gingival tissues, which resulted in a finely minced consistency. Following a phosphate-buffered saline (PBS) (Gibco BRL, CA, USA) filtering step, cell pellets were isolated from the tissues and cultured in Dulbecco's modified Eagle's medium/F-12 Ham medium (DMEM/F12, Sigma-Aldrich) supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, USA) [49]. The cells employed in this analysis were in their fourth passage.

2.2. GMSC Characterization

2.2.1. Surface Marker Analysis

Cultured GMSCs were analyzed using flow cytometry for the quantitative surface marker identification of CD73, CD90, CD105, CD34, and CD45. About 1 × 105 cultivated cells were examined using a flow cytometer (Beckman Coulter, Fullerton, CA, USA) after being treated with monoclonal antibodies against the surface markers [50].

2.2.2. Multilineage Differentiation Potential

The multilineage differentiation potential of the cultured cells was determined using a differentiation kit (Human mesenchymal stem cell functional identification kit, R&D systems, Minneapolis, MN, USA). Products of the differentiated cells were stained with Alizarin Red S (Sigma Aldrich, Steinheim, Germany) for osteogenic differentiation, Oil red O stain (Sigma Aldrich, Steinheim, Germany) for adipogenic differentiation, and Alcian Blue (Sigma Aldrich, Steinheim, Germany) for chondrogenic differentiation (N = 5) [51].

2.3. LLLT Protocol

Cultured cells were irradiated using a GaAIAs Diode laser device (K2 mobile laser, Hulaser, Seoul, Republic of Korea) with a wavelength of 980 nm in a continuous mode. Laser radiation was applied in two different energy densities: group I received 1.5 J/cm^2 and group II received 3 J/cm^2 in single doses for 60 s. The irradiation procedures were performed in dark conditions to prevent any dispersion of light [52].

2.4. Cell Viability

The cell viability of GMSCs after laser radiation of different energy densities was evaluated using MTT assays on days 1, 3, and 7 of the culture (N = 3). The results

are reported as the viability percentage against the control group (cells without laser radiation) [53].

2.5. Osteo/Odontogenic Differentiation of GMSCs

To induce osteo/odontogenic differentiation, irradiated GMSCs (N = 3) were cultured in osteo/odontogenic media consisting of DMEM (Sigma Aldrich, Steinheim, Germany) supplemented with 0.1 µM dexamethasone (Sigma Aldrich, Steinheim, Germany), 10 mM β-glycerophosphate (Merck, Darmstadt, Germany), and 50 µg/mL ascorbic acid (Sigma Aldrich, Steinheim, Germany) for 14 days. Non-radiated cells cultured in osteogenic media were considered a positive control [51].

2.6. Alizarin Red S Staining

Following 2 weeks in osteogenic media, GMSCs (N = 3) were stained with Alizarin Red S dye (Sigma-Aldrich, Steinheim, Germany), adhering to the guidelines provided by the manufacturer. The mineralized nodules were examined, and images were captured using an inverted microscope (Olympus, Tokyo, Japan) following the removal of any excess dye. The mineralized nodules were measured via their absorbance at 405 nm after the red dye was mixed with 10% glacial acetic acid (Sigma-Aldrich) [54].

2.7. Quantitative Real-Time Polymerase Chain Reaction

Total mRNA was extracted from GMSCs (N = 3) using a QIAGEN RNA extraction kit (QIAGEN GmbH, Hilden, Germany) following the manufacturer's instructions. q-Real-time Polymerase Chain Reaction (q-RT PCR) quantification was performed using SYBR Green premix (Qiagen). The mRNA expression levels were normalized against β-actin, and all experiments were performed in triplicate [51]. The primer sequences used were as follows:

DMP1: 5′-AGGAAGTCTCGCATCT CAGAG-3′ (forward) and 5′-TGGAGTTGCTGTTT-TCTGTAGAG-3′ (reverse);
DSPP: 5′-TCACAAGGGAGAAGGGAATG-3′ (forward) and 5′-TGCCATTTGCTGTGATGTTT-3′ (reverse);
Runx2: 5′-CACTGGCGCTGCAACAAGA-3′ (forward) and 5′-CATTCCGGAGCTCAGCA-GAATAA-3′ (reverse);
OC: 5′-CAGCAAAGGTGCAGCCTTG-3′ (forward) and 5′-TGGGGCTCCCAGCCATTG-3′ (reverse);
β-actin: 5′-CCATCGTCCACCGCAAAT-3′ (forward) and 5′-CCTGTAACAACGCATCTCATA-3′ (reverse).

2.8. Statistical Analysis

The results for cell viability and osteo/odontogenic differentiation are presented as means ± standard deviations and were obtained through experiments conducted in triplicate. The Shapiro–Wilk test was used to assess the normality of the data. To determine the statistical significance between the experimental groups, a two-way ANOVA with Tukey's post hoc test was used. A value of $p < 0.05$ was considered significant. Graphpad Prism 9.0 (GraphPad Software, San Diego, CA, USA) was used for the statistical analysis.

3. Results

3.1. Morphological Characterization, Identification, and Multi-Differentiation Potential of GMSCs

A surface antigen analysis via the flow cytometry of GMSCs revealed the positive expression of CD73, CD105, and CD90, confirming the stemness of the cells. It was found that GMSCs do not express the hematopoietic markers CD34 and CD45 (Figure 1). A morphological examination of GMSCs at the fourth passage showed that the cells acquired a spindle-shaped appearance typical of MSCs. Moreover, trilineage differentiation (osteo/adipo/chondrogenic) potential was confirmed after the positive expression of the respective stains (Figure 2).

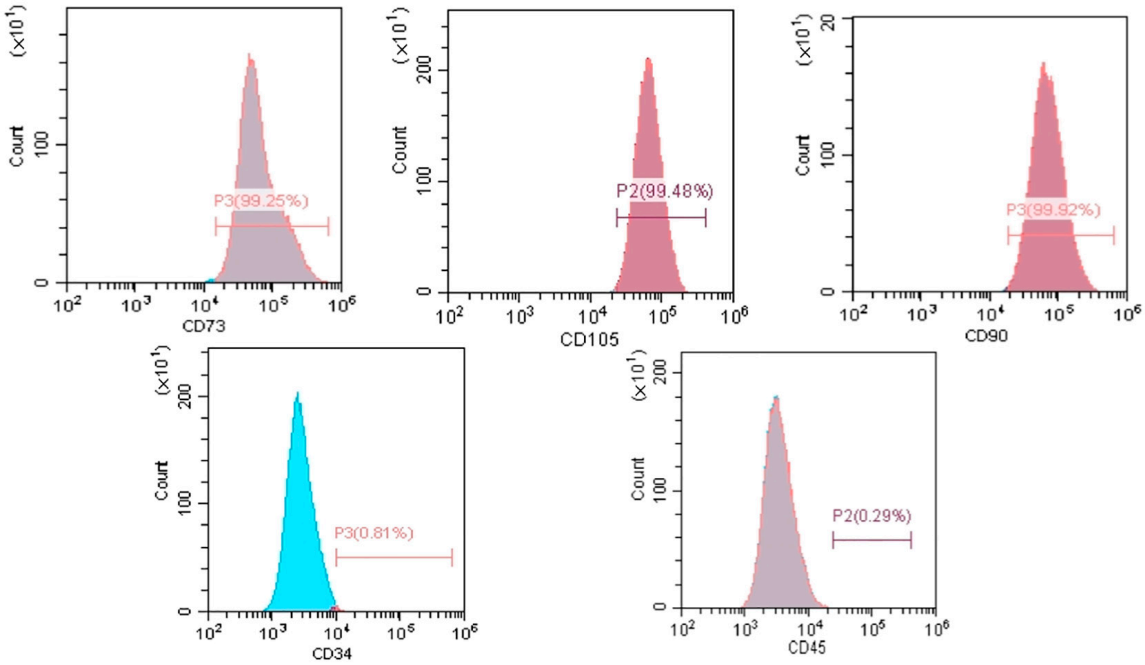

Figure 1. The surface genes of GMSCs were analyzed using flow cytometry. CD73, CD90, and CD105 were found to be positively expressed, while the hematopoietic markers CD34 and CD45 were not expressed.

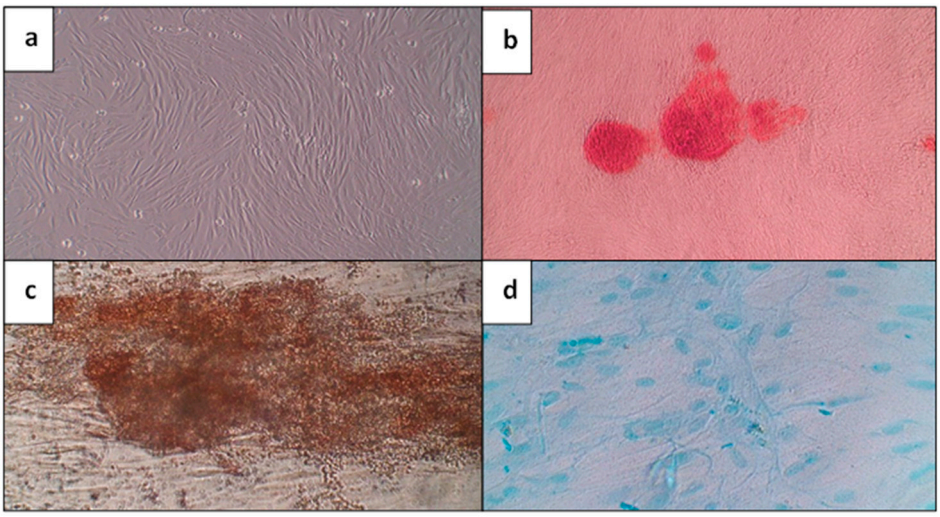

Figure 2. (**a**) Morphological characterization of GMSCs at 4th passage. The multilineage differentiation potential of GMSCs to (**b**) osteocytes (confirmed with Alizarin Red S stain), (**c**) adipocytes (confirmed with Oil red O stain), and (**d**) chondrocytes (confirmed with Alcian Blue stain).

3.2. MTT Assay

To evaluate the cytotoxic effects of both power densities of LLLT on GMSC viability, an MTT assay was conducted. Figure 3 shows that the groups had no significant difference after 24 h. After 72 h, it was observed that both parameters of LLLT resulted in a significant increase ($p < 0.05$) in cell viability. This indicates that LLLT did not have any negative impact on the viability of GMSCs. On day 7, the GMSCs treated with 1.5 J/cm^2 LLLT had a significantly higher absorption value ($p < 0.05$) than the GMSCs treated with 3.5 J/cm^2 LLLT (Figure 3).

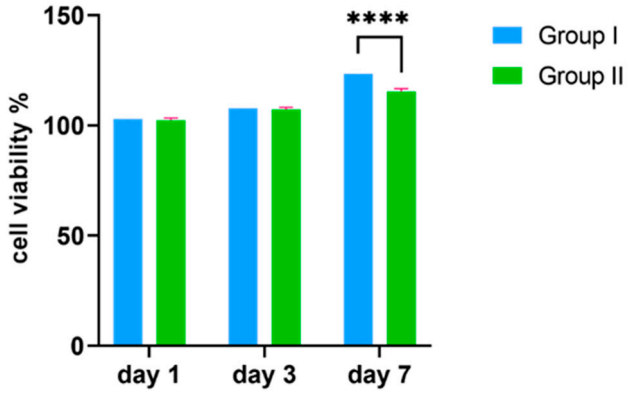

Figure 3. Effect of LLLT on GMSC viability on days 1, 3, and 7 (evaluated using MTT assay). **** indicates a significant difference ($p < 0.0001$).

3.3. Alizarin Red S Assay

The Alizarin Red S stain was used to assess calcium deposition and mineralization in the GMSC samples after 2 weeks of culture in osteogenic media. After the staining procedure, the presence of calcium ions was detected by forming a red-orange color. In addition, in both experimental groups, strong and distinct red staining was observed, indicating a high level of calcium deposition. This suggests that LLLT promoted the osteogenic differentiation of the cells. A quantitative analysis of Alizarin Red S staining showed a significant difference in calcium deposition between the group I and II control groups. In addition, the lower energy density (1.5 J/cm^2) demonstrated higher levels than the high energy density, highlighting the lower power's effectiveness in promoting mineralization (Figure 4).

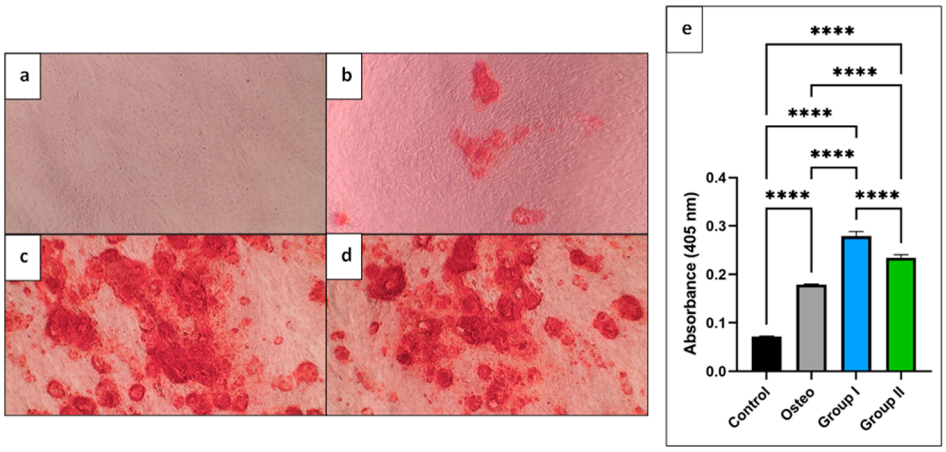

Figure 4. Effect of LLLT on osteogenic differentiation of GMSCs. Representative images of cells stained with Alizarin Red S dye after 14 days: (**a**) control (GMSCs in normal culture), (**b**) osteogenic group (without LLLT), (**c**) group I (1.5 J/cm^2), and (**d**) group II (3 J/cm^2). (**e**) A bar chart demonstrating quantitative results of Alizarin Red S stain absorbance rate. **** indicates significance ($p < 0.0001$) for each group compared to the control group and differences between groups.

3.4. q-RT PCR

The expression levels of osteogenic and odontogenic markers were determined to detect the effect of 1.5 J/cm^2 and 3 J/cm^2 LLLT on the osteo/odontogenic differentiation of GMSCs. In both groups that received LLLT, the levels of DMP1, DSPP, and RUNX2 expression were noticeably higher than in the control group. Additionally, there was a significantly greater expression of these markers (with a p-value less than 0.05) in the 1.5 J/cm^2 LLLT group compared to the 3 J/cm^2 group. The mRNA expression of OCN was significantly increased ($p < 0.05$) in both LLLT parameter groups compared to the

untreated groups. However, there was no significant difference ($p > 0.05$) found in the OCN expression between the two LLLT groups (Figure 5).

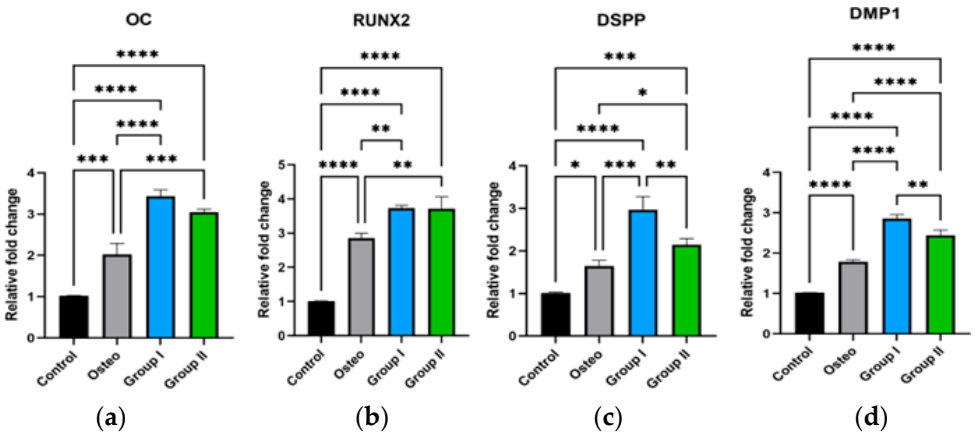

Figure 5. Expression of (**a**) OC, (**b**) RUNX2, (**c**) DSPP, and (**d**) DMP1 genes. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$ for each group compared to the control group and differences between groups.

4. Discussion

GMSCs have been proven to be effective in the treatment of various diseases and in the regenerative processes of damaged tissues [16,55]. However, the potential of GMSCs for oral regenerative procedures is yet to be thoroughly studied, and a limited number of studies have investigated these cells' osteo/odontogenic differentiation ability. Moreover, studies utilizing LLLT and identifying the best laser protocol for stimulating GMSCs toward osteo/odontogenic differentiation still need to be made available. Accordingly, the present study aimed to evaluate the cytocompatibility and osteo/odontogenic differentiation potential of GMSCs after stimulation using two different PBM parameters.

GMSCs were isolated from gingival tissues, and the positive expression of MSC cell surface markers CD73, CD90, and CD105 was confirmed following a phenotypic analysis using flow cytometry. Numerous studies previously described this phenotypic profile to be characteristic of MSCs, ensuring the successful isolation of GMSCs [56,57].

In the present study, the bio-stimulation of GMSCs was examined after LLLT using different energy densities. The laser type used for LLLT in this study was a 980 nm diode laser, which can be available in dental offices because of its relatively low cost and ease of handling. Moreover, a diode laser with a wavelength ranging from 600 to 1200 nm and energy densities ranging from 0.4 to 4 J/cm^2 has been proven to have bio-stimulatory effects on different stem cells, while energies greater than 10 J/cm^2 might have cytotoxic effects [44]. Therefore, a single application of a diode laser with a 980 nm wavelength for 60 s using two energy densities, 1.5 J and 3 J, was examined in the present study.

The current study used an MTT assay to examine the cell viability and proliferation rates of GMSCs following LLLT. The results showed a significant increase ($p < 0.05$) in cell viability following the application of both energies, indicating that LLLT with the tested parameters was non-toxic to GMSCs and stimulated their proliferation. This effect may be attributed to the enhancement of cellular metabolism by LLLT and the increased production of adenosine triphosphate (ATP) [58], which promoted the function and proliferation of GMSCs.

The MTT results on day 7 showed that lower energy doses (1.5 J/cm^2) resulted in better viability of GMSCs compared to higher doses (3 J/cm^2). High-energy doses of laser irradiation may cause cellular stress and damage and inhibit metabolism, thereby negatively affecting cell viability and function. On the other hand, GMSC viability was improved by exposure to lower energy doses because they were less cytotoxic and promoted GMSC growth. These findings corroborate previous studies that found low-energy-density lasers

to be more effective than higher dosages at encouraging stem cell development. Large energy doses have been shown to have deleterious effects on cell photoreceptors, resulting in metabolic suppression and cell death. Higher doses of laser irradiation can cause more stress and damage to cells, which may reduce their ability to survive and function [59,60]. In order to maintain better viability, GMSCs may be able to withstand lower energy dosages that are less cytotoxic.

The current study also looked at how varying LLLT energy densities affected the ability of GMSCs to differentiate into osteo/odontogenic tissues. It is critical to remember that precise laser irradiation parameters, such as the wavelength, energy density, and exposure period, can significantly impact how odontogenic differentiation turns out [57,61]. Successful odontogenic differentiation also depends on the choice of the stem cell source, culture conditions, and the presence of the right growth factors or signaling molecules [12,62]. LLLT has been investigated for its potential to promote the osteo/odontogenic differentiation of stem cells, such as DPSCs [63], SCAP [57], and PDLSCs [64]. GMSCs were found to form calcified nodules with positive Alizarin Red S staining in both experimental groups. The quantitative absorption values of the Alizarin Red S staining revealed a significantly higher value in group I than in group II. Once again, the lower energy dose caused a better biological response by GMSCs for odontogenic differentiation. Different energy doses of laser irradiation can activate other signaling pathways within cells. Lower energy doses may selectively activate specific pathways involved in odontogenic differentiation, leading to a more focused and efficient cellular response. Higher energy doses, on the other hand, may activate additional or different pathways that could interfere with or dampen the desired odontogenic differentiation [65,66].

During the osteogenic and odontogenic differentiation process, GMSCs experience changes in the patterns of gene expression that they exhibit, including the upregulation of certain markers. The expression of osteogenic and odontogenic genes was analyzed using quantitative real-time PCR for a deeper evaluation of the osteo/odontogenic differentiation. Both experimental groups showed significant levels of gene expression, which was indicative of the commitment and maturation of GMSCs in the direction of the osteoblast and odontoblast lineages.

When compared to the control and osteogenic groups, the expression of Runt-related transcription factor 2 (Runx2), which is one of the earliest markers of osteogenic differentiation and plays a crucial role in the commitment of mesenchymal stem cells to the osteoblast lineage [67], was significantly increased in both laser groups. This contrasted with the groups that received osteogenic treatment. However, a decrease in Runx2 expression was observed in the high-energy group (3 J/cm^2), which aligns with previous findings. The expression of Runx2 was significantly increased following laser irradiation at 0.4 and 1.9 J/cm^2, as demonstrated in previous research. However, a decrease in Runx2 expression in osteoblasts was found after using a laser at 3 J/cm^2, which was consistent with the current study in which group II had significantly lower expression of RUNX2 than group I.

In this study, high upregulation of osteocalcin (OC) in groups I and II, compared to the control group and osteogenic group (positive control), indicated the maturation of osteoblasts. OC is a protein that mature osteoblasts produce and is associated with their terminal differentiation [68]. Previous studies have shown an upregulation of OC following LLLT at different energy densities [69,70]. Cevik et al. [71] applied LLLT with different wavelengths and energy densities to BMMSCs and found that LLLT promoted osteogenesis. Chen et al. [72] demonstrated that LLLT significantly increased the expression of the osteogenesis-related genes RUNX2, ALP, and OC at low energy levels of LLLT. The authors also revealed that the inhibition of the osteogenic markers was evident after using high-power energy densities, which agrees with the present results.

Dentin sialophosphoprotein (DSPP) is thought to be a unique marker for odontogenic differentiation [73], and it plays a vital role in the regeneration of dentin. Both bone and dentin contain DSPP in varying amounts. However, dentin contains a substantially larger concentration of it than bone. Dentin matrix protein-1 (DMP1) is yet another protein that is

essential to the process of odontogenesis and has a significant impact on the mineralization of dentin [74]. The current work confirmed the effect that LLLT has in promoting the odontogenic differentiation of GMSCs by showing that the genes DSPP and DMP1 were highly elevated in both experimental groups. In addition, a laser with a lower energy (1.5 J/cm^2) exhibited considerably greater gene expression of DMP1 and DSPP than a laser with a higher energy (3 J/cm^2), which suggests that lasers with lower energy can have a more significant influence in encouraging the odontogenic differentiation of GMSCs. The stimulation of the odontogenic differentiation of GMSCs by LLLT may be inferred from the elevated expression of DSPP and DMP1, both of which are markers of this process. Despite this, more study is required to ascertain the histological structure of the newly created tissue.

Additionally, some recently introduced compounds have been demonstrated to have a significant influence on the oral environment. Probiotics [75], lysates [76], and post-biotics [77] can modify clinical and microbiological parameters in periodontal patients, so these products should also be considered in future clinical trials, as adjuvants and in combination with LLLT.

We recognize that individual variability plays a crucial role in assessing treatment outcomes, and we intend to explore this aspect in our subsequent research. By analyzing individual cells, we aim to identify any variations in responses that may contribute to different treatment outcomes among individuals. This approach will provide a more comprehensive understanding of LLLT's efficacy and its potential implications.

On the other hand, this study was an in vitro experiment, which limited its ability to completely replicate the complex in vivo environment. Results obtained in a controlled laboratory environment may not necessarily translate to the same clinical outcomes.

5. Conclusions

Overall, this study showed that GMSCs have great potential to be widely used in tissue engineering and the regeneration of tissues. Within the limitations of the in vitro experiments, the proliferative capacity of GMSCs was stimulated and their osteo/odontogenic differentiation potential was promoted following LLLT. Lower-energy LLLT had a more stimulatory effect on GMSCs than higher-energy LLLT. These findings emphasize the importance of optimizing the laser parameters for LLLT therapy to achieve the desired outcomes in oral regenerative procedures involving GMSCs.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Khademhosseini, A.; Langer, R. A decade of progress in tissue engineering. *Nat. Protoc.* **2016**, *11*, 1775–1781. [CrossRef] [PubMed]
2. Moysidou, C.-M.; Barberio, C.; Owens, R.M. Advances in Engineering Human Tissue Models. *Front. Bioeng. Biotechnol.* **2021**, *8*, 620962. [CrossRef]

3. Caddeo, S.; Boffito, M.; Sartori, S. Tissue Engineering Approaches in the Design of Healthy and Pathological In Vitro Tissue Models. *Front. Bioeng. Biotechnol.* **2017**, *5*, 40. [CrossRef] [PubMed]
4. Roato, I.; Chinigò, G.; Genova, T.; Munaron, L.; Mussano, F. Oral Cavity as a Source of Mesenchymal Stem Cells Useful for Regenerative Medicine in Dentistry. *Biomedicines* **2021**, *9*, 1085. [CrossRef] [PubMed]
5. Gronthos, S.; Mankani, M.; Brahim, J.; Robey, P.G.; Shi, S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 13625–13630. [CrossRef] [PubMed]
6. Sonoyama, W.; Liu, Y.; Yamaza, T.; Tuan, R.S.; Wang, S.; Shi, S.; Huang, G.T. Characterization of the *Apical papilla* and its residing stem cells from human immature permanent teeth: A pilot study. *J. Endod.* **2008**, *34*, 166–171. [CrossRef]
7. Morsczeck, C.; Götz, W.; Schierholz, J.; Zeilhofer, F.; Kühn, U.; Möhl, C.; Sippel, C.; Hoffmann, K.H. Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. *Matrix Biol.* **2005**, *24*, 155–165. [CrossRef]
8. Seo, B.M.; Miura, M.; Gronthos, S.; Bartold, P.M.; Batouli, S.; Brahim, J.; Young, M.; Robey, P.G.; Wang, C.Y.; Shi, S. Investigation of multipotent postnatal stem cells from human periodontal ligament. *Lancet* **2004**, *364*, 149–155. [CrossRef]
9. Costela-Ruiz, V.J.; Melguizo-Rodríguez, L.; Bellotti, C.; Illescas-Montes, R.; Stanco, D.; Arciola, C.R.; Lucarelli, E. Different Sources of Mesenchymal Stem Cells for Tissue Regeneration: A Guide to Identifying the Most Favorable One in Orthopedics and Dentistry Applications. *Int. J. Mol. Sci.* **2022**, *23*, 6356. [CrossRef]
10. Campanella, V. Dental Stem Cells: Current research and future applications. *Eur. J. Paediatr. Dent.* **2018**, *19*, 257. [CrossRef]
11. Lee, Y.C.; Chan, Y.H.; Hsieh, S.C.; Lew, W.Z.; Feng, S.W. Comparing the Osteogenic Potentials and Bone Regeneration Capacities of Bone Marrow and Dental Pulp Mesenchymal Stem Cells in a Rabbit Calvarial Bone Defect Model. *Int. J. Mol. Sci.* **2019**, *20*, 5015. [CrossRef] [PubMed]
12. Soudi, A.; Yazdanian, M.; Ranjbar, R.; Tebyanian, H.; Yazdanian, A.; Tahmasebi, E.; Keshvad, A.; Seifalian, A. Role and application of stem cells in dental regeneration: A comprehensive overview. *EXCLI J.* **2021**, *20*, 454–489. [CrossRef]
13. Grawish, M.E. Gingival-derived mesenchymal stem cells: An endless resource for regenerative dentistry. *World J. Stem Cells* **2018**, *10*, 116–118. [CrossRef] [PubMed]
14. Gan, L.; Liu, Y.; Cui, D.; Pan, Y.; Zheng, L.; Wan, M. Dental Tissue-Derived Human Mesenchymal Stem Cells and Their Potential in Therapeutic Application. *Stem Cells Int.* **2020**, *2020*, 8864572. [CrossRef] [PubMed]
15. Fonticoli, L.; Della Rocca, Y.; Rajan, T.S.; Murmura, G.; Trubiani, O.; Oliva, S.; Pizzicannella, J.; Marconi, G.D.; Diomede, F. A Narrative Review: Gingival Stem Cells as a Limitless Reservoir for Regenerative Medicine. *Int. J. Mol. Sci.* **2022**, *23*, 4135. [CrossRef] [PubMed]
16. Kim, D.; Lee, A.E.; Xu, Q.; Zhang, Q.; Le, A.D. Gingiva-Derived Mesenchymal Stem Cells: Potential Application in Tissue Engineering and Regenerative Medicine—A Comprehensive Review. *Front. Immunol.* **2021**, *12*, 667221. [CrossRef] [PubMed]
17. Ge, S.; Mrozik, K.M.; Menicanin, D.; Gronthos, S.; Bartold, P.M. Isolation and characterization of mesenchymal stem cell-like cells from healthy and inflamed gingival tissue: Potential use for clinical therapy. *Regen. Med.* **2012**, *7*, 819–832. [CrossRef]
18. Jauregui, C.; Yoganarasimha, S.; Madurantakam, P. Mesenchymal Stem Cells Derived from Healthy and Diseased Human Gingiva Support Osteogenesis on Electrospun Polycaprolactone Scaffolds. *Bioengineering* **2018**, *5*, 8. [CrossRef]
19. Gao, Y.; Zhao, G.; Li, D.; Chen, X.; Pang, J.; Ke, J. Isolation and Multiple Differentiation Potential Assessment of Human Gingival Mesenchymal Stem Cells. *Int. J. Mol. Sci.* **2014**, *15*, 20982–20996. [CrossRef]
20. Tomar, G.B.; Srivastava, R.K.; Gupta, N.; Barhanpurkar, A.P.; Pote, S.T.; Jhaveri, H.M.; Mishra, G.C.; Wani, M.R. Human gingiva-derived mesenchymal stem cells are superior to bone marrow-derived mesenchymal stem cells for cell therapy in regenerative medicine. *Biochem. Biophys. Res. Commun.* **2010**, *393*, 377–383. [CrossRef]
21. Li, J.; Xu, S.Q.; Zhang, K.; Zhang, W.J.; Liu, H.L.; Xu, Z.; Li, H.; Lou, J.N.; Ge, L.H.; Xu, B.H. Treatment of gingival defects with gingival mesenchymal stem cells derived from human fetal gingival tissue in a rat model. *Stem Cell. Res. Ther.* **2018**, *9*, 27. [CrossRef]
22. Li, D.; Zou, X.Y.; El-Ayachi, I.; Romero, L.O.; Yu, Z.; Iglesias-Linares, A.; Cordero-Morales, J.F.; Huang, G.T. Human Dental Pulp Stem Cells and Gingival Mesenchymal Stem Cells Display Action Potential Capacity In Vitro after Neuronogenic Differentiation. *Stem Cell. Rev. Rep.* **2019**, *15*, 67–81. [CrossRef]
23. Murugan Girija, D.; Kalachaveedu, M.; Ranga Rao, S.; Subbarayan, R. Transdifferentiation of human gingival mesenchymal stem cells into functional keratinocytes by *Acalypha indica* in three-dimensional microenvironment. *J. Cell. Physiol.* **2018**, *233*, 8450–8457. [CrossRef] [PubMed]
24. Smojver, I.; Katalinić, I.; Bjelica, R.; Gabrić, D.; Matišić, V.; Molnar, V.; Primorac, D. Mesenchymal Stem Cells Based Treatment in Dental Medicine: A Narrative Review. *Int. J. Mol. Sci.* **2022**, *23*, 1662. [CrossRef]
25. Andrukhov, O.; Behm, C.; Blufstein, A.; Rausch-Fan, X. Immunomodulatory properties of dental tissue-derived mesenchymal stem cells: Implication in disease and tissue regeneration. *World J. Stem Cells* **2019**, *11*, 604–617. [CrossRef] [PubMed]
26. Shafiee, A.; Cavalcanti, A.S.; Saidy, N.T.; Schneidereit, D.; Friedrich, O.; Ravichandran, A.; De-Juan-Pardo, E.M.; Hutmacher, D.W. Convergence of 3D printed biomimetic wound dressings and adult stem cell therapy. *Biomaterials* **2021**, *268*, 120558. [CrossRef] [PubMed]
27. Gao, Y.; Duan, R.; Li, H.; Jiang, L.; Tao, T.; Liu, X.; Zhu, L.; Li, Z.; Chen, B.; Zheng, S.; et al. Single-cell analysis of immune cells on gingiva-derived mesenchymal stem cells in experimental autoimmune uveitis. *iScience* **2023**, *26*, 106729. [CrossRef]
28. Zhang, Q.; Nguyen, P.D.; Shi, S.; Burrell, J.C.; Cullen, D.K.; Le, A.D. 3D bio-printed scaffold-free nerve constructs with human gingiva-derived mesenchymal stem cells promote rat facial nerve regeneration. *Sci. Rep.* **2018**, *8*, 6634. [CrossRef]

29. Sun, W.; Wang, Z.; Xu, Q.; Sun, H.; Liu, X.; Yang, J.; Hong, R. The treatment of systematically transplanted gingival mesenchymal stem cells in periodontitis in mice. *Exp. Ther. Med.* **2019**, *17*, 2199–2205. [CrossRef]
30. Hasani-Sadrabadi, M.M.; Sarrion, P.; Pouraghaei, S.; Chau, Y.; Ansari, S.; Li, S.; Aghaloo, T.; Moshaverinia, A. An engineered cell-laden adhesive hydrogel promotes craniofacial bone tissue regeneration in rats. *Sci. Transl. Med.* **2020**, *12*, eaay6853. [CrossRef]
31. Zhang, Q.; Nguyen, A.L.; Shi, S.; Hill, C.; Wilder-Smith, P.; Krasieva, T.B.; Le, A.D. Three-dimensional spheroid culture of human gingiva-derived mesenchymal stem cells enhances mitigation of chemotherapy-induced oral mucositis. *Stem Cells Dev.* **2012**, *21*, 937–947. [CrossRef] [PubMed]
32. Abd El-Latif, N.; Abdulrahman, M.; Helal, M.; Grawish, M.E. Regenerative capacity of allogenic gingival margin- derived stem cells with fibrin glue on albino rats' partially dissected submandibular salivary glands. *Arch. Oral. Biol.* **2017**, *82*, 302–309. [CrossRef] [PubMed]
33. Choudhery, M.S. Strategies to improve regenerative potential of mesenchymal stem cells. *World J. Stem Cells* **2021**, *13*, 1845–1862. [CrossRef]
34. Subba, T.A.; Varma, S.; Thomas, B.; Rao, S.; Kumar, M.; Talwar, A.; Shashidhar, K. Comparison of Cellular and Differentiation Characteristics of Mesenchymal Stem Cells Derived from Human Gingiva and Periodontal Ligament. *J. Int. Soc. Prev. Community Dent.* **2022**, *12*, 235–244. [CrossRef]
35. Theocharidou, A.; Bakopoulou, A.; Kontonasaki, E.; Papachristou, E.; Hadjichristou, C.; Bousnaki, M.; Theodorou, G.; Papadopoulou, L.; Kantiranis, N.; Paraskevopoulos, K.; et al. Odontogenic differentiation and biomineralization potential of dental pulp stem cells inside Mg-based bioceramic scaffolds under low-level laser treatment. *Lasers Med. Sci.* **2017**, *32*, 201–210. [CrossRef]
36. 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*, 941–948. [CrossRef]
37. El Nawam, H.; El Backly, R.; Zaky, A.; Abdallah, A. Low-level laser therapy affects dentinogenesis and angiogenesis of in vitro 3D cultures of dentin-pulp complex. *Lasers Med. Sci.* **2019**, *34*, 1689–1698. [CrossRef]
38. Avci, P.; Gupta, A.; Sadasivam, M.; Vecchio, D.; Pam, Z.; Pam, N.; Hamblin, M.R. Low-level laser (light) therapy (LLLT) in skin: Stimulating, healing, restoring. *Semin. Cutan. Med. Surg.* **2013**, *32*, 41–52.
39. Tim, C.R.; Bossini, P.S.; Kido, H.W.; Malavazi, I.; von Zeska Kress, M.R.; Carazzolle, M.F.; Parizotto, N.A.; Rennó, A.C. Effects of low level laser therapy on inflammatory and angiogenic gene expression during the process of bone healing: A microarray analysis. *J. Photochem. Photobiol. B: Biol.* **2016**, *154*, 8–15. [CrossRef]
40. Berni, M.; Brancato, A.M.; Torriani, C.; Bina, V.; Annunziata, S.; Cornella, E.; Trucchi, M.; Jannelli, E.; Mosconi, M.; Gastaldi, G.; et al. The Role of Low-Level Laser Therapy in Bone Healing: Systematic Review. *Int. J. Mol. Sci.* **2023**, *24*, 7094. [CrossRef] [PubMed]
41. Zecha, J.A.; Raber-Durlacher, J.E.; Nair, R.G.; Epstein, J.B.; Elad, S.; Hamblin, M.R.; Barasch, A.; Migliorati, C.A.; Milstein, D.M.; Genot, M.T.; et al. Low-level laser therapy/photobiomodulation in the management of side effects of chemoradiation therapy in head and neck cancer: Part 2: Proposed applications and treatment protocols. *Support. Care Cancer* **2016**, *24*, 2793–2805. [CrossRef]
42. Cardoso Bezerra, S.J.; Fioranelli Vieira, G.; Eduardo Cde, P.; de Freitas, P.M.; Aranha, A.C. Laser Phototherapy (660 nm) Can Be Beneficial for Reducing Gingival Inflammation in Prosthodontics. *Case Rep. Dent.* **2015**, *2015*, 132656. [CrossRef]
43. Najeeb, S.; Khurshid, Z.; Zafar, M.S.; Ajlal, S. Applications of Light Amplification by Stimulated Emission of Radiation (Lasers) for Restorative Dentistry. *Med. Princ. Pract.* **2016**, *25*, 201–211. [CrossRef] [PubMed]
44. Fernandes, A.P.; Junqueira Mde, A.; Marques, N.C.; Machado, M.A.; Santos, C.F.; Oliveira, T.M.; Sakai, V.T. Effects of low-level laser therapy on stem cells from human exfoliated deciduous teeth. *J. Appl. Oral. Sci.* **2016**, *24*, 332–337. [CrossRef] [PubMed]
45. Rahmati, A.; Abbasi, R.; Najafi, R.; Rezaei-soufi, L.; Karkehabadi, H. Effect of diode low level laser and red light emitting diode irradiation on cell proliferation and osteogenic/odontogenic differentiation of stem cells from the apical papilla. *BMC Oral. Health* **2022**, *22*, 543. [CrossRef]
46. Rathod, A.; Jaiswal, P.; Bajaj, P.; Kale, B.; Masurkar, D. Implementation of Low-Level Laser Therapy in Dentistry: A Review. *Cureus* **2022**, *14*, e28799. [CrossRef]
47. Meesuk, L.; Suwanprateeb, J.; Thammarakcharoen, F.; Tantrawatpan, C.; Kheolamai, P.; Palang, I.; Tantikanlayaporn, D.; Manochantr, S. Osteogenic differentiation and proliferation potentials of human bone marrow and umbilical cord-derived mesenchymal stem cells on the 3D-printed hydroxyapatite scaffolds. *Sci. Rep.* **2022**, *12*, 19509. [CrossRef]
48. Widholz, B.; Tsitlakidis, S.; Reible, B.; Moghaddam, A.; Westhauser, F. Pooling of Patient-Derived Mesenchymal Stromal Cells Reduces Inter-Individual Confounder-Associated Variation without Negative Impact on Cell Viability, Proliferation and Osteogenic Differentiation. *Cells* **2019**, *8*, 633. [CrossRef]
49. Du, L.; Yang, P.; Ge, S. Isolation and characterization of human gingiva-derived mesenchymal stem cells using limiting dilution method. *J. Dent. Sci.* **2016**, *11*, 304–314. [CrossRef] [PubMed]
50. Naji, A.; Eitoku, M.; Favier, B.; Deschaseaux, F.; Rouas-Freiss, N.; Suganuma, N. Biological functions of mesenchymal stem cells and clinical implications. *Cell. Mol. Life Sci.* **2019**, *76*, 3323–3348. [CrossRef] [PubMed]
51. Saber, S.; Raafat, S.; Elashiry, M.; El-Banna, A.; Schäfer, E. Effect of Different Sealers on the Cytocompatibility and Osteogenic Potential of Human Periodontal Ligament Stem Cells: An In Vitro Study. *J. Clin. Med.* **2023**, *12*, 2344. [CrossRef] [PubMed]

52. Ballini, A.; Mastrangelo, F.; Gastaldi, G.; Tettamanti, L.; Bukvic, N.; Cantore, S.; Cocco, T.; Saini, R.; Desiate, A.; Gherlone, E.; et al. Osteogenic differentiation and gene expression of dental pulp stem cells under low-level laser irradiation: A good promise for tissue engineering. *J. Biol. Regul. Homeost. Agents* **2015**, *29*, 813–822.
53. Saghaei Bagheri, H.; Rasta, S.H.; Mohammadi, S.M.; Rahimi, A.A.R.; Movassaghpour, A.; Nozad Charoudeh, H. Low-Level Laser Irradiation Modulated Viability of Normal and Tumor Human Lymphocytes In Vitro. *J. Lasers Med. Sci.* **2020**, *11*, 174–180. [CrossRef] [PubMed]
54. Rodríguez-Lozano, F.J.; López-García, S.; García-Bernal, D.; Sanz, J.L.; Lozano, A.; Pecci-Lloret, M.P.; Melo, M.; López-Ginés, C.; Forner, L. Cytocompatibility and bioactive properties of the new dual-curing resin-modified calcium silicate-based material for vital pulp therapy. *Clin. Oral. Investig.* **2021**, *25*, 5009–5024. [CrossRef] [PubMed]
55. Liu, Z.; Yang, S.; Li, X.; Wang, S.; Zhang, T.; Huo, N.; Duan, R.; Shi, Q.; Zhang, J.; Xu, J. Local transplantation of GMSC-derived exosomes to promote vascularized diabetic wound healing by regulating the Wnt/β-catenin pathways. *Nanoscale Adv.* **2023**, *5*, 916–926. [CrossRef]
56. Mushahary, D.; Spittler, A.; Kasper, C.; Weber, V.; Charwat, V. Isolation, cultivation, and characterization of human mesenchymal stem cells. *Cytom. Part A* **2018**, *93*, 19–31. [CrossRef]
57. Gutiérrez, D.; Rouabhia, M.; Ortiz, J.; Gaviria, D.; Alfonso, C.; Muñoz, A.; Inostroza, C. Low-Level Laser Irradiation Promotes Proliferation and Differentiation on *Apical papilla* Stem Cells. *J. Lasers Med. Sci.* **2021**, *12*, e75. [CrossRef]
58. Tam, S.Y.; Tam, V.C.W.; Ramkumar, S.; Khaw, M.L.; Law, H.K.W.; Lee, S.W.Y. Review on the Cellular Mechanisms of Low-Level Laser Therapy Use in Oncology. *Front. Oncol.* **2020**, *10*, 1255. [CrossRef]
59. Gao, X.; Chen, T.; Xing, D.; Wang, F.; Pei, Y.; Wei, X. Single cell analysis of PKC activation during proliferation and apoptosis induced by laser irradiation. *J. Cell. Physiol.* **2006**, *206*, 441–448. [CrossRef]
60. Rola, P.; Włodarczak, S.; Lesiak, M.; Doroszko, A.; Włodarczak, A. Changes in Cell Biology under the Influence of Low-Level Laser Therapy. *Photonics* **2022**, *9*, 502. [CrossRef]
61. Cios, A.; Ciepielak, M.; Szymański, Ł.; Lewicka, A.; Cierniak, S.; Stankiewicz, W.; Mendrycka, M.; Lewicki, S. Effect of Different Wavelengths of Laser Irradiation on the Skin Cells. *Int. J. Mol. Sci.* **2021**, *22*, 2437. [CrossRef] [PubMed]
62. Li, B.; Ouchi, T.; Cao, Y.; Zhao, Z.; Men, Y. Dental-Derived Mesenchymal Stem Cells: State of the Art. *Front. Cell. Dev. Biol.* **2021**, *9*, 654559. [CrossRef] [PubMed]
63. Amid, R.; Kadkhodazadeh, M.; Gilvari Sarshari, M.; Parhizkar, A.; Mojahedi, M. Effects of Two Protocols of Low-Level Laser Therapy on the Proliferation and Differentiation of Human Dental Pulp Stem Cells on Sandblasted Titanium Discs: An In Vitro Study. *J. Lasers Med. Sci.* **2022**, *13*, e1. [CrossRef] [PubMed]
64. Borzabadi-Farahani, A. Effect of low-level laser irradiation on proliferation of human dental mesenchymal stem cells; a systemic review. *J. Photochem. Photobiol. B: Biol.* **2016**, *162*, 577–582. [CrossRef]
65. Chen, J.; Sang, Y.; Li, J.; Zhao, T.; Liu, B.; Xie, S.; Sun, W. Low-level controllable blue LEDs irradiation enhances human dental pulp stem cells osteogenic differentiation via transient receptor potential vanilloid 1. *J. Photochem. Photobiol. B: Biol.* **2022**, *233*, 112472. [CrossRef]
66. Sharma, S.K.; Sardana, S.; Hamblin, M.R. Role of opsins and light or heat activated transient receptor potential ion channels in the mechanisms of photobiomodulation and infrared therapy. *J. Photochem. Photobiol.* **2023**, *13*, 100160. [CrossRef]
67. Komori, T. Regulation of Proliferation, Differentiation and Functions of Osteoblasts by Runx2. *Int. J. Mol. Sci.* **2019**, *20*, 1694. [CrossRef]
68. Komori, T. Functions of Osteocalcin in Bone, Pancreas, Testis, and Muscle. *Int. J. Mol. Sci.* **2020**, *21*, 7513. [CrossRef]
69. Marques, L.; Holgado, L.A.; Francischone, L.A.; Ximenez, J.P.; Okamoto, R.; Kinoshita, A. New LLLT protocol to speed up the bone healing process-histometric and immunohistochemical analysis in rat calvarial bone defect. *Lasers Med. Sci.* **2015**, *30*, 1225–1230. [CrossRef]
70. de Oliveira, G.J.P.L.; Aroni, M.A.T.; Pinotti, F.E.; Marcantonio, E.; Marcantonio, R.A.C. Low-level laser therapy (LLLT) in sites grafted with osteoconductive bone substitutes improves osseointegration. *Lasers Med. Sci.* **2020**, *35*, 1519–1529. [CrossRef]
71. Yaralı Çevik, Z.B.; Karaman, O.; Topaloğlu, N. Photobiomodulation therapy at red and near-infrared wavelengths for osteogenic differentiation in the scaffold-free microtissues. *J. Photochem. Photobiol. B: Biol.* **2023**, *238*, 112615. [CrossRef] [PubMed]
72. Chen, H.; Chen, Y.; Wang, Y.; Li, T.; Liu, P.; Han, Z.; Sun, Y.; Liu, Y. Study on the Osteogenic Differentiation of hUCMSCs in Silk Fibroin/Polycaprolactone Membrane Under the Intervention of Photobiomodulation. *IEEE J. Sel. Top. Quantum Electron.* **2023**, *29*, 3200367. [CrossRef]
73. Figueredo, C.A.; Abdelhay, N.; Ganatra, S.; Gibson, M.P. The role of Dentin Sialophosphoprotein (DSPP) in craniofacial development. *J. Oral. Biol. Craniofac Res.* **2022**, *12*, 673–678. [CrossRef] [PubMed]
74. Dussold, C.; Gerber, C.; White, S.; Wang, X.; Qi, L.; Francis, C.; Capella, M.; Courbon, G.; Wang, J.; Li, C.; et al. DMP1 prevents osteocyte alterations, FGF23 elevation and left ventricular hypertrophy in mice with chronic kidney disease. *Bone Res.* **2019**, *7*, 12. [CrossRef]
75. Butera, A.; Pascadopoli, M.; Pellegrini, M.; Gallo, S.; Zampetti, P.; Scribante, A. Oral Microbiota in Patients with Peri-Implant Disease: A Narrative Review. *Appl. Sci.* **2022**, *12*, 3250. [CrossRef]

76. Vale, G.C.; Mayer, M.P.A. Effect of probiotic *Lactobacillus rhamnosus* by-products on gingival epithelial cells challenged with Porphyromonas gingivalis. *Arch. Oral. Biol.* **2021**, *128*, 105174. [CrossRef]
77. Butera, A.; Pascadopoli, M.; Pellegrini, M.; Gallo, S.; Zampetti, P.; Cuggia, G.; Scribante, A. Domiciliary Use of Chlorhexidine vs. Postbiotic Gels in Patients with Peri-Implant Mucositis: A Split-Mouth Randomized Clinical Trial. *Appl. Sci.* **2022**, *12*, 2800. [CrossRef]