INTRODUCTION

The orthodontic treatment aims to align the teeth position into the correct dental arch. However, the duration of orthodontic treatment is long enough which becomes a burden for the patient.\(^1\)\(^,\)\(^2\) In addition, long duration treatment may increase the risk of gingivitis, root resorption, and dental caries.\(^3\)\(^-\)\(^5\) Therefore, it is necessary to shorten the length of orthodontic treatment.

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Narmada IB, Rubianto M, Putra ST. The effect of low-level light therapy on orthodontic tooth movement rate, heat shock protein 70, and matrix metalloproteinase 8 expression: Animal study. Dent Res J 2020;17:73-9.
duration by accelerating the remodeling process during orthodontic treatment. Various studies reported the injections of prostaglandins (PGs – PGE2), administration of 1,25-dihydroxyvitamin D3, parathyroid hormone, ultrasound waves, and osteocalcin administration around the apical area as an attempt to accelerate orthodontic tooth movement (OTM), but this effort still has not given the expected outcomes. Clinical studies among patients with fixed orthodontic appliances irradiated with low-level light therapy (LLLT) of 100 mW can accelerate canine retraction by maintaining the stability of periodontal ligament (PDL). Mechanical orthodontic force for OTM using the concept of stress cell was a routine performed in malocclusion treatments. The administration of mechanical force strength may cause inflammatory responses in PDL, dental pulp, and alveolar bone. Mechanical force can also activate inflammatory cells, resulting in various chemical mediators release such as PGs, interleukin-1 (IL-1), IL-6, and tumor necrosis factor-alpha (TNF-α) and stimulating osteoclast differentiation. The mechanical orthodontic force on tooth leads to PDLs and alveolar bone remodeling, due to the interaction process between bone resorption and bone deformation. There is an increase of vascular endothelial growth factor and macrophage colony-stimulating factors and followed by the accumulation of matrix metalloproteinase 8 (MMP-8) produced by polymorphonuclear leukocyte cells, gingival fibroblast cells, and bone and plasma cells. MMPs play an important role in the physiological re-modeling of the periodontium as well as in response to mechanical forces during orthodontics. The inhibition of MMPs by synthetic MMP inhibitors has been shown to reduce orthodontic tooth movement. Experimental orthodontic tooth movement in animals, mostly rats, shows an increased expression of MMP-1, -2, -8, -9, and -13 and tissue inhibitor of metalloproteinases-1 and -3 in the PDL and alveolar bone. Ingman et al. confirmed that there was an increasing of MMP-8 in the gingival crevicular fluid (GCF) of orthodontic patients.

Heat shock proteins (HSPs) can be expressed by all types of cells and they play a protective role against a variety of harmful factors, including orthodontic force. Furthermore, strong orthodontic force induced apoptosis of PDL fibroblasts. It was confirmed that HSP-70, which serves in maintaining homeostasis, can inhibit apoptosis by interfering with the function of apoptosis-inducing factor. HSP-70 has the ability to provide resistance to the reported stress-induced apoptosis, and its expression exists in the PDL throughout life. In addition, there is an increase of HSP-70 expression, to maintain homeostasis, anti-apoptosis, and protecting cells from pathological stress including OTM.

So far, the explanation of LLLT mechanism effect on OTM acceleration is still unclear. Through the knowledge of molecular biology, it is possible to observe the mechanical effect of LLLT on OTM acceleration, which allows development of LLLT as a supplementary instrument to accelerate the osteogenesis process. This study aims to determine the effect of LLLT on OTM rate and HSP-70 and MMP-8 expression.

**MATERIALS AND METHODS**

**Ethical approval**

All experimental procedures were approved by the Board for Animal Experiments, Faculty of Dental Medicine, Airlangga University.

**Animals**

This was an experimental laboratories research with posttest only control group design, using 24 healthy, 3–4 months old, weighing 300–500 g male Cavia porcellus (guinea pig) with complete and healthy tooth structure. The experimental animals were evaluated clinically and placed in the appropriate environment, with foods and drinks given ad libitum for 14 × 24 h before being randomly divided into three groups (n = 8): control group (K) without orthodontic force and LLLT; treatment group 1 (T1) with orthodontic force, and treatment group 2 (T2) with orthodontic force and LLLT.

**Experimental design**

The experimental animals in the T1 and T2 groups were anesthetized using an injection of 50 mg/kg intraperitoneal pentobarbital sodium anesthesia before single-wing bracket installation (Zhejiang Protect Medical Equipment Co, Ltd., China) on the labial surfaces of both maxillary central incisors with direct attachment technique, before being inserted with close coil spring of NiTi Sentaloy (GAC, International, Bohemia, USA) 0.009 inch in diameter between maxillary central incisors using 10 g/cm² orthodontic force for 14 days to gain the distal movement of central incisors [Figure 1]. As for the T2 group, light emitting
Figure 1: Photograph showing the experimental animals with single-wing on the labial surfaces of both maxillary central incisors.

Measurement of tooth movement and immunohistochemistry assay

On day 14, all experimental animals were sacrificed using intraperitoneal injection of 250 mg/kg pentobarbital sodium. Furthermore, maxilla along with the teeth was resected to measure the central incisors distal movement gap and to prepare the specimens for HSP-70 and MMP-8 expression immunohistochemistry (IHC) examination.

The impression of maxillary central incisors was made to measure the distal movement gap with the use of individual trays containing hydrophilic vinyl polysiloxane impression material (EXAFAST Injection Type, GC Co., Tokyo, Japan). The samples were fixed in 4% paraformaldehyde after the impressions were obtained. The amount of teeth movement was evaluated by measuring the closing distance between the central incisors in the impression under a stereoscopic microscope [VH-7000; Keyence, Osaka, Japan; Figure 2b]. For each mouse, the measurement was taken four times, and the mean value was used.

For IHC test, collected tissues were blocked with paraffin before being cut and fixed to object glass. The samples were analyzed by IHC staining, using monoclonal-antibody (MoAb) anti-HSP-70 and MoAb anti-MMP-8 (BioRad, Hercules, USA). The results were examined under digital microscope at x400, equipped with a Nikon microscope OPTIPHOT (Nikon, Tokyo, Japan).

Statistical analysis

The collected data were analyzed using SPSS version 20 (IBM, New York, USA) by means of analysis of variance (ANOVA), followed by Turkey’s HSD test to determine the differences between groups. Nonnormal distributed data would be analyzed using Kruskal–Wallis test, followed by Mann–Whitney test with $P < 0.05$ being performed.

RESULTS

To determine the effect of LLLT on OTM rate, the gap between central incisors was measured before and after the treatment. The results suggested that the movement of maxillary central incisors in the T2 group ($1.0063 \pm 0.45108$ mm) was faster compared to the T1 ($0.825 \pm 0.378$ mm) and K ($0 \pm 0$ mm) groups ($P = 0.00 < 0.05$).

The result of IHC to examine HSP-70 expression can be seen in Figure 2a-c, where T1 groups show more positive expression (brown colored) compared to T2 group, while K group shows a negative expression by giving no color reaction. The result of MMP-8 expression can be seen in Figure 3, where K groups show a minimal expression compared to T1 and T2 groups.

Mean and standard deviation of HSP-70 and MMP-8 expression on the PDL fibroblasts of compressive...
and tensile side of groups K, T1, and T2 can be seen in Table 1. The HSP-70 expression on the tensile side ($P < 0.05 = 0.038$) and the compressive side ($P < 0.05 = 0.036$) was not normally distributed; therefore, the Mann–Whitney (one-tailed) test was used and $P < 0.05 (P = 0.00)$ was found. The results showed that there was a significant decrease of HSP-70 expression in T2 group compared to T1 group in the tensile and compressive sides.

In contrast, MMP-8 expression data in the tensile side was found to be normally distributed ($P > 0.05 = 2.00$) with nonhomogeneous variance ($P < 0.05 = 0.031$), so $t$-test was used. The result of $t$-test analysis showed that there was significant decrease of MMP-8 expression in T2 group compared to T1 group ($P = 0.001$). However, as the MMP-8 expression data on the compressive side ($P > 0.05 = 0.378$) was homogenous, the one-way ANOVA analysis was used. There is a significant decrease of MMP-8 expression in T2 group compared to T1 group ($P < 0.05 = 0.002$).

**DISCUSSION**

Orthodontic force applied to the tooth is a routine orthodontic treatment, but it can cause discomfort and pain and even can lead to dental and oral health disorders. In this study, the subjects were divided into three groups: K group without orthodontic force and LLLT, T1 group with 10 g/cm$^2$ of orthodontic force, and T2 group with orthodontic force and 4 J/cm$^2$ of LLLT, which was the optimal dose with minimal side effects.$^{[25,26]}$ This treatment was performed for 14 days to observe the rate of central incisors distal movement and cellular or molecular biological responses.

PDLs are supportive tissues attached the periapical area in the alveolar bone and have an important function in tooth movement. PDL disease will cause tooth mobility which may negatively impact orthodontic treatment. Fibroblasts, as structural cells of the extra cellular matrix, also have the same important role as the sensory cells and nerve cells found in the PDLs. Orthodontic force will produce cellular stress, resulting in both structural and functional changes. The orthodontic force response is evaluated through the rate of central incisor distal movement and through the molecular changes of PDL fibroblasts by measuring the expression number of HSP-70 and MMP-8, due to LLLT.

In this study, there was a significant increase of central incisor gap between the T2 groups compared with the T1 group. The results of this study proved the ability of LLLT in accelerating the rate of orthodontic teeth movement. This is in accordance with a study by Cruz et al. and Kim et al. which stated that clinical trials using LLLT in orthodontic treatment can improve the acceleration of tooth movement resulting in shortening of orthodontic treatment duration.$^{[10,27]}$ The effect of LLLT in expanding the median palatine suture of rat using orthodontic mechanical force showed 20%–40% higher maxillary bone regeneration rate compared to the group without LLLT intervention.$^{[28]}$ Kawasaki and Shimizu also stated that administration of LLLT could accelerate 30% orthodontic molar tooth movement compared to without LLLT, through increasing bone-forming activity.$^{[29]}$
Previous studies proved that LLLT could accelerate the rate of tooth movement.\cite{10,29} LLLT with wavelengths of 860 nm and 850 nm were used in most of the studies, but other studies used lower wavelength lasers. This could be a reason for different amounts of tooth movement. The visible wavelengths such as 630 nm did not result in an increased rate of OTM because the penetration depth of infrared is higher than visible waves and laser must stimulate bone and PDL cells.\cite{30,31}

HSP plays a dominant role in maintaining cell homeostasis to withstand stress, as anti-inflammatory and antiapoptosis.\cite{32} HSP-70 is a conservative protein found in the basal state and will increase through the activation of heat shock transcription factor (HSF-1).

In this study, OTM was done using 10 g/cm² orthodontic force through the application of NiTi coil spring between maxillary central incisors. This force can cause oxidative stress in PDL which activated HSF-1 and then led to HSP-70 increase in the cytoplasm. This increase resulted in the excretion of HSP-70 from cell, to form eHSP-70.\cite{33-35}

There was a significant decrease in HSP-70 expression in the T2 group compared to T1 group in the tensile and compressive side, which proved that LLLT could affect the fibroblast of periodontal tissue applied with orthodontic force. LLLT can accelerate the healing process and reduce the damage through biostimulation induction on chromophore in mitochondria, as well as increase the adenosine triphosphate and reactive oxygen species production. LLLT may also increase the proliferation and migration of fibroblasts by modulating cytokines, growth factors, and inflammatory mediators, followed by the increase of tissue oxygenation.\cite{36}

In normal circumstances, the MMP matrix is an important mediator of regeneration, remodeling, and tissue development. However, in pathological conditions, there is an increase in MMP activity as an important mediator of inflammatory tissue damage.\cite{37}

Several studies have suggested that the tooth movement by orthodontic force in the tensile and compressive sides of PDLs will increase the excretion of acute inflammatory mediators, such as cytokines, IL-1, IL-6, TNF-α, and PGE on GCF.\cite{38} In the inflammatory state, the increased expression of destructive NF-kB and enzyme nitric oxide in the PDL tissue will activate pro-MMP-8 in fibroblast cells. Research conducted by Susilowati et al. showed that there was an increase in MMP-8 expression on GCF examination in OTM with fixed appliances compared to the control group.\cite{39} This is consistent with this study where there is an increase in MMP-8 expression in the T1 group compared with group K.

However, there was a significant decrease in MMP-8 expression in the tensile and compressive sides in the T2 group compared with the T1 group. The results show that LLLT intervention in orthodontic force process can decrease MMP-8 expression.

MMP-8 is stored in the secretory granulocytes and inflammatory lesions during migration. It is possible to be regarded as a surrogate marker of the number of neutrophils in the side and as a marker of the severity of inflammation. In vitro irradiation of peripheral neutrophils affects neutrophils.\cite{40}

**CONCLUSION**

The conclusions of this study were LILT irradiation intervention during orthodontic treatment can accelerate OTM rate and decrease HSP-70 and MMP-8 expression both in tension and compressive side. Thus, LILT irradiation interventions can be used as adjuvant therapy to shorten orthodontic treatment duration.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

**REFERENCES**

1. Proffit WR, Fields HW, Sarver DM. Contemporary Orthodontic. 4th ed. St. Louis: Mosby Elsevier; 2007. p. 331-41.
2. Mitchell L. An Introduction to Orthodontics. 4th ed. Oxford: Oxford University Press; 2013. p. 2-3.
3. Zhang M, McGrath C, Hägg U. Changes in oral health-related quality of life during fixed orthodontic appliance therapy. Am J Orthod Dentofacial Orthop 2008;133:25-9.
4. Liu Z, McGrath C, Hägg U. The impact of malocclusion/orthodontic treatment need on the quality of life. A systematic review. Angle Orthod 2009;79:585-91.
5. Wishney M. Potential risks of orthodontic therapy: A critical review and conceptual framework. Aust Dent J 2017;62 Suppl 1:86-96.
6. Yamasaki K. The role of cyclic AMP, calcium, and prostaglandins
in the induction of osteoclastic bone resorption associated with experimental tooth movement. J Dent Res 1983;62:877-81.
7. Takano-Yamamoto T, Kawakami M, Kobayashi Y, Yamashiro T, Sakuda M. The effect of local application of 1,25-dihydroxycholecalciferol on osteoclast numbers in orthodontically treated rats. J Dent Res 1992;71:53-9.
8. Dahhas FY, El-Bialy T, Afify AR, Hassan AH. Effects of low-intensity pulsed ultrasound on orthodontic tooth movement and orthodontically induced inflammatory root resorption in ovariectomized osteoporotic rats. Ultrasound Med Biol 2016;42:808-14.
9. Hashimoto F, Kobayashi Y, Mataka S, Kobayashi K, Kato Y, Sakai H, et al. Administration of osteocalcin accelerates orthodontic tooth movement induced by a closed coil spring in rats. Eur J Orthod 2001;23:535-45.
10. 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.
11. Yousef M, Ashkar S, Hamade G, Gutknecht N, Lampert F, Mir M, et al. The effect of low-level laser therapy during orthodontic movement: A preliminary study. Lasers Med Sci 2008;23:27-33.
12. Fujita S, Yamaguchi M, Utsunomiya T, Yamamoto H, Kasai K. Low-energy laser stimulates tooth movement velocity via expression of RANK and RANKL. Orthod Craniofac Res 2008;11:143-55.
13. Apajalahti S, Sofas T, Ingman T. Matrix metalloproteinase -2, -8, -9, and -13 in gingival crevicular fluid of short root anomaly patients. Eur J Orthod 2003;25:365-9.
14. Jiang C, Li Z, Quan H, Xiao L, Zhao J, Jiang C, et al. Osteoimmunology in orthodontic tooth movement. Oral Dis 2015;21:694-704.
15. Yamaguchi M, Garlet GP. Biological Mechanisms of Tooth Movement. 2nd ed. UK: Wiley Blackwell; 2015. p. 121-37.
16. Ren Y, Maltha JC, Van’t Hof MA, Von Den Hoff JW, Kuipers-Jagtman AM, Zhang D, et al. Cytokine levels in crevicular fluid are less responsive to orthodontic force in adults than in juveniles. J Clin Periodontol 2002;29:757-62.
17. d’Apuzzo F, Cappabianca S, Ciavarella D, Monsurro A, Silvestrini-Biavati A, Perillo L, et al. Biomarkers of periodontal tissue remodeling during orthodontic tooth movement in mice and men: Overview and clinical relevance. ScientificWorldJournal 2013;2013:105873.
18. Alhadlaq AM. Biomarkers of orthodontic tooth movement in gingival crevicular fluid: A Systematic review. J Contemp Dent Pract 2015;16:578-87.
19. Pereira PV, Asquino N, Apellaniz D, Bueno RL, Tapia G, Bologa MR. Metalloproteinases (MMPs) of the extracellular matrix in dentistry. Odontoestomatologica 2016;18:19-28.
20. Bildt MM, Bloemen M, Kuipers-Jagtman AM, Von den Hoff JW. Matrix metalloproteinases and tissue inhibitors of metalloproteinases in gingival crevicular fluid during orthodontic tooth movement. Eur J Orthod 2009;31:529-35.
21. Takahashi I, Nishimura M, Onodera K, Bae JW, Mitani H, Okazaki M, et al. Expression of MMP-8 and MMP-13 genes in the periodontal ligament during tooth movement in rats. J Dent Res 2003;82:646-51.
38. Uematsu S, Mogi M, Deguchi T. Interleukin (IL)-1 beta, IL-6, tumor necrosis factor-alpha, epidermal growth factor, and beta 2-microglobulin levels are elevated in gingival crevicular fluid during human orthodontic tooth movement. J Dent Res 1996;75:562-7.

39. Susilowati S, Nasir M, Mudjari M, Hamid T. Expression of matrix metalloproteinase-8 gene in fixed orthodontic patients. J Majalah Kedokt Gig 2011;44:54-8.

40. Fujimaki Y, Shimoyama T, Liu Q, Umeda T, Nakaji S, Sugawara K, et al. Low-level laser irradiation attenuates production of reactive oxygen species by human neutrophils. J Clin Laser Med Surg 2003;21:165-70.