

# Comparison of Single and Multiple Low-Level Laser Applications After Rapid Palatal Expansion on Bone Regeneration in Rats



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## Abstract

**Introduction:** This study was performed to compare the effects of single and multiple irradiations of low-level laser therapy (LLLT) on bone regeneration in a mid-palatal suture following rapid palatal expansion (RPE).

**Methods:** In this animal study, 40 male Wistar rats underwent RPE for 7 days and were divided into 4 groups including A: single LLLT on day 7, B: Multiple LLLT on days 7, 9, 11, 13 and 15, C: control (no LLLT), and D: sacrificed on day 7. Animals in group D were used to determine the amount of suture expansion. LLLT was done by a diode laser set at an 808 nm wavelength with a useful power output of 100 mW and duration of 0.1 ms. LLLT was applied to three points. After three weeks of retention, the rats were sacrificed and beheaded and the maxilla was evaluated by occlusal radiography,  $\mu$ -CT, and histomorphometric analyses. A comparison of the mean measurements between the groups was performed using ANOVA and the Tukey post hoc test.

**Results:** Based on occlusal radiography and  $\mu$ CT, bone density in group B was significantly higher than group A and group C ( $P < 0.05$ ). There was no significant difference in bone density between group A and group C ( $P > 0.05$ ). Mean suture width (MSW) in group B was significantly lesser than the control group ( $P = 0.027$ ) while there was no significant difference between MSW in groups A and B ( $P = 0.116$ ) and groups A and C ( $P = 0.317$ ).

**Conclusion:** It may be concluded that multiple low-power laser irradiation improves bone regeneration after RPE while single irradiation does not have a positive effect.

**Keywords:** Low-level light therapy; Palatal expansion; Bone regeneration.



## Introduction

Posterior crossbite is one of the most important and most prevalent transverse abnormalities in the primary and mixed dentition period.<sup>1</sup> It can be due to either palatal tipping of the posterior teeth, or maxillary skeletal constriction, or a combination of both.<sup>2</sup> Maxillary constriction could cause bilateral posterior crossbite or premature tooth contact in centric relation resulting in a functional shift to a pseudo unilateral posterior crossbite.<sup>3</sup> In the cases of skeletal constriction, the treatment modality includes maxillary expansion, which can be performed until adolescence with tooth-borne expansion appliances called rapid palatal expansion (RPE). In patients with mature mid palatal suture, expansion could be done with

bone-borne appliances.<sup>4,5</sup>

The high rate of the relapse of intermolar width which is about 40% has compromised the maxillary expansion stability.<sup>6</sup> This makes 6-9 months of retention necessary for the mid palatal bone to mature.<sup>7</sup> Therefore, accelerating and improving bone formation through and after palatal expansion can help to reduce retention period relapse.<sup>8</sup> Several methods including laser therapy,<sup>8</sup> drugs such as lithium chloride,<sup>9</sup> strontium ranelate,<sup>10</sup> stem cells,<sup>11</sup> plasma rich platelet,<sup>12</sup> and palatal mucoperiostomy<sup>13</sup> have been used to increase bone regeneration following RPE.

Several studies on the impacts of low-level laser therapy (LLLT) on osteogenesis have suggested an increase in bone formation.<sup>14-17</sup> The LLLT increases the activity of

osteoblasts by increasing alkaline phosphatase activity.<sup>18</sup> It also improves the quality of bone formation.<sup>16</sup> The other effects include cell proliferation and increased numbers of osteoclast in the laser irradiation zone.<sup>19</sup> Saito et al<sup>8</sup> found that with the expansion of the palate of rats, there was a direct relationship between the rate of bone formation and the overall laser dose, timing, and frequency of irradiations. Although several studies on animal and human models have shown the applicability of LLLT after RPE,<sup>8,20,21</sup> to the extent of our knowledge, there are few studies which have compared the effects of single irradiation and multiple irradiations of LLLT following RPE during the retention period. The objectives of this investigation were to assess the effects of single and multiple irradiations on bone regeneration after RPE in rats.

## Materials and Methods

### Animals

A total of 40 wild type Wistar male rats which were fit and healthy were used for this experiment. They were kept at a temperature of around 23 degrees in clean cages controlled daily by an expert operator. Each rat weighed around 200 g  $\pm$  20 g at the time of the experiment.

### Palatal Expansion

Expansion springs were made by a trained operator with 0.014-inch stainless steel wire (American orthodontics, Washington, USA) with one helix for activation and two retention heads in a V-shape form. The spring was adjusted to produce 50 $\pm$ 5 g force measured with a digital gauge.

Animals were sedated with the intraperitoneal injection of 50 mg/kg animal ketamine (100 mg/mL, Alfasan, Woerden, The Netherlands) and 10 mg/kg of xylazine (20 mg/mL, Alfasan, Woerden, The Netherlands) prior to the placement of the springs. After the anesthesia, first, an elastic separator was placed between two anterior maxillary incisors for 10 min and then removed. After that, the incisors were etched with 37% acid phosphoric gel (Morvabon™, Tehran, Iran) for 30 seconds. The teeth were irrigated with sterile water and dried with air spray until the chalky enamel was visible. After that, resin adhesive bonding (3M™ Adper™ Single Bond Plus Adhesive Refill, 3M ESPE, Irvine, CA, USA) was applied with a micro-brush and cured for 20 seconds (VALO™ Cordless LED Curing Light, Ultradent Inc. South Jordan, UT, USA). Next, the restorative resin composites (Filtek™ Supreme Ultra Universal Restorative, ESPE, Irvine, CA, USA) were used to adhere the spring loops to the central incisors. Then, the resin composites were cured for 30 seconds. During this process, a transparent matrix was placed between the incisors, and care was taken to prevent the incisors from adhering together. The spring was activated by placing a 139 plier (American Orthodontics, 001-E140) in the helix to keep apart the two arms.

### Retention

The expansion springs were left at the place for 7 days. Ten samples were sacrificed at that point to measure the amount of mid-palatal suture expansion without retention (group D). The other samples underwent a retention period for further 21 days. For this purpose, after sedation, a proper amount of restorative composite was placed between incisors following the conventional bonding protocol. Then the springs were cut with a fine cutter.

### Low-Level Laser Therapy

Thirty animals continued the study during the retention period. The rats were distributed randomly into three groups of ten, including group A: single LLLT on day 7, group B: multiple LLLT on days 7, 9, 11, 13, and 15, and group C: control with no LLLT. In order to provide similar conditions for all groups, all animals were sedated moderately using half amounts of anesthesia on days 9, 11, 13 and 15.

A diode laser (whitening lase II, DMC®, Sao Paulo, Brazil) was set to irradiate as follows: an 808 nm wavelength with a useful power output of 100 mJ/cm<sup>2</sup> and 0.1 msec. The beam delivery (Figure 1) was done by an optical fiber, which was placed in three points in contact with palatal mucosa in the following order: (1) Anterior part of the two incisors (The most anterior part of the mid-palatal suture), (2) Posterior of the incisors, and (3) Molar region.

### Occlusal Radiography

Three weeks after the retention period (day 28), the rats were sacrificed with CO<sub>2</sub> exposure. The rats were beheaded. Then the samples were immersed in formalin 10%. After one month, the occlusal radiograph images were taken with an intraoral digital x-ray unit (Planmeca Prostyle™, Helsinki, Finland) in the Fantom department (Fantom Department, School of Dentistry, Shahid

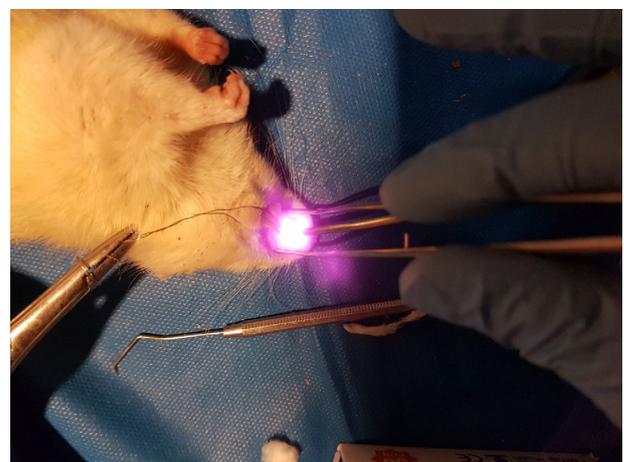


Figure 1. Laser Irradiation to the Mid-palate of the Sedated Rat.

Beheshti University of Medical Sciences). The device was set at 63 kVp and 0.06 seconds. The images were processed with Owandy XIO StandAlone (Owandy Radiology®, Croissy-Beaubourg, France). For the calibration of the occlusal images, an aluminum step wedge was used. First, the boundaries of the expanded suture were determined in group D (animals with expansion only and no retention) using ImageJ software (U. S. National Institutes of Health, Bethesda, Maryland, USA) (Figure 2). Then, in other groups, the radiographic gray scale of the specified area was measured by Adobe Photoshop software (Adobe Photoshop CS4, Adobe, Philadelphia, USA). The gray scale values were compared to the gray scale values of the step wedge. As the step wedge was made of aluminum (Al), the density of bone at the suture area was calculated as mm-Al. Therefore, the mean values of the palatal bone suture density were calculated as mm-Al for each group and then the groups were compared to each other.

### Micro-Computed Tomography

In this study, we used an in vivo micro-computed tomography ( $\mu$ -CT) scanner (LOTUS-inVivo, Behin Negareh Co., Tehran, Iran). LOTUS-inVivo had a cone-beam micro-focus X-ray source and a flat panel detector. In order to obtain the best possible image quality, the tube voltage was set at 80 kV and its current was 100  $\mu$ A. Also, the power of the scan was 8 W through a rotation of 360° around the vertical axis and a rotation step of 0.3°. The total scan duration was 30 minutes. The slice thicknesses of the reconstructed images were set to 25  $\mu$ m. The process of all the protocol settings was controlled by LOTUS-inVivo-ACQ software. The acquired 3D data were reconstructed using LOTUS inVivo-REC by a standard Feldkamp, Davis, Kress (FDK) algorithm.<sup>22</sup>

For each sample, the section showing a midpalatal suture was selected. The suture area was first determined in the samples of group D using ImageJ software. The radiodensity of the suture was measured based on the Hounsfield scale using ImageJ software.<sup>23</sup>

### Histology

Following radiographic assessment, the specimens were decalcified in a 10% Formic acid (Surgipath's Decalcifier I, Buffalo Grove, NY, USA). Then, they were embedded in paraffin. After the fixation, the sections were carried out in 5  $\mu$ m. Finally, specimens underwent H&E staining.

The samples were observed with the light microscope (E400, Nikon, Tokyo, Japan) with a magnification of  $\times 40$ . Dimensions of the sutures were measured by ImageJ software. For each section, a central suture line was drawn between two sides of the suture (Figure 3). The measurements were carried out at 5 areas of the suture to determine mean suture width (MSW).

### Statistical Analysis

A comparison of the mean measurements between the

groups was performed using ANOVA and the Tukey post hoc test. The data were analyzed using SPSS version 25 with a significant level of 0.05.

### Results

In this research, 40 rats were evaluated and they were divided into four groups as groups A, B, C and group D (Table 1). Based on the occlusal radiography, the mean  $\pm$  SD of the bone density in groups A, B, C, and D was  $5.26 \pm 0.96$ ,  $6.91 \pm 0.86$ ,  $4.73 \pm 1.20$ , and  $2.72 \pm 0.48$  (mm-Al) respectively. Bone density in group B (multiple exposure) was significantly more than group A (single exposure,  $P=0.024$ ) and group C (control,  $P=0.003$ ). The bone density between group A and group C did not show a significant difference ( $P=0.752$ ) while it was significantly lower in group D compared to the other groups ( $P \leq 0.005$ ).

The results of  $\mu$ CT imaging revealed that the mean ( $\pm$  standard deviation) of the bone density in groups A, B, C, and D was  $140.83 \pm 26.24$ ,  $202.06 \pm 31.44$ ,  $125.83 \pm 37.35$ ,



Figure 2. The radiographic image of the expanded suture.

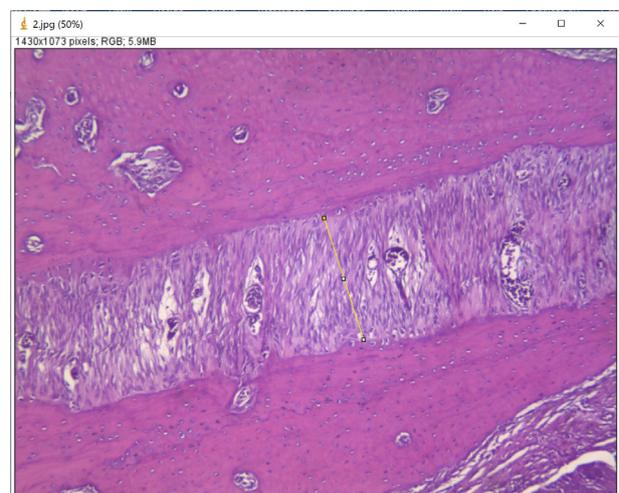


Figure 3. Measurement of Suture Width.

**Table 1.** Results of Mean Bone Density Values in Occlusal Radiography (mm-Al), micro-CT (Hounsfield Unit) and Histomorphometric Analysis Values in Groups A-D

Mean Values	Group A	Group B	Group C	Group D
Bone density (mm-Al)	5.26±0.96	6.91±0.86	4.73±1.20	2.72±0.48
Bone density (Hounsfield unit)	140.83±26.24	202.06±31.44	125.83±37.35	36.92±25.97
MSW values	0.18±0.05	0.13±0.08	0.28±0.10	0.39±0.08

Note. Group A: single LLLT on day 7; Group B: multiple LLLT on days 7, 9, 11, 13, and 15; Group C: control groups with no LLLT; Group D: Expansion without any retention.

and 36.92±25.97 according to the Hounsfield unit respectively. Similar to the occlusal radiography, the Hounsfield unit in group B was significantly higher than group A ( $P=0.012$ ) and group C ( $P=0.002$ ). The results did not show a significant difference in the Hounsfield unit between group A and group C ( $P=0.831$ ). The Hounsfield unit of group D was significantly lower than the other experimental groups ( $P<0.001$ ) (Figure 4).

The histomorphometric analysis results showed that the mean ( $\pm$  standard deviation) suture width in groups A, B, C and D was 0.18±0.05, 0.13±0.08, 0.28±0.10, and 0.39±0.08 (mm) respectively. MSW in group B was significantly lower than the control group ( $P=0.022$ ) while there was no significant difference between MSW in groups A and B ( $P=0.724$ ) and groups A and C ( $P=0.176$ ). MSW in group D was significantly larger than group A ( $P=0.001$ ) and group B ( $P<0.001$ ) with no significant difference with group C ( $P=0.094$ ).

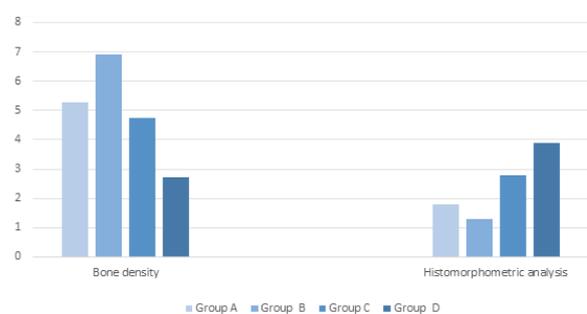
## Discussion

Laser irradiation is user-friendly, painless, without certain side effects, and financially affordable and it takes a little time. There are several studies about the impacts of Low-power laser therapy on bone formation with a basis of non-quantitative histological or radiological surveillance.<sup>24-26</sup> They have concluded that LLLT almost provides environmental conditions in such a way that it accelerates bone healing rather than osteosynthesis. As the previous studies showed that low-power laser irradiation can raise the rate of bone regeneration,<sup>8,24,27</sup> the question is what dose of LLLT has favorable impacts on midpalatal suture bone repair. The purpose of this research was to assess the effects of single- and multiple-dose irradiations of the LLLT on the rate and quality of bone formation in the mid-palatal suture after expansion. The quantitative assessment of the mean bone density, the mean Hounsfield unit and the MSW showed that multiple irradiations are associated with higher bone formation and smaller suture distance. However, in the single-dose irradiation group compared to the control group, the results didn't have a significant difference.

One of the advantages of the present study was the inclusion of the samples that were sacrificed just after the expansion without any retention device or time (group D). This group was used as a scale to estimate the amount

of suture expansion following RPE. New bone formation during the retention period in the experimental and control groups was measured within the expanded suture zone.

Several studies have discussed the importance of using an adequate energy level, yet there has not been an established ideal LLLT protocol for bone promotion.<sup>24, 27</sup> Evaluating 48 rabbits, Fekrazad et al<sup>18</sup> showed a significant difference in bone formation using the gallium-aluminum-arsenide (GaAlAs) laser. The laser irradiation protocol was a wavelength of 810 nm, a power density of 0.2 W/cm<sup>2</sup> and a fluency of 4 J/cm<sup>2</sup> and the irradiation was done every other day for 3 weeks. Also, they concluded that there was not any evidence of an interactive effect when applied in conjunction with mesenchymal stem cells. Saito et al<sup>8</sup> used 100 mW irradiation of the GaAlAs diode laser at different time points in rats. They compared irradiation in three time points as follows: 7 days, 3 days and 1 day; they concluded that irradiation ahead of time of expansion (days 0 to 2) was most useful, whereas the later period (days 4 to 6) and the one-time irradiation didn't have any impact on bone regeneration. These results were in accordance with the current study. Pretel et al<sup>28</sup> studied the effects of LLLT on bone repair in rats. They used single laser irradiation with a GaAlAs semiconductor diode laser device. They reported that the use of LLLT had a biostimulating effect on bone remodeling and caused the tissue to come back to normal conditions at the earlier periods. However, there were no differences between the groups after 60 days from the first irradiation, which



**Figure 4.** The Comparison Chart of Bone Density and MSW Values in Groups A-D. (Group A: single LLLT on day 7; Group B: multiple LLLT on days 7, 9, 11, 13, and 15; Group C: control groups with no LLLT; Group D: Expansion without any retention.)

means finally there is no difference between the control group and the single irradiation group. Our results were similar to those of that study and we also showed single irradiation LLLT had no effect. While most studies have reported that single-dose irradiation has no effect, Bloise et al<sup>29</sup> and Renno et al<sup>30</sup> have reported that single LLL irradiation is helpful for osteoblast proliferation. Cepera et al<sup>31</sup> evaluated the impact of LLLT on bone regeneration in a clinical trial. They used LLLT in five time points and concluded that the low-power laser, associated with RPE, impressed the bone regeneration process of the suture and improved healing. The results of the multiple irradiation group in the current study also showed significantly more bone formation. However, single-dose irradiation did not show a significant difference. This is perhaps because of insufficient irradiation timing as Saito et al<sup>8</sup> have declared. Also, the low-power laser and the type of laser (diode laser) in the current study caused a lack of significant effect following single-dose exposure.

$\mu$ CT evaluation showed that the Hounsfield unit amounts of the multiple exposure group were significantly more than the single exposure and control groups. This means that the palatal suture was more calcified in the multiple exposure group and no significant difference was observed between the calcification of the single exposure group and the control group.

From a molecular point of view, Li et al<sup>15</sup> surveyed the molecular signaling of LLLT on pre-osteoblast cells and indicated that laser therapy improved the reproduction of MC3T3-E1 cells through the hedgehog signaling pathway. They showed that laser irradiation promoted Ihh, Ptch, Smo and Gli expressions on both mRNA and protein levels during osteoblastic reproduction in MC3T3-E1Cs at 3.75 J/cm<sup>2</sup>. However, they declared that the detailed mechanisms of the hedgehog signaling pathway are still unknown. Fávoro-Pípi et al<sup>32</sup> assessed the impacts of LLLT on the expression of osteogenic genes and found that laser irradiation produced an upregulation of BMP-4, ALP, and Runx 2 after surgery.

The current study had some limitations. This study was performed on rats. Further studies on bigger animals like monkeys and human studies are necessary to confirm these findings. Furthermore, human studies are much more important to evaluate the amounts of clinical effects and whether they have significant clinical positive effects.

## Conclusion

Within the limitations of this study, it can be concluded that the irradiation at five timepoints could improve bone regeneration after RPE while single irradiation does not seem to have positive effects. This was the first report comparing different LLLT protocols. Further human studies are required to show the clinical importance of these findings.

## Ethical Considerations

This study was carried out according to legal and ethical specifications for animal experiments and approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (Code: IR.sbmu.drc.rec.1397.070).

## Conflicts of Interests

The authors declare no conflict of interest.

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