# Effect of 940nm Low Level Laser Therapy on Bone Remodelling During Orthodontic Tooth Movement in Rats

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**Abstract**

Bone remodeling is essential for successful orthodontics treatments. This study investigates the effect of 940 nm low level laser with different powers on orthodontic tooth movement in rats.

21 male, 6-week-old Sprague Dawley rats were used. A force of 10*g* was applied to the molars to induce tooth movement. The rats were grouped into 3 main experimental groups according to the power delivered: 100, 200 and 300 mW for 6 min/day. A positive and negative control groups were used. The Irradiation was performed once a day on days 0-7, then the experiment was ended in the same day. To determine the amount of tooth movement, plaster models of the maxillae were made. The models were imaged and analysed. Histological examination was performed after staining with (haematoxylin and eosin) and (Alizarin red and Alcian blue) stain. RT-PCR was also performed to elucidate the gene expression of *RANK, RANKL, OPG* and *RUNX-2* in the area of treatment.

The amount of tooth movement, the Histological bone remodelling and the RT-PCR was significantly greater in the treatment groups than that in the control group. Among the treatment groups, the 100 mW group was the highest and the 300 mW group was the lowest. These findings suggest that 940 nm low level laser treatment can facilitate the velocity of tooth movement and improve the quality of bone remodelling during orthodontic tooth movement in rats.

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## Introduction

Quality bone regeneration, which leads to the improvement of bone remodeling with limiting treatment time, is essential for orthodontic treatment. In order to improve bone regeneration and increase the amount of tooth movement, biochemical methods have been employed involving medicines.[1,2] However, these biochemical methods were accompanied with painful procedures or with effects on body metabolism, making their application difficult.[3]

Recently, the biostimulation effect of low level laser treatment (LLLT) is used to reduce the discomfort and pain that is triggered by trauma or even by the forces applied from the orthodontic appliance on teeth.[4-6] It is thought that this stimulation could also increase bone repair by promoting better bone tissue remodelling, which can be considered a way to accelerate, increase the amount of tooth movement and evolve the final stages of orthodontic treatment.[7-9] LLLT irradiation facilitates the turnover of connective tissues with acceleration of bone remodeling process by stimulating osteoblast and osteoclast cell proliferation and function during orthodontic tooth movement.[10-14]

The therapeutic methods that uses lasers

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under 500 mW power are considered as low-level laser therapies that have a biostimulatory effect on the tissue.[3] LLLT stimulation mechanism is related to the stimulation of inter and intra cells activities. LLLT stimulates mitochondria to increase the production of Adenosine diphosphate (ADP) in an increase in reactive oxygen species, which influences redox signaling, affecting intracellular homeostasis or the proliferation of cells.[15] The final enzyme in the production of Adenosine triphosphate (ATP) by the mitochondria, cytochrome c oxidase, does appear to accept energy from laser-level lights, making it a possible candidate for mediating the properties of laser therapy.[16] Furthermore, laser irradiation also affects hydrogen ion levels in the cell. This, coupled with an increase in ATP, causes activation of other membrane ion carriers such as sodium and potassium, and alters the flow of calcium between mitochondria and cytoplasm. The variation of such parameters is a necessary component in the control of cells activity.[17]

The 940 nm LLLT is increasingly used in oral treatments. However, its effects on orthodontic tooth movement have not been well investigated. The primary interest of this study is to understand the impact of the use of 940 nm LLLT with orthodontic treatment on bone remodeling in rats.

## Materials and methods

### Animals housing

The animal experimental protocol in this study was approved by the Ethics Committee for Animal Experiments in Universiti Sains Malaysia (USM) with the number of USM/Animal Ethics Approval/2012/(77) (396). The sample size of rats was calculated by PS software version 3.0.10. The experiment used 21 Sprague Dawley rats (ARASC, USM, Malaysia), 6-week-old, weighing 180 ± 10 g. They were kept in the animal house of USM in separate cages in a 12-hour light/dark environment at a constant temperature of 23°C and provided with nutrients. The health status of each rat was evaluated daily. Also, the body weight monitored biweekly starting 1 week before the experiments, the body weight was not allowed to drop more than 15% during the experiment.

### Experimental tooth movement

The animals were anesthetized with an intramuscular (IM) injection of ketamine hydrochloride (PUTNEY, USA) and xylazine hydrochloride (PROXYLAZ, Belgium) with 1 mg/kg body weight prior to; appliance placement, LLLT application and teeth impression tacking.

Experimental tooth movement was performed according to the method of Fujita *et al.* (8) and Yamaguchi *et al.* [9] with a closed-coil spring (wire size: 0.005 inch, diameter: 1/12 inch) (3M Unitek, USA) ligated to the maxillary first molar by a 0.008 inch ligature wire (3M Unitek, USA).[8, 9] The other side of the coil spring was also ligated to the maxillary incisors using the same ligature wire. The upper first molar was moved mesially by the closed-coil spring with a force of 10 g. Figure 1 shows the orthodontic appliance in situ.

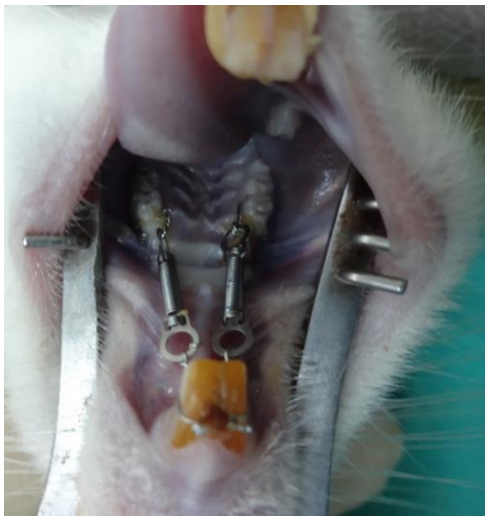

**Figure 1.** Orthodontic Appliance in Rat After Placement.

### Laser irradiation

The rats were grouped into 3 main experimental groups according to the power delivered: 100, 200 and 300 mW for 6 min/day with energy densities of 45.85 $J/cm^2$, 91.79 $J/cm^2$ and 137.57 $J/cm^2$ respectively. The laser device used was Ga-Al-As diode laser (ezlase, USA) with a wavelength at 940 nm in a continuous mode of operation. The working powers and exposure time were obtained from our previous *in vitro* study.[18] Each experimental group for this study consisted of 6 rats with a total of 18 rats for all experimental groups. In addition, positive and negative controls were used. The positive orthodontic control group was without the use of

LLLT in the other side of the experimental rat's mouth as an incomplete block split-mouth design. The negative non-treatment control was 3 rats without any intervention. In the experimental groups, the laser beam was delivered by placing the end of the optical fiber tip in contact with the mesial, buccal, and palatal sides of the gingiva, located in the area of orthodontic movement as recommended by Fujita et al.[8] and Yamaguchi et al.[9] Irradiation was performed once a day on days 0-7 (a total of eight times), then the experiment was ended in the same day.

**Measurement of tooth movement**

To determine the amount of tooth movement, impressions for rat's maxilla were taken before orthodontic appliances placement and in the end of the experimental time point. Then, study models were made. The models were used to determine the amount of tooth movement by comparing before and after study models for all animals of the experiment. The models' image was taken by medical image analysis system (JVC, USA), using Leica Material Workstation analysis software version 3.2.1. Measurement was made to the distance between first molar central fossa and second molar mesial surface to determine tooth movement in animals of the experiment as done by Yamaguchi *et al.*[9] Figure 2 shows the study models formation.

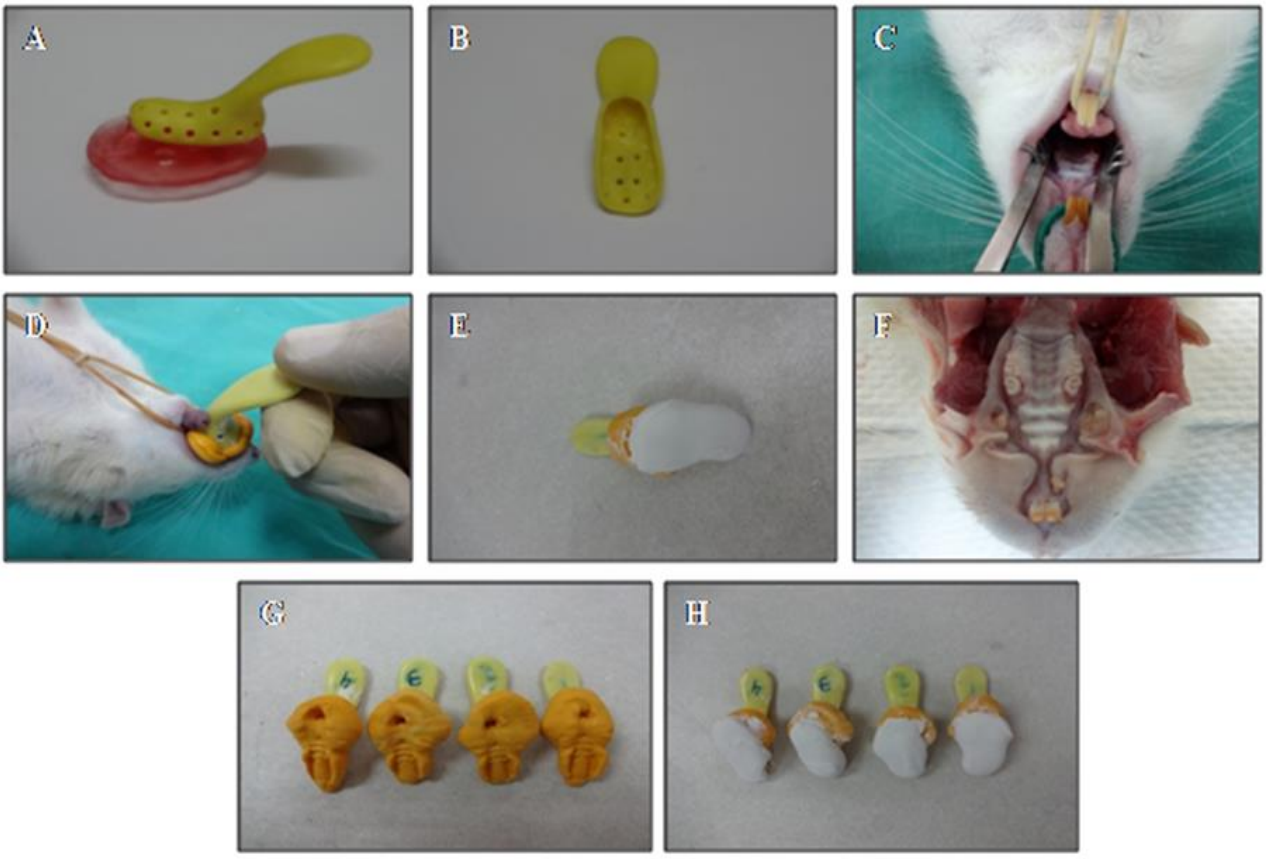


**Figure 2.** Rats Impressions and Study Models Formation. (A) Fabrication of Impression Try, (B) Impression Tray Ready to be used, (C) Rat in the Position for Taking Impression, (D) Taking 1st Impression, (E) Making 1st Study Model, (F) Rat Maxilla after Animal Sacrificed and Orthodontic Appliance was Removed, (G) Taking 2nd Impression, (H) Making 2nd Study Model.

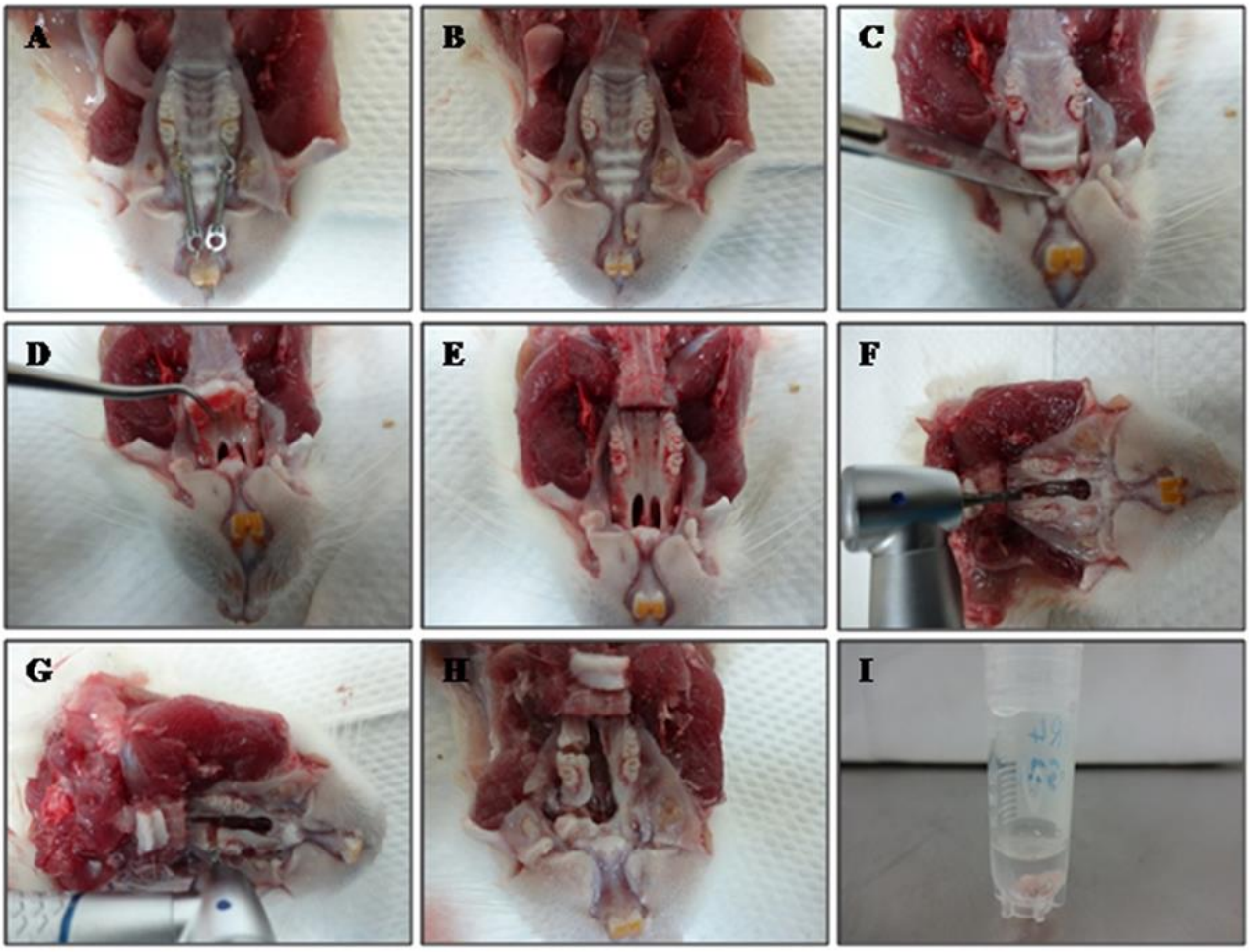


**Figure 3.** Procedure of Tissue Dissection. (A) Maxilla Detached from Mandible, (B) Orthodontic Appliance Removed, (C) Cutting Gingiva, (D) and (E) Gingival Detachment, (F), (G) and (H) Cutting the Area of Interest, (I) Preserving Tissue Specimens.

| Target Gene | Primers | Melting $T_m$ (°C) | Product size | Gene bank accession no. |
|---|---|---|---|---|
| RANKL | Forward:5'-acgcagatttgcaggactcgac-3'<br>Reverse:5'-ttcgtgctccctcctttcatc-3' | 59.5<br>57.6 | 493 bp | AF019048 |
| RANK | Forward:5'-ttaagccagtgcttcacggg-3'<br>Reverse:5'-acgtagaccacgatgatgtcgc-3' | 57.4<br>59.5 | 497 bp | AF018253 |
| OPG | Forward:5'-tggcacacgagtgatgaatgcg-3'<br>Reverse:5'-gctggaaagtttgctcttgcg-3' | 59.5<br>57.6 | 537 bp | U94330 |
| RUNX2 | Forward:5'-gaaccaagaaggcacagaca-3'<br>Reverse:5'-tccaccaccctgttgctgta-3' | 55.4<br>55.4 | 452 bp | NM053470.2 |
| GAPDH | Forward:5'-accacagtccatgccatcac-3'<br>Reverse:5'-tccaccaccctgttgctgta-3' | 57.4<br>57.4 | 452 bp | NM017008 |

**Table 1.** The Primer Sequences, Melting Temperature, Their Respective Product Size, and Gene Bank Accession Number.

**Tissue preparation**

At the end of each experimental period, rats were terminated by using 100% $CO_2$ inhalation as recommended be Jawad *et al.*[18] After that the maxilla was immediately dissected using surgical instruments and low speed hand piece. The cut on the tissue was 2 mm mesial and distal to the first molar which was the area of interest. Then, the tissue specimens were immersed in preservative solution as shown in Figure 3.

### RNA isolation and RT-PCR

A 3 tissue specimens from each experimental and control group were subjected to RT-PCR. TRIzol reagent kit (Ambion, USA) was used to dissolve the tissue samples in order to extract the RNA. The NCBI BLAST server was used to determine primer specificity for the expression of the following genes: NF-$_K$B ligands (*RANKL*), NF-$_K$B (*RANK*), Osteoprotegerin (*OPG*), Runt-related transcription factor 2 (*RUNX2*) and Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*). The primer sequences, melting temperature, product size, and gene bank accession number are shown in Table 1. One step reverse transcription-polymerase chain reaction (RT-PCR) was performed following the QIAGEN One Step RT-PCR kit (QIAGEN, Germany) manufacturer's protocol.

### Tissue processing

A 3 tissue specimens from each experimental group and positive orthodontic control were decalcified in 10 % disodium ethylenediamine tetra-acetic acid (pH 7.4) solution for 4 weeks. Then, the specimens were casted by paraffin wax. The microtome was used to slice sample into 4µm continuous sections in the horizontal direction and mounted on glass slides to be used for further staining.

### Hematoxylin and Eosin generalized staining

The slides were deparaffinised and rinsed in distilled water. Then, stained in Hematoxylin (Merck, Germany). Slides were counterstained in Eosin (Sigma-Aldrich, USA) and dehydrated, cleared and mounted. Slides were viewed with slide scanner (Zeiss, Germany).

### Alizarin red and Alcian blue specialised staining

Alizarin Red was used to identify calcium in tissue sections such as bone and the alcian blue was used to identify mucosubstances of connective tissues. The first step for staining is deparaffinization. Then, rehydration and Staining with alcian blue solution (Sigma-Aldrich, USA). Slides washed and stained in alizarin red stain solution (Sigma-Aldrich, USA). Slides were dehydrated, cleared and mounted. Slides were viewed with slide scanner (Zeiss, Germany).

### Statistical Analysis

The statistical analyses of the data for all experiments were performed using Statistical Package of Social Science (SPSS) software version 22 (Armonk, NY: IBM Corp. Released 2013). For teeth movement measurement and gene expression analysis, the assumptions of normality and homogeneity of variances were checked and were not fulfilled; Kruskal-Wallis Test was used. The pairwise comparisons were analysed through Mann-Whitney test and Bonferroni correction. A value of $P < 0.05$ was considered statistically significant.

## Results

### Measurement of tooth movement

The result shows that there was an increased in the amount of tooth movement of treatment groups in relation to control. When comparing between treatment groups, the 100 mW LLLT group was the highest as shown in Figure 4 and 5.

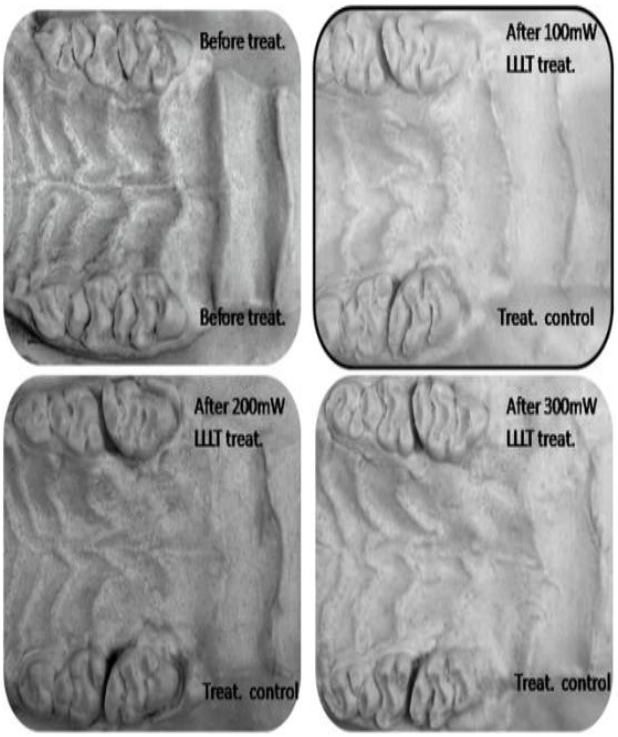


**Figure 4.** Study Model Images of LLLT Effect on Orthodontic Tooth Movement in Rats at Day 7.

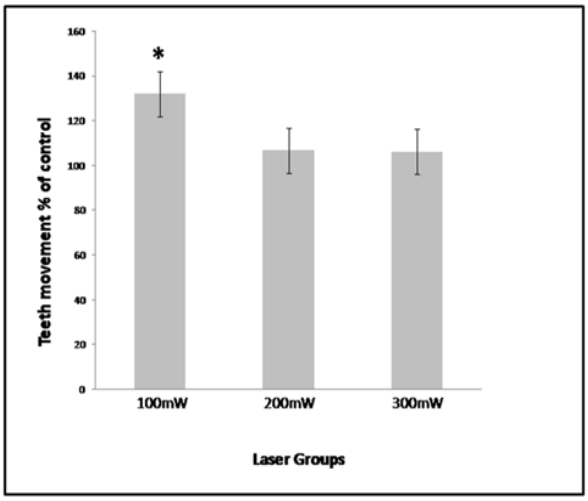


**Figure 5.** Effect of LLLT on Orthodontic Tooth Movement in Rats at Day 7. The Rats Were Orthodonticaly Treated with Presence and Absence of Daily LLL Treatment. The Orthodontic Control = 100%. The Data Are Shown as the Mean ± SEM of Experiments. *P ≤ 0.05 Between Groups.

### Gene expression analysis

In order to substantiate the signal changes of RNA level in bone cells with the different treatment groups, the gene expression of *RANKL*, *RANK, OPG* and *RUNX2* were normalized by *GAPDH* and examined by semi-quantitative RT-PCR at day 7. For these gene expressions, the RNA was up-regulated in 100 mW treatment group in relation to control and other treatment groups as shown in Figure 6.

### Histological evaluation

For histological evaluation, the histology slides were prepared from the area of interest tissue samples and stained with ether haematoxylin and eosin or alizarin red and alcian blue specialised staining to detect changes in bone. The 100 mW LLLT group showed beater amount of interseptal bone between the roots of the orthodontically moving teeth in addition to the intelligible amount of osteoclast resorption lacunae as compared to the control and the other treatment groups. While, the 300 mW LLLT group showed a diminished interseptal bone between the roots of the orthodontically moving teeth, as shown in Figures 7 and 8.

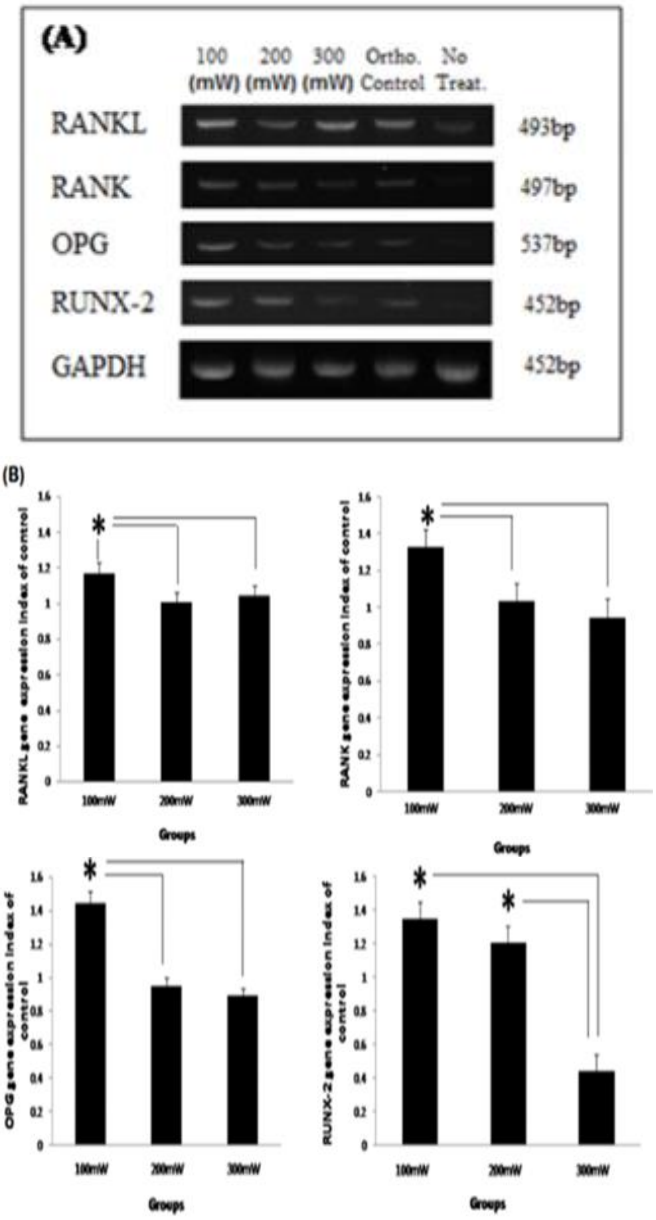


**Figure 6.** Effect of LLLT on Gene Expression of Rat's Maxilla at Day 7. The Gene Expression for *RANKL, RANK, OPG* and *RUNX2* was Determined using Semi-Quantitative RT-PCR and Normalized by *GAPDH* for the Following Groups: Control; LLLT (100 Mw); LLLT (200 Mw); LLLT (300 Mw). The Data are Shown as the Mean ± SEM of Three Separate Experiments. *P ≤ 0.05 Between Groups.

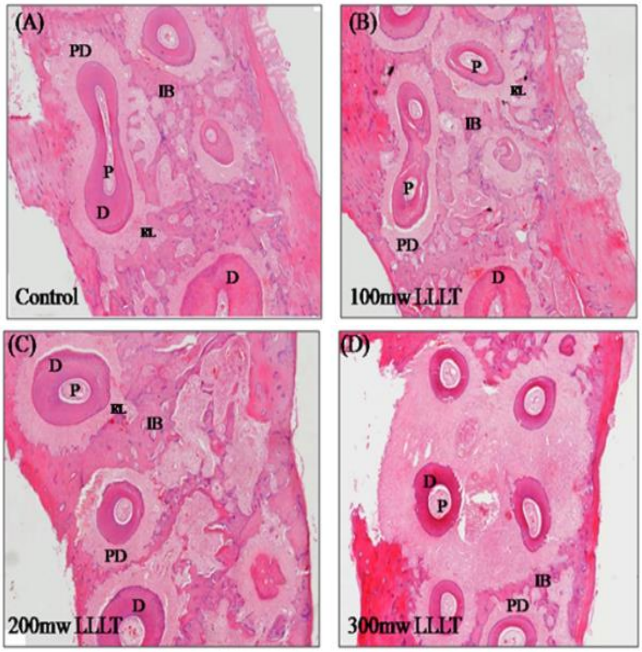


**Figure 7.** Light Micrographs of Rat's Maxilla Portions with Orthodontically Induced Tooth Movement for 7 Days, Sections Stained with H&E. ×10. (A) Control, (B) Treated with 100 Mw LLLT, (C) Treated with 200 Mw LLLT and (D) Treated with 300 Mw LLLT. *P*: Pulp, *IB*: Interseptal Bone, *PD*: Periodontal Ligament, *D*: Dentin, *RL*: Resorption Lacunae.

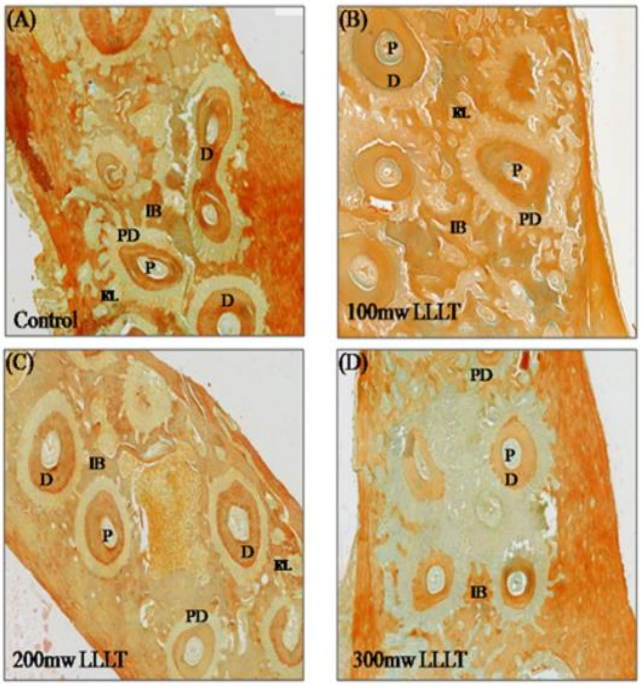


**Figure 8.** Light Micrographs of Rat's Maxilla Portions with Orthodontically Induced Tooth Movement for 7 Days Sections Stained with Alizarin Red and Alcian Blue Specialised Staining. ×10. (A) Control, (B) Treated With 100 Mw LLLT, (C) Treated with 200 Mw LLLT and (D) Treated With 300 Mw LLLT. *P*: Pulp, *IB*: Interseptal Bone, *PD*: Periodontal Ligament, *D*: Dentin, *RL*: Resorption Lacunae.

## Discussion

The purpose of this study was to compare the effects of 940 nm LLLT different powers on orthodontic tooth movement. Clinical measurement, gene expressions and histological evaluation were used in order to determine the best stimulation parameters for *in vivo* in rats. For gene expressions, the *RANKL, RANK, OPG* and *RUNX2* were used. The *OPG* and *RUNX2* gene expressions are considered as early markers for osteoblastic activity and bone formation.[8] On the other hand, *RANKL* and *RANK* are considered as early markers for osteoclastic activity and bone resorption.[13] The results for clinical tooth movement are in consistent with Yamaguchi *et al.* who stated that the amount of tooth movement was significantly greater in 100 mW irradiated group as compared to that in non-irradiated group.[9] Similarly, Fujita *et al.* reported that 100 mW of LLLT stimulated the velocity of tooth movement in rats as compared to control.8 Kawasaki and Shimizu[11] showed that orthodontic movement of laser-irradiated rat teeth of 96 mW was faster when compared with that in non-irradiated rats due to an increase in bone formation at the tension side and in the number of osteoclasts at the compression side because of cellular stimulation promoted by low energy laser irradiation.

For RT-PCR, gene expressions of *RANKL, RANK, OPG* and *RUNX2* in bone, the gene expressions were up-regulated in 100 mW treatment group in relation to control and other treatment groups. This result agrees with Fujita *et al.*[8] and Yamaguchi *et al.*[19] who demonstrated that 100 mW LLLT irradiation stimulated *RANK/RANKL/OPG* and the macrophage colony-stimulating factor (*M-CSF*) and its receptor (c-Fms) expressions that led to increase in the velocity of tooth movement. In addition, Altan *et al.*[13] determined that low-level laser therapy increased *RANKL* release and the osteoclastic activity by exacerbating the inflammatory response to orthodontic forces through triggering of inflammatory cytokines to *RANKL* release which regulated osteoclastic cell activity.

For histological evaluation, 100 mW LLLT group showed more amount of interseptal bone between the roots of the orthodontically moving teeth in addition to reasonable amount of resorption lacunae as compared to the control and other treatment groups as an indication of active bone remodelling process. This can be attributed to LLLT stimulation to cells metabolic activities though stimulating cells mitochondrial energy cycle.[20] This result is consistent with Fujita *et al.*[8] who found that at day 7 after initiation of tooth movement and irradiation of 100 mW LLLT, the fibroblasts in the PDL were increased. Furthermore, on the surface of the alveolar bone, bone resorption lacunae with multinucleate osteoclasts were recognized and the number of multinucleate osteoclasts was found to be significantly increased in the irradiation group compared with the non-irradiation group. Also, this is in consistent with Kim *et al.*[14] who stated that 96 mW LLLT irradiation facilitated the remodelling of connective tissues during tooth movement in rats. The result of this study also is in agreement with Kawasaki *et al.*[11] and Yoshida *et al.*[12] who concluded that 100 mW of LLLT irradiation stimulated tooth movement in rats accompanied with an acceleration of alveolar bone remodelling as indicated by increases in the number of osteoclasts, cellular proliferation of periodontal ligament cells, and mineralized bone formation.

Distinctly, 300 mW LLLT group showed diminished interseptal bone between the roots of the orthodontically moved teeth and replaced by granulation tissues as a sign of bone destruction which indicated that 300 mW LLLT power was too high to be used.

## Conclussion

The findings of this study suggest that the use of LLLT of 100 mW power for 6 min/day can increase clinical orthodontic tooth movement, up-regulate tissue gene expressions and actively improve bone remodeling in the area undergoing orthodontic tooth movement in rats.

## References

1. Yamasaki K, Shibata Y, Fukuhara T. The effect of prostaglandins on experimental tooth movement in monkeys (Macaca fuscata). J Dent Res. 1982;61:1444-46.
2. 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.
3. Kim YD, Kim SS, Kim SJ, Kwon DW, Jeon ES, Son WS. Low-level laser irradiation facilitates fibronectin and collagen type I turnover during tooth movement in rats. Lasers Med Sci. 2010;25:25-31.
4. 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.
5. Youssef M, Ashkar S, Hamade E, Gutknecht N, Lampert F, Mir M. The effect of low-level laser therapy during orthodontic movement: a preliminary study. Lasers Med Sci. 2008;23:27-33.
6. Zahra SE, Elkasi AA, Eldin MS, Vandevska-Radunovic V. The effect of low level laser therapy (LLLT) on bone remodelling after median diastema closure: A one year and half follow-up study. Orthod Waves. 2009;68: 116-22.
7. Limpanichkul W, Godfrey K, Srisuk N, Rattanayatikul C. Effects of low-level laser therapy on the rate of orthodontic tooth movement. Orthod Craniofac Res. 2006;9:38-43.
8. 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.
9. Yamaguchi M, Hayashi M, Fujita S, Yoshida T, Utsunomiya T, Yamamoto H, et al. Low-energy laser irradiation facilitates the velocity of tooth movement and the expressions of matrix metalloproteinase-9, cathepsin K, and alpha (v) beta (3) integrin in rats. European J Orthod. 2010;32:131-39.
10. Ozawa Y, Shimizu N, Kariya G, Abiko Y. Low-energy laser irradiation stimulates bone nodule formation at early stages of cell culture in rat calvarial cells. Bone 1998;22: 347-54.
11. Kawasaki K, Shimizu N. Effects of low-energy laser irradiation on bone remodeling during experimental tooth movement in rats. Lasers Surg Med. 2000;26:282-91.
12. Yoshida T, Yamaguchi M, Utsunomiya T, Kato M, Arai Y, Kaneda T, et al. Low - energy laser irradiation accelerates the velocity of tooth movement via stimulation of the alveolar bone remodeling. Orthod Craniofac Res. 2009;12:289-98.
13. Altan BA, Sokucu O, Ozkut MM, Inan S. Metrical and histological investigation of the effects of low-level laser therapy on orthodontic tooth movement. Lasers Med Sci. 2010;27:131-40.
14. Kim SJ, Moon SU, Kang SG, Park YG. Effects of low-level laser therapy after Corticision on tooth movement and paradental remodeling. Lasers Surg Med. 2009;41:524-33.
15. Tafur J, Mills PJ. Low-intensity light therapy: Exploring the role of redox mechanisms. Photomedicine Laser Surg. 2008;26:323-28.
16. Karu TI. Mitochondrial Signaling in Mammalian Cells Activated by Red and Near - IR Radiation. Photochemistry Photobiology. 2008;84:1091-99.
17. Fitz-Ritson D. Lasers and their therapeutic application in chiropractic. J Canadian Chiropractic Assoc. 2001;45:26.
18. Jawad MM, Husein A, Azlina A, Alam MK, Hassan R, Shaari R. Effect of 940 nm low-level laser therapy on osteogenesis in vitro. J Biomed Optics. 2013;18:128001-01.
19. Yamaguchi M, Fujita S, Yoshida T, Oikawa K, Utsunomiya T, Yamamoto H, et al. Low-energy laser irradiation stimulates the tooth movement velocity via expression of M-CSF and c-fms. Orthod Waves. 2007;66:139-48.
20. Sun G, Tunér J. Low-level laser therapy in dentistry. Dent Clinics North Am. 2004;48:1061-76.