Journal of Dental Sciences 19 (2024) 1452–1460

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jds.com

Original Article

The effect of low-level laser therapy on osteoclast differentiation: Clinical implications for tooth movement and bone density

Chun-Yi Huang [a,b], Huynh Hoai Thuong Le [c], Hsiao-Chi Tsai [d,e], Chih-Hsin Tang [c,e,f**], Jian-Hong Yu [a,b*]

[a] School of Dentistry, College of Dentistry, China Medical University, Taichung, Taiwan
[b] Department of Orthodontics, China Medical University Hospital Medical Center, Taichung, Taiwan
[c] Graduate Institute of Biomedical Sciences, College of Medicine, China Medical University, Taichung, Taiwan
[d] Division of Hematology and Oncology, Department of Internal Medicine, China Medical University Hospital, Taichung, Taiwan
[e] Department of Pharmacology, School of Medicine, College of Medicine, China Medical University, Taichung, Taiwan
[f] Department of Medical Laboratory Science and Biotechnology, College of Medical and Health Science, Asia University, Taichung, Taiwan

Received 16 March 2024; Final revision received 25 March 2024
Available online 30 March 2024

KEYWORDS
Bone density;
Low level laser therapy;
Orthodontic tooth movement;
Osteoclast cell

Abstract *Background/purpose:* Osteoclast differentiation is crucial for orchestrating both tooth movement and the maintenance of bone density. Therefore, the current study sought to explore the impact of low-level laser therapy (LLLT) on osteoclast differentiation, functional gene expression, molecular signaling pathways, and orthodontic tooth movement in clinical settings.
Materials and methods: The RAW 264.7 cell line served as the precursor for osteoclasts, and these cells underwent irradiation using a 808-nm LLLT. Osteoclast differentiation was assessed through tartrate-resistant acid phosphatase (TRAP) staining. Functional gene expression levels were evaluated using real-time quantitative polymerase chain reaction (RT-qPCR) while signaling molecules were examined through Western blot analysis. In the clinical study, 12 participants were enrolled. Their tooth movement was monitored using a TRIOS desktop scanner.

* Corresponding author. School of Dentistry, College of Dentistry, China Medical University, No. 91, Xueshi Road, North District, Taichung, 40402, Taiwan.
** Corresponding author. Graduate Institute of Biomedical Sciences, College of Medicine, China Medical University, No. 91, Xueshi Road, North District, Taichung, 40402, Taiwan.
E-mail addresses: chtang@mail.cmu.edu.tw (C.-H. Tang), kenkoyu@hotmail.com, kenkoyu@mail.cmu.edu.tw (J.-H. Yu).

https://doi.org/10.1016/j.jds.2024.03.023

Bone density measurements were conducted using Mimics software, which processed cone-beam computed tomography (CBCT) images exported in Digital Imaging and Communications in Medicine (DICOM) format.

Results: We found that LLLT effectively promoted receptor activator of nuclear factor-κB ligand (RANKL)-dependent osteoclast differentiation and the expression of osteoclast functional genes, including matrix metallopeptidase 9 (MMP9), nuclear factor of activated T-cells cytoplasmic 1(NFATc1), tartrate-resistant acid phosphatase (TRAP) and cathepsin K (CTSK) in RAW264.7 cells. Clinically, the cumulative tooth movement over 90 days was significantly higher in the laser group than in the control group.

Conclusion: Our research demonstrates that LLLT not only significantly promotes osteoclast differentiation but is also a valuable adjunct in orthodontic therapy.

Introduction

The health of teeth is vital for eating and speaking and significantly affecting well-being.[1] Poor alignment usually causes discomfort and self-esteem issues and necessitates orthodontic correction.[2,3] However, this process often lasts 2–3 years and can introduce complications like tooth decay and gum deterioration.[4,5] Therefore, exploring non-invasive methods to shorten treatment time and enhance outcomes is crucial.[6,7] A key process in shortening treatment time is increasing orthodontic tooth movement, which depends on osteoclast differentiation.[8,9] Osteoclast differentiation plays a pivotal role in tooth movement and the regulation of bone density.[10,11] During orthodontic tooth movement, the force applied to a tooth creates pressure in the surrounding alveolar bone, and osteoclasts are responsible for resorbing bone in the path of the moving tooth.[10,11]

Receptor activator of nuclear factor kappa-B ligand (RANKL) plays an essential role in the differentiation, activation, and survival of osteoclasts, the cells accountable for bone resorption.[12] When RANKL binds to its receptor, RANK, on osteoclast precursors, it triggers downstream signaling pathways that enhance osteoclast differentiation and activity.[13] In the context of orthodontic tooth movement, applying force to teeth increases RANKL expression, thereby stimulating osteoclastogenesis and bone resorption on the compression side of the tooth.[12,13] Osteoclast differentiation was divided into two main pathways: RANKL-independent and RANKL-dependent.[14] The RANKL-independent pathway can be activated by various factors, including inflammatory cytokines and hormones, which influence osteoclastogenesis through alternative signaling pathways, allowing for osteoclast differentiation under conditions without RANKL.[15,16] Conversely, the RANKL-dependent pathway involves a complex orchestration of specific proteins critical for bone resorption. These include matrix metalloproteinase 9 (MMP9), which aids in remodeling the extracellular matrix, enabling osteoclasts to efficiently resorb bone;[17] tartrate-resistant acid phosphatase (TRAP), which participates in the dephosphorylation processes within resorption sites;[18] cathepsin K (CTSK), essential for degrading the organic matrix of bone;[19,20] and nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1), a key transcription factor that upregulates genes crucial for osteoclast differentiation, including those encoding MMP9, TRAP, and CTSK.[15] These proteins play pivotal roles in facilitating the efficient resorption of bone, highlighting the sophisticated regulatory mechanisms involved in osteoclast differentiation and activity, especially in processes such as orthodontic tooth movement.[21]

Low-level laser therapy (LLLT) represents an innovative, non-invasive approach that can trigger cell proliferation without causing tissue damage by providing energy to cells.[22] LLLT has been considered a potentially useful method for accelerating orthodontic tooth movement through its influence on cellular activities, including osteoclast differentiation.[22] Previous research has demonstrated LLLT's potential effectiveness in expediting orthodontic tooth movement in rats.[23] However, the detailed mechanisms of LLLT in bone resorption and osteoclast differentiation remain unclear, and there are limitations to its use in human clinicals, including the need to specify laser wavelength, power, and frequency.[24] Consequently, the objectives of this research were to assess the underlying mechanisms and efficacy of LLLT in orthodontic tooth movement and to identify the most effective laser parameters.

Material and methods

Cell culture

In this study, the RAW 264.7 cell line was utilized as osteoclast precursor cells. This cell was purchased from the American Type Culture Collection (ATCC) (Manassas, VA, USA). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco-Life Technologies, Gaithersburg, MD, USA), supplemented with 10% fetal bovine serum (FBS) (Sigma-Aldrich, St. Louis, MO, USA), 100 μg/mL of penicillin, and 100 μg/mL of streptomycin (Thermo Scientific, Waltham, MA, USA). The culture conditions were maintained at 37 °C in an atmosphere containing 5% CO_2.

Laser parameters

In this experiment, we utilized the "TRANS" Portable Laser Phototherapy Device (model TI-816-3E2) from Transverse Industries Co., based in Taipei, Taiwan. This device features a continuous output wavelength of 808 $\pm$ 10 nm and an output power of 300 mW. It is equipped with an LCD screen display and has dimensions of approximately L230$\pm$5 mm, W30$\pm$5 mm, and H27$\pm$5 mm.

Low-laser level treatment of macrophage cell

RAW 264.7 cells were initially seeded in 12-well plates at a concentration of 5000 cells per well and allowed to adhere for 24 h. Following this, the cells were cultured in the presence of 50 ng/ml RANKL (Abcam Limited, Boston, MA, USA) and subsequently stimulated with a wavelength of 808 nm and a power of 150 mW at an energy density of 4 J/cm2 for 5 days. Cells that were not treated with laser irradiation served as the sham-irradiated control group. After the treatment, cells were harvested for TRAP staining, reverse transcription-quantitative polymerase chain reaction (RT-qPCR), and Western blot analysis.

Staining

To assess osteoclast differentiation, we performed TRAP staining. The cells were fixed in 10% formalin for 10 min, followed by staining to detect TRAP activity using a commercially available TRAP staining kit (Sigma-Aldrich), as per the manufacturer's guidelines. Cells that were TRAP-positive and multinucleated, containing more than three nuclei, were classified as osteoclasts. These differentiated cells were then examined under a microscope and quantified using ImageJ software.

Reverse transcription-quantitative polymerase chain reaction analysis

Total mRNA was isolated using the Total RNA Miniprep Purification Kit (GMBiolab, Taichung, Taiwan), and reverse transcription was performed using the MMLV Reverse Transcription Kit (Invitrogen, Taipei, Taiwan) following the manufacturer's instructions. The PCR replication was performed using specific primer sets for detect MMP9, NFATc1, TRAP and CTSK expression. The PCR program included an initial denaturation at 95 °C for 10 min, followed by 35 cycles of 94 °C:1 min, 60 °C: 1min, 72 °C: 2 min. The glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was used as an internal control to ensure sample loading consistency and mRNA integrity.

The primer sequences used in this study as follows:

MMP9: GGACCCGAAGCGGACATTG and CGTCGTCGAAATG GGCATCT.

NFATc1: GACCCGGAGTTCGACTTCG and TGACACTAGG GGACACATAACTG.

TRAP: ATGGGCGCTGACTTCATCAT and GGTCTCCTGG AACCTCTTGT.

CTSK: AGTAGCCACGCTTCCTATCC and CCATGGGTAGC AGCAGAAAC.

GAPDH: AAGCCCATCACCATCTTCCAG and AGGGGCCATC CACAGTCTTCT.

Western blot

Proteins were separated using a 10% SDS-PAGE gel and subsequently electroblotted onto polyvinylidene difluoride membranes. The membranes were blocked with 1 $\times$ Tris-buffered saline containing 0.1% Tween 20 and 2% bovine serum albumin (BSA) for 1 h at room temperature. This was followed by incubation with the rabbit anti-human antibodies against p-p65 or p65 (Santa Cruz Biotechnology, Dallas, TX, USA) at a 1:1000 concentration overnight at 4 °C, and then with horseradish peroxidase-conjugated donkey anti-rabbit secondary antibody (Santa Cruz Biotechnology) at a 1:3000 concentration for 1 h at room temperature. The membranes were developed using enhanced chemiluminescence, imaged with a LAS-4000 Luminescent Image Analyzer (Fujifilm, Tokyo, Japan), and the results were analyzed using ImageJ software.

Clinical subjects

This study recruited 12 subjects and all subjects provided informed consent before study. The inclusion criteria were: (a) age over 18 years old; (b) good oral hygiene; (c) the requirement for bilateral canine retraction into the extracted premolar sites; (d) no significant medical history; (e) no history of mental or neurological disorders. The exclusion criteria included: long-term medical treatment that could interfere with bone metabolism, premature loss of the first molar leading to the absorption of more than half of the alveolar ridge width, pregnancy or lactation, previous malignancy, and mental illness. The study was approved by the China Medical University Hospital Institutional Review Board (CMUH110-REC2-138) and conducted in compliance with national legislation and the Declaration of Helsinki guidelines.

Clinical data collection

Each subject underwent monthly visits during which their tooth movement was meticulously tracked using a TRIOS desktop scanner (TRIOS3, 3shape A/S, Copenhagen, Denmark). To ensure minimal errors, all intraoral scans were consistently conducted by the same skilled orthodontist. These scans yielded 3D digital models of the subjects' dentition, which were then stored as STL files. Subsequent analysis of these files was carried out using the Blender software (Blender, Blender Institute, Netherlands), facilitating both convenient and precise measurement of the distances involved in tooth movement. Additionally, cone-beam computed tomography (CBCT) was employed throughout the experiment to provide detailed insights into the condition of both teeth and alveolar bone.

Alveolar bone density was assessed using Mimics software, which analyzed CBCT files exported in DICOM format. This analysis involved measuring the density changes in the alveolar bone surrounding the teeth. The software distinguished the alveolar bone from the teeth by identifying variations in grayscale values, correlating these values with bone density for thorough analysis and comparison.

Additionally, X-ray images taken before and after orthodontic treatment were utilized. These images facilitated the evaluation of changes in alveolar bone density resulting from the application of low-energy laser treatment.

Statistical analysis

Data were analyzed using SAS software (SAS, Inc., Cary, NC, USA). Statistical significance was assessed between two groups using Student's *t* test. Results were presented as the mean ± standard deviation. $P < 0.05$ was considered to indicate statistical significance.

Results

Laser promotes osteoclast differentiation

Osteoclast differentiation can occur through two distinct pathways: RANKL-dependent and RANKL-independent.[14] Our study initially sought to investigate the role of LLLT in facilitating osteoclast differentiation and to determine whether it follows the RANKL-dependent or independent pathway. We cultured osteoclast precursor cells with and without RANKL (50 ng/ml) and subsequently applied LLLT for 5 days. As depicted in Fig. 1A and B, we observed a dose-dependent increase in osteoclast formation in the group treated with both RANKL and LLLT. In contrast, the group treated only with LLLT exhibited no mature, multi-nucleated osteoclast cells (Fig. 1B). We also noted that the numbers of osteoclast cells at 60 and 120 s were similar (Fig. 1B), indicating that the stimulation times of 60 and 120 s have a similar effect. Therefore, we opted for 60 s in the follow-up experiment. These results indicate that LLLT primarily stimulates osteoclast differentiation through a RANKL-dependent mechanism.

Laser promotes osteoclast functional genes

To characterize the functional aspects of osteoclast differentiation promoted by LLLT, we investigated the expression of NFATc1 (the master transcriptional factor), MMP9 and TRAP (the osteoclast phenotype molecular), as well as CTSK (the bone resorption-related molecular). These molecules are widely acknowledged as specific osteoclast markers.[19,25] We cultured osteoclast precursor cells with RANKL (50 ng/ml) and subsequently applied LLLT for 5 days. In line with our expectations, we observed an upregulation level of mRNA in *NFATc1*, *MMP9*, *TRAP*, and *CTSK* following LLLT exposure (Fig. 2A-D). These data suggests that LLLT not only facilitates osteoclast differentiation but also augments their functional activity, thereby contributing to the process of osteoclastogenesis.

Laser-mediated osteoclastogenesis

The functional importance of p65 nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathways in osteoclastogenesis has been extensively studied[26–28] which lead to the expression of downstream master transcriptional factors, such as NFATc1.[29] We therefore examined the activation of p65 in RAW264.7 cells treated with LLLT by Western blot. Our findings revealed that stimulation through LLLT significantly induced nearly a 2-fold increase in p65 phosphorylation (Fig. 3A and B). These observations suggest that LLLT promotes osteoclastogenesis primarily by activating the NF-κB signaling pathway.

Laser promotes cumulative tooth movement in clinical

We then sought to apply the observations from cell experiments to clinical practice. To this end, we recruited 12 subjects who underwent LLLT irradiation, and meticulously documented the outcomes using an intraoral digital scanner at each monthly follow-up visit. The data revealed that after three months of LLLT treatment, canine retraction on the LLLT-treated side measured 4.06 ± 1.03 mm, compared to 2.48 ± 0.88 mm on the control side (Fig. 4A and B). These findings demonstrate that LLLT significantly enhances tooth retraction in a clinical setting.

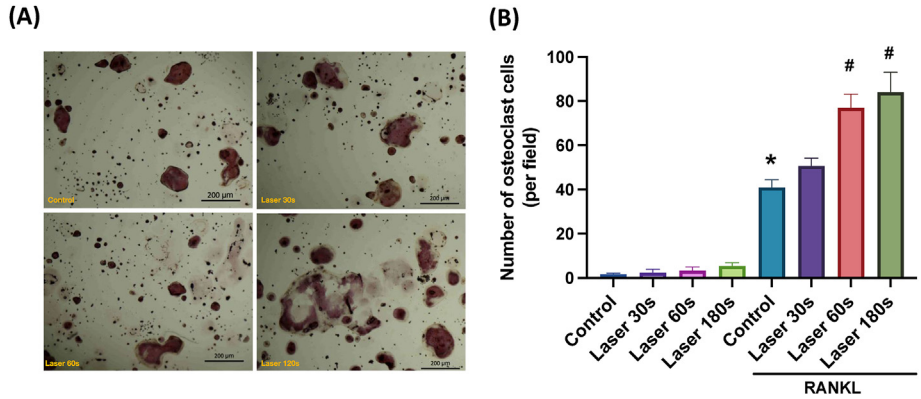

Figure 1 Low-level laser therapy promotes RANKL-dependent osteoclast differentiation. RAW 264.7 cells underwent low-level laser therapy for varying durations (0, 30s, 60s, or 120s) and were co-cultured with or without RANKL (50 ng/mL) for 5 days to assess osteoclast differentiation. (A) Mature osteoclasts were identified using the TRAP assay, and (B) the count of TRAP-positive osteoclasts was quantified (scale bars: 200 μm). All data are presented as mean ± standard deviation (SD) from triplicate experiments. $^{*}P < 0.05$ indicates a statistically significant difference compared to the control group; $^{\#}P < 0.05$ compared to the RANKL-treated control group.

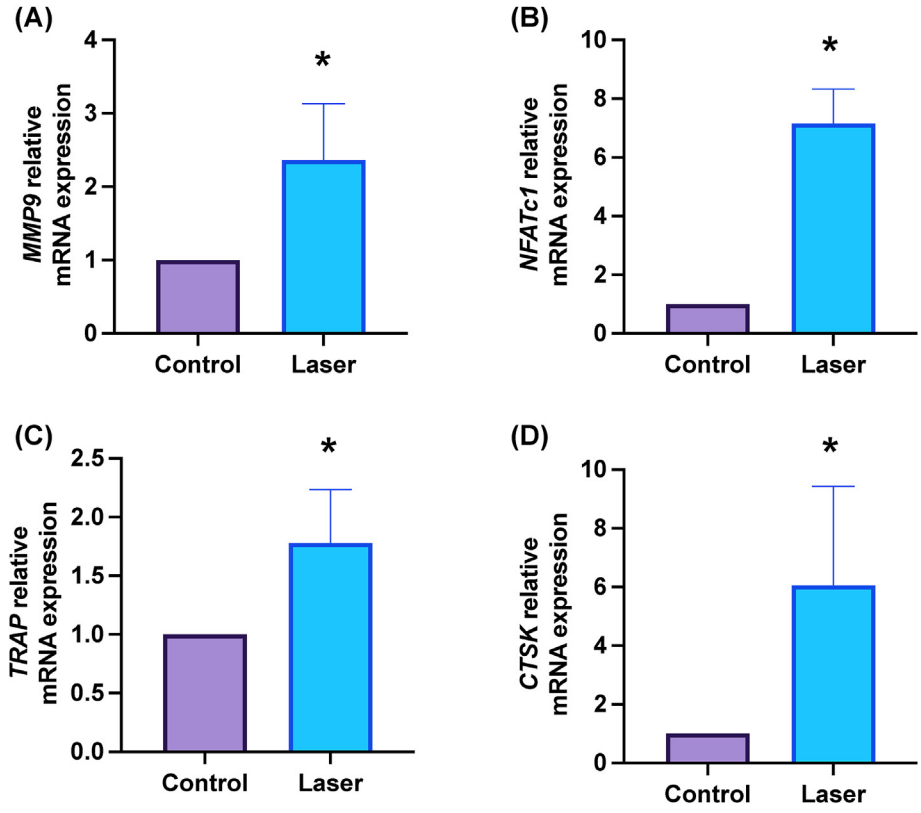

Figure 2 Low-level laser therapy promotes osteoclast functional genes in RAW264.7 cells. RAW 264.7 cells were subjected to laser irradiation for 120 s and then co-cultured with RANKL (50 ng/mL) for 5 days to evaluate osteoclast differentiation. The osteoclast phenotype genes (A) *MMP9*, (B) *NFATc1*, and (C) *TRAP*, as well as (D) bone resorption-related gene *CTSK* mRNA expressions were detected by RT-qPCR. All data are presented as mean ± standard deviation (SD) from triplicate experiments. **P* < 0.05 indicates a statistically significant difference compared to the control group.

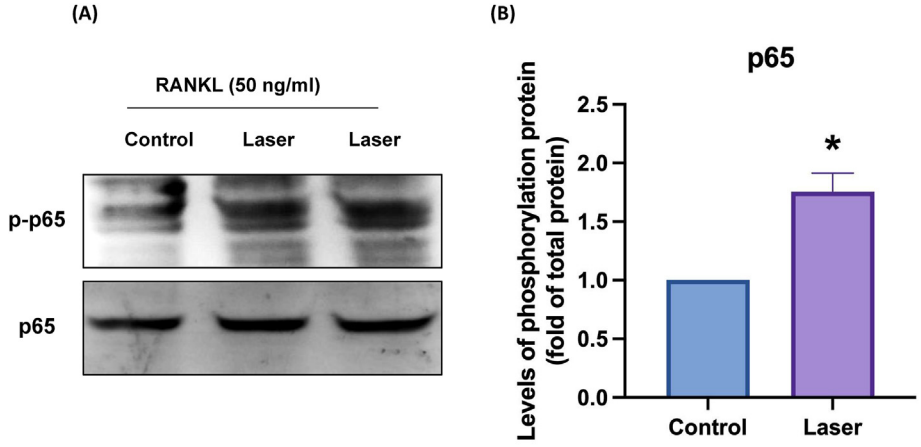

Figure 3 NF-κB was involved in Low-level laser therapy -mediated osteoclastogenesis. RAW 264.7 cells were subjected to laser irradiation for 120 s and then co-cultured with RANKL (50 ng/mL) for 5 days to assess osteoclast differentiation. (A) The phosphorylation of p65 NF-κB was evaluated by Western blot. (B) The band intensities were quantified using ImageJ software. All data are presented as mean ± standard deviation (SD) from triplicate experiments. **P* < 0.05 indicates a statistically significant difference compared to the control group.

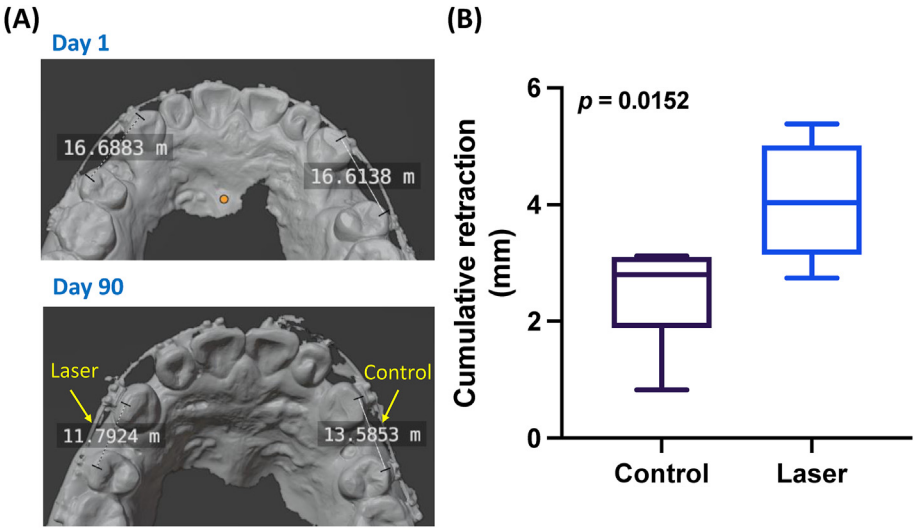

Figure 4 Low-level laser therapy promotes Cumulative tooth movement in clinical. (A) The distance between the canine and the second premolar was measured on both day 1 and day 90 to assess the retraction velocity with and without LLLT treatment. (B) The cumulative retraction of the canines. *$P < 0.05$ indicates a statistically significant difference compared to the control group.

Laser increased bone density loss and tooth movement in clinical

Alveolar bone density around the teeth is a critical indicator for assessing improvements in the speed of tooth movement.[30] In this study, we measured the bone density of each tooth at three specific regions (cervical, intermediate, and apical) on day 1 and day 90 to determine the percentage of bone density loss. Our results showed a more pronounced decrease in bone density around the teeth subjected to LLLT, which facilitates tooth movement (Fig. 5A). Additionally, the velocity of tooth movement was monitored monthly. By the 3rd month, the distance of tooth movement on the LLLT-treated side was measured at 1.96 ± 0.60 mm, compared to 1.17 ± 0.27 mm on the control side (Fig. 5B), indicating that the LLLT group experienced significantly greater distalization than the control group. These findings demonstrate that LLLT treatment offers clinical advantages, especially in shortening the duration of orthodontic treatments.

Discussion

Poor dental alignment, often associated with a reduced quality of life, can lead to discomfort and difficulties in eating, thus emphasizing the importance of orthodontic treatments for correction.[2,3] However, these treatments typically involve a prolonged process. As a result, there is a pressing need to investigate noninvasive approaches that can shorten the duration of orthodontic treatments, thereby reducing risks and improving patient outcomes.[4] In our study, we discovered that LLLT significantly promotes

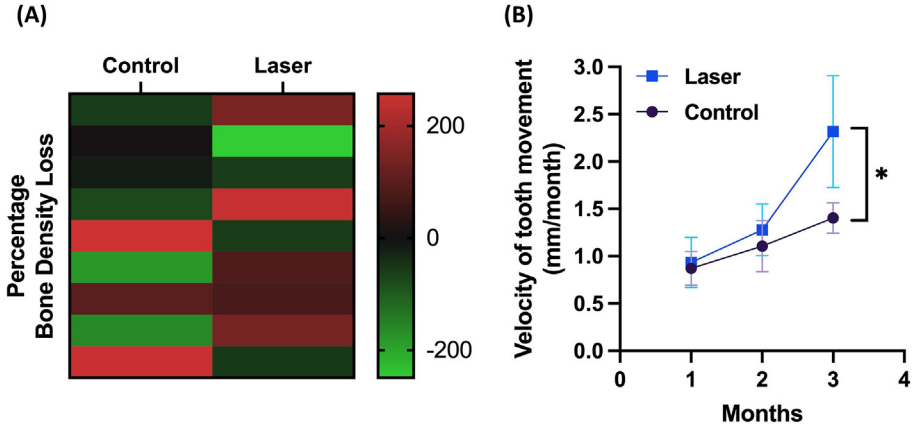

Figure 5 Low-level laser therapy increased bone density loss and tooth movement in clinical. (A) Bone density was measured using Mimics 3D analysis software on day 1 and day 90. The percentage of bone density loss was calculated using the formula: bone density on day 90 divided by bone density on day 1. (B) The distance of tooth movement was monitored monthly. To calculate the velocity of tooth movement, we subtracted the distance measured in the previous month from the measurement of the subsequent month. *$P < 0.05$ indicates a statistically significant difference compared to the control group.

the formation of osteoclasts, crucial for bone resorption, both in cellular experiments and clinical settings. These findings underscore the potential of LLLT as an effective supplement in orthodontic therapy, offering a promising avenue to enhance treatment efficiency and patient experience. Osteoclasts are pivotal in initiating tooth movement.[8] They are specialized cells that dissolve bone, creating the necessary space for tooth movement.[8] Osteoclast differentiation can occur through two distinct pathways: RANKL-dependent and RANKL-independent.[14] Our findings indicate that, in the absence of RANKL, LLLT does not initiate osteoclast differentiation, suggesting that LLLT cannot substitute for RANKL in this process. Recent research underscores that LLLT enhances RANKL release during the orthodontic movement of teeth.[31–33] In our study, we observed that LLLT enhances osteoclast differentiation more effectively in conjunction with RANKL than RANKL alone. Notably, an irradiation duration of just 60 s proved to be sufficient.

The pathway of osteoclast differentiation is a complex process, and specific proteins (including MMP9, NFATc1, TRAP, and CTSK) playing crucial roles in various functions of osteoclasts differentiation and bone remodeling.[25,34] Earlier investigations have revealed that the application of RANKL and LLLT irradiation notably enhances the expression of genes NFATc1 and TRAP in RAW 264.7 cells.[35] Furthermore, LLLT combined with platelet-rich fibrin has been shown to facilitate the formation of mineralized nodules in the apical papilla.[36] Additionally, while the expressions of RANKL and macrophage colony-stimulating factor were upregulated, osteoprotegerin (OPG) levels remain largely unchanged by LLLT exposure.[37] These findings align with the observations in our research. Our study documented an upregulation in MMP9, NFATc1, TRAP, and CTSK expression, which is linked to bone resorption, after LLLT exposure. These results suggest that LLLT may potentiate osteoclast differentiation and bone resorption through the modulation of key gene expressions, offering a potential therapeutic avenue for conditions characterized by bone degradation or abnormal bone remodeling.

NFATc1 acts as a master modulator for osteoclast activation and maturation, the activation of NFATc1 is dependent on the up-stream molecular including p38, JNK, ERK, and p65.[38,39] It has previously been reported that low-level Er:YAG irradiation induces the phosphorylation of extracellular ERK without activating JNK.[40] Another study reported that low-intensity pulsed ultrasound disrupts the phosphorylation of ERK, subsequently inhibiting the activation of osteoclastic transcription factors, such as c-Fos and NFATc1.[41] Song et al. have shown that LLLT promotes the transfer of p65 from the cytoplasm to the nucleus.[19] These studies indicated that p65 maybe the most important molecular of LLLT-related osteoclast differentiation. These results are in line with our findings, which revealed that LLLT significantly induced nearly a 2-fold increase in p65. This suggests that LLLT promotes osteoclastogenesis not only by stimulating osteoclast differentiation but also by enhancing their functional activity, primarily through the activation of the NF-κB signaling pathway.

LLLT has been utilized in various medical fields for over a decade, offering benefits such as pain and inflammation reduction, and enhanced wound healing.[42] With its numerous adjustable parameters, LLLT can produce diverse effects.[43] Notably, low-dosage LLLT is considered safe for clinical use and often yields more accurate results than traditional treatments.[43] Several clinical trials have explored the effectiveness of LLLT in orthodontic treatments. Cruz et al. conducted a self-controlled study and discovered that canine retraction was significantly faster following LLL irradiation (780 nm, 20 mW, 5 J/cm2), with a 34% increase in velocity over 60 days in the laser group compared to the control group.[44,45] In a separate study, Zheng and Yang reported that the cumulative tooth movement over 28 days was markedly greater in the laser group, accompanied by decreases in OPG levels and increases in IL-1β and RANKL levels in gingival crevicular fluid (GCF) samples from the experimental sides.[46] In alignment with these studies, our research indicates that LLLT can significantly enhance cumulative tooth movement and increase both bone density loss and tooth movement. These findings position LLLT as a valuable and effective tool in orthodontic treatments.

In conclusion, our study has demonstrated that LLLT significantly enhances the formation of osteoclasts, which are vital for bone resorption. LLLT effectively promotes RANKL-dependent osteoclast differentiation. It has been observed to stimulate the expression of osteoclast functional genes, including MMP9, NFATc1, TRAP, and CTSK in RAW264.7 cells (Fig. 6). Additionally, the involvement of

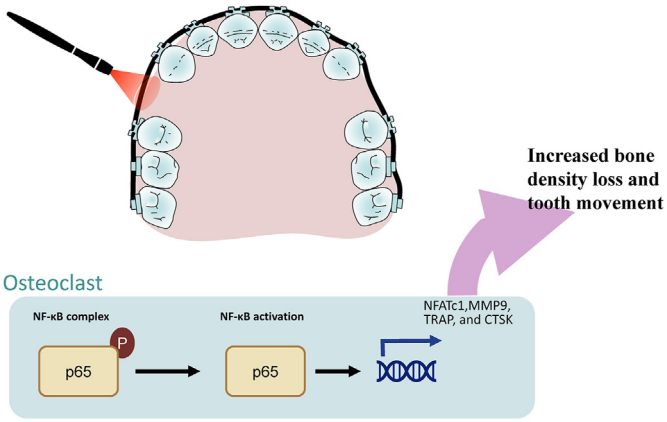

Figure 6 The mechanism of Low-level laser therapy in enhancing osteoclast differentiation and dental treatment outcomes.

the NF-κB pathway in LLLT-mediated osteoclastogenesis has been established. Clinically, LLLT has been shown to augment cumulative tooth movement, as well as increase bone density loss and tooth movement, making it a promising tool in orthodontic treatments. The changes in bone density following orthodontic treatment with LLLT will be the focus of our future experiments. Overall, our findings highlight the potential of LLLT as a valuable adjunct in orthodontic therapy.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

References

1. Tonetti MS, Bottenberg P, Conrads G, et al. Dental caries and periodontal diseases in the ageing population: call to action to protect and enhance oral health and well-being as an essential component of healthy ageing—Consensus report of group 4 of the joint EFP/ORCA workshop on the boundaries between caries and periodontal diseases. *J Clin Periodontol* 2017;44:S135—44.
2. Närhi L, Tolvanen M, Pirttiniemi P, et al. Malocclusion severity and its associations with oral health-related quality of life in an adult population. *Eur J Orthod* 2022;44:37784.
3. Sicari F, Merlo EM, Gentile G, et al. Body image and psychological impact of dental appearance in adolescents with malocclusion: a preliminary exploratory study. *Children* 2023;10:1691.
4. Abid MF, Alhuwaizi AF, Al-Attar AM. Do orthodontists aim to decrease the duration of fixed appliance treatment? *J Orthod Sci* 2021;10:6.
5. Alam MK, Kanwal B, Abutayyem H, et al. Complications arising due to orthodontic treatment—A systematic review and meta-analysis. *Appl Sci* 2023;13:4035.
6. Gabada D, Reche A, Saoji KP, et al. Accelerated orthodontics: stepping into the future orthodontics. *Cureus* 2023;15:e46824.
7. Sangle R, Parab M, Gujare A, et al. Effective techniques and emerging alternatives in orthodontic tooth movement: a systematic review. *Med Novel Technol Devices* 2023;20:100274.
8. Jeon HH, Teixeira H, Tsai A. Mechanistic insight into orthodontic tooth movement based on animal studies: a critical review. *J Clin Med* 2021;10:1733.
9. Tsolakis IA, Christopoulou I, Sitaras S, et al. Molecular and biological aspects of orthodontic tooth movement: possibilities for bioengineering intervention—a narrative review. *Bioengineering* 2023;10:1275.
10. Omi M, Mishina Y. Role of osteoclasts in oral homeostasis and jawbone diseases. *Oral Sci Int* 2021;18:14—27.
11. Omi M, Mishina Y. Roles of osteoclasts in alveolar bone remodeling. *Genesis* 2022;60:e23490.
12. Boyce BF, Li J, Yao Z, et al. Nuclear factor-kappa B regulation of osteoclastogenesis and osteoblastogenesis. *Endocrinol Metab (Seoul)* 2023;38:504—21.
13. Infante M, Fabi A, Cognetti F, et al. RANKL/RANK/OPG system beyond bone remodeling: involvement in breast cancer and clinical perspectives. *J Exp Clin Cancer Res* 2019;38:12.
14. Elango J, Bao B, Wu W. The hidden secrets of soluble RANKL in bone biology. *Cytokine* 2021;144:155559.
15. Feng W, Guo J, Li M. RANKL-independent modulation of osteoclastogenesis. *J Oral Biosci* 2019;61:16—21.
16. Sabokbar A, Mahoney D, Hemingway F, et al. Non-canonical (RANKL-independent) pathways of osteoclast differentiation and their role in musculoskeletal diseases. *Clin Rev Allergy Immunol* 2016;51:16—26.
17. Qian Y, Huang HZ. The role of RANKL and MMP- 9 in the bone resorption caused by ameloblastoma. *J Oral Pathol Med* 2010;39:592—8.
18. Ma Z, Yu R, Zhao J, et al. Constant hypoxia inhibits osteoclast differentiation and bone resorption by regulating phosphorylation of JNK and IκBα. *Inflamm Res* 2019;68:157—66.
19. Lampiasi N, Russo R, Kireev I, et al. Osteoclast differentiation from murine RAW 264.7 cells stimulated by RANKL: timing and behavior. *Biology* 2021;10:117.
20. Hasegawa Y, Tanaka Y, Shaver C, et al. RANKL dependent differentiation of pulmonary osteoclast-like cells in silica-induced pulmonary fibrosis. In: *Lung fibrosis: insights from the bench*. American Thoracic Society, 2020. A2253-A53.
21. AlQranei MS, Chellaiah MA. Osteoclastogenesis in periodontal diseases: possible mediators and mechanisms. *J Oral Biosci* 2020;62:123—30.
22. Alhazmi YA, Aljabri MY, Raafat SN, et al. Exploring the effects of low-level laser therapy on the cytocompatibility and osteo/odontogenic potential of gingival-derived mesenchymal stem cells: preliminary report. *Appl Sci* 2023;13:8490.
23. Suzuki SS, Garcez AS, Suzuki H, et al. Low-level laser therapy stimulates bone metabolism and inhibits root resorption during tooth movement in a rodent model. *J Biophotonics* 2016;9:1222—35.
24. Mistry D, Dalci O, Papageorgiou SN, et al. The effects of a clinically feasible application of low-level laser therapy on the rate of orthodontic tooth movement: a triple-blind, split-mouth, randomized controlled trial. *Am J Orthod Dentofacial Orthop* 2020;157:444—53.
25. Niu Q, Gao J, Wang L, et al. Regulation of differentiation and generation of osteoclasts in rheumatoid arthritis. *Front Immunol* 2022;13:1034050.
26. Cong Q, Jia H, Li P, et al. p38α MAPK regulates proliferation and differentiation of osteoclast progenitors and bone remodeling in an aging-dependent manner. *Sci Rep* 2017;7:45964.
27. Rashed F, Kamijyo S, Shimizu Y, et al. The effects of receptor activator of NF-κB ligand-binding peptides on bone resorption and bone formation. *Front Cell Dev Biol* 2021;9:648084.
28. Xu S, Cao X, Yu Z, et al. Nicorandil inhibits osteoclast formation base on NF-κB and p-38 MAPK signaling pathways and relieves ovariectomy-induced bone loss. *Front Pharmacol* 2021;12:726361.
29. Xu H, Chen F, Liu T, et al. Ellagic acid blocks RANKL-RANK interaction and suppresses RANKL-induced osteoclastogenesis by inhibiting RANK signaling pathways. *Chem Biol Interact* 2020;331:109235.
30. Guo R, Zhang L, Hu M, et al. Alveolar bone changes in maxillary and mandibular anterior teeth during orthodontic treatment: a systematic review and meta-analysis. *Orthod Craniofac Res* 2021;24:165—79.
31. Sedky Y, Refaat W, Gutknecht N, et al. Comparison between the effect of low-level laser therapy and corticotomy-facilitated orthodontics on RANKL release during orthodontic tooth movement: a randomized controlled trial. *Lasers Dent Sci* 2019;3:99—109.
32. Domínguez A, Gómez C, Palma JC. Effects of low-level laser therapy on orthodontics: rate of tooth movement, pain, and release of RANKL and OPG in GCF. *Laser Med Sci* 2015;30:915—23.
33. Milligan M, Arudchelvan Y, Gong SG. Effects of two wattages of low-level laser therapy on orthodontic tooth movement. *Arch Oral Biol* 2017;80:62—8.
34. Zhu G, Chen W, Tang CY, et al. Knockout and double knockout of cathepsin K and MMP9 reveals a novel function of cathepsin K as a regulator of osteoclast gene expression and bone homeostasis. *Int J Biol Sci* 2022;18:5522—38.

35. Song JM, Park BS, Shin SH, et al. Low-level laser irradiation stimulates RANKL-induced osteoclastogenesis via the MAPK pathway in RAW 264.7 cells. *Appl Sci* 2021;11:5360.
36. Ramírez DG, Inostroza C, Rouabhia M, et al. Osteogenic potential of apical papilla stem cells mediated by platelet-rich fibrin and low-level laser. *Odontology* 2024;112:399—407.
37. Shin SH, Kim KH, Choi NR, et al. Effect of low-level laser therapy on bisphosphonate-treated osteoblasts. *Maxillofac Plast Reconstr Surg* 2016;38:48.
38. Nguyen J, Kelly S, Wood R, et al. A synthetic peptide, CK2.3, inhibits RANKLinduced osteoclastogenesis through BMPRIa and ERK signaling pathway. *J Dev Biol* 2020;8:12.
39. Zhao Q, Shao J, Chen W, et al. Osteoclast differentiation and gene regulation. *Front Biosci (Landmark Ed)* 2007;12:2519—29.
40. Aleksic V, Aoki A, Iwasaki K, et al. Low-level Er:YAG laser irradiation enhances osteoblast proliferation through activation of MAPK/ERK. *Laser Med Sci* 2010;25:559—69.
41. Meng J, Hong J, Zhao C, et al. Low-intensity pulsed ultrasound inhibits RANKL-induced osteoclast formation via modulating ERK-c-Fos-NFATc1 signaling cascades. *Am J Transl Res* 2018;10: 2901—10.
42. Barolet AC, Villarreal AM, Jfri A, et al. Low-intensity visible and near-infrared light-induced cell signaling pathways in the skin: a comprehensive review. *Photobiomodul Photomed Laser Surg* 2023;41:147—66.
43. Jung HJ, Chung YJ, Choi YS, et al. Clinical efficacy and safety of low-level laser therapy in patients with perennial allergic rhinitis: a randomized, double-blind, placebo-controlled trial. *J Clin Med* 2021;10:772.
44. Jivrajani SJ, Bhad Patil WA. Effect of low intensity laser therapy (LILT) on MMP-9 expression in gingival crevicular fluid and rate of orthodontic tooth movement in patients undergoing canine retraction: a randomized controlled trial. *Int Orthod* 2020;18:330—9.
45. Cruz DR, Kohara EK, Ribeiro MS, Wetter NU. Effects of low-intensity laser therapy on the orthodontic movement velocity of human teeth: a preliminary study. *Lasers Surg Med* 2004;35: 117—20.
46. Zheng J, Yang K. Clinical research: low-level laser therapy in accelerating orthodontic tooth movement. *BMC Oral Health* 2021;21:324.