

# ROOT SURFACE TREATMENT USING DIODE LASER IN TOOTH REPLANTATION (EXPERIMENTAL STUDY IN RATS)

Karim Y Hafez<sup>1\*</sup> BDS, Sahar S. Karam<sup>2</sup> PhD, Hanaa M. Ali<sup>3</sup> PhD

## ABSTRACT

**INTRODUCTION:** Avulsion of teeth radically affects the integrity of the tooth and the supporting tissue, and impairs the natural dentition as a whole; thus, all efforts must be directed to the replantation of traumatically avulsed teeth. However, its success is limited due to the occurrence of external root resorption. The challenge is to follow an effective protocol that reduces the occurrence of root resorption and allow for reestablishment of the natural architecture of the periodontal tissue. Biomodulation provided by low level laser therapy provides a promising tool in managing the inflammatory process and augmentation of the reparative process.

**OBJECTIVES:** Was to evaluate the effect of low-level laser therapy (LLLT) on the healing process of rat teeth replanted after delayed replantation.

**MATERIALS AND METHODS:** Thirty healthy Albino male rats had their maxillary left incisors extracted; According to the root surface treatment before the replantation, the teeth were assigned randomly to two groups (n = 15): G1 (control) – no root surface treatment; G2 (study group)-laser diode treatment on the root surface. The teeth were replanted into their respective sockets after 30 minutes, mimicking the natural timing it would take to replant an avulsed tooth. G2 were treated with LLLT performed on the buccal and palatal mucosa every 48 hours for 15 days. The rats were sacrificed after 15, 30 and 45 days of replantation. The specimens were processed for histological and histomorphometric analysis to determine the average root resorption areas and to evaluate the histological events.

**RESULTS:** Histomorphometric analysis showed that the study group (Laser irradiated group) showed lower areas of root resorption and ankylosis, than in control group (non-irradiated group); with a significant statistical difference ( $p > 0.05$ ). Histological assessment showed restoration of periodontal ligament attachment and increased vascularity in the study group.

**CONCLUSIONS:** Laser irradiation reduces external root resorption and ankylosis in delayed tooth replantation.

**KEYWORDS:** LLLT, Replantation, Ankylosis, Root resorption, Biomodulation.

- 1- Demonstrator of oral biology Faculty of Dentistry, Alexandria University.  
2- Professor of oral biology and head of oral biology department, Faculty of Dentistry, Alexandria University.  
3- Professor of oral biology Faculty of Dentistry, Alexandria University.

\* Corresponding author:  
E-mail: dr.karimyasser13@gmail.com

## INTRODUCTION

Tooth avulsion is the complete displacement of the tooth from its alveolar socket. 0.5% to 16% fall victim to traumatic orofacial injuries (1); most of this population are in the age of 7 to 9. Most avulsion cases are in this range of age due to the resilient nature of the bone at this age. The maxillary central incisors are the main target to avulsion after trauma (2); given its placement and position. This type of trauma must be readily accounted for as it may not only result in avulsion, but may result in damage to the supporting tissues (3, 4).

The complexity in treatment of avulsion treatment resides in readiness of the replantation procedure, which is stated to be in the range of 1 to 4 hours after avulsion (1, 5, 6), the longer it takes the less the chances of survival of the tissues of retaining vitality, and the slimmer the chances of a successful replantation procedure (7-10); studies have shown that the best possible prognosis is replantation within 5 minutes (11), and the storage of the avulsed tooth in a suitable storage medium, such as, salt solution, coconut water, milk, saliva, calcitonin and alendronate. Thus the treatment plan must take into account the time and storage medium of the avulsed tooth, as well as the age of the patient and the post-implantation care (1).

In the case of delayed replantation of avulsed teeth, which is probable due to the unknown circumstances of the trauma, the healing process is mainly by repair (1). This is

due to the necrosis of the periodontal ligament cells; and this healing process comes about by the replacement resorption. This pathology involves resorption of the root surface, termed external root resorption which is characterized by removal of dentine from the root surface and may be evident radiographically, and replacement of the space created by the surrounding alveolar bone (12). Replacement resorption is a by-product of the inflammatory response to eradicate the affected tissue and commence with the reparative process, another unwanted result of this process is ankylosis, which is the pathological fusion of the cemental or dentinal surface of the root surface to the alveolar bone (13), this may result in infra-occlusion of the tooth in relation to occlusal plane due to the continual growth in younger patients (14). This presents unfavorable treatment outcomes, which raises the question of whether replantation of avulsed teeth could result in favorable and predictable outcomes, or present other clinical difficulties that would render it ineffective (15). Modulation of the inflammatory process is the key to reduction of the resorptive process; this is where the biomodulation property of low level laser therapy (LLLT) comes into effect (16).

Effects of LLLT can be explained by light absorption within the mitochondria (16, 17). Given its presence in the cells in abundance, the mitochondria are the main source of ATP, the power house of the cell, which is needed by the cell for vital processes such as Krebs cycle and glycolysis,

this ATP is generated from oxygen and pyruvate. Under stressful conditions the mitochondria produce nitric oxide (18-20), which can displace oxygen from binding to Cytochrome c oxidase (CcO) (an enzyme required for electron transport chain). The production of nitric oxide results in reduced ATP production and raised oxidative stress, thus activating transcription factor, NF- $\kappa$ B, which consequently leads to inflammation (18-23).

At cellular level photo biomodulation by red to near infrared light, results in activation of mitochondria; as growing evidence prove that CoA enzyme is the acceptor of light in this range of light (17, 24-26). Given that CoA is a vital membrane protein which contains four redox active metal centers and has strong affinity for this range of light ; its absorbance results in release of nitric acid from CoA thus increasing ATP synthesis, which in turn reduces the oxidative stress (27-29). Due to the increase in ATP synthesis and the release of nitric acid, the cell signaling state is altered either directly or indirectly. This affects components of the cytosol, the cell membrane, and nuclear functions that control gene transcription and subsequently regulate cellular responses such as proliferation, migration, necrosis and inflammation. Tissues and cells that have not absorbed the photons can be affected indirectly by bioactive molecules released from irradiated cells. This can subsequently have systematic effects via autocrine, paracrine and endocrine mechanisms (27-30).

Added to the benefit of reduction of inflammation by low level laser irradiation, it has shown that it also has the ability to improve lymphatic flow and energize the lymphocytic system. A systematic review of eight clinical trials of LLLT for post-mastectomy lymphoedema concludes that "There is moderate to strong evidence for the effectiveness of LLLT for the management of breast cancer related lymphoedema" (31). The enhancement in the lymphatic flow may be attributed to the improvement in the micro circulation (32).

The promising bio stimulatory effect of laser diode irradiation provides a viable tool in prevention of root resorption and ankylosis in replanted teeth (26), and considering the few reports on the effect of LLLT following replantation of teeth, and that its effect on root surface of avulsed teeth is not yet fully understood. The aim of this study was aimed to investigate the effect of root surface treatment with LLLT on replanted teeth with extended extra-oral time.

## MATERIALS AND METHODS

This study was conducted following the ethical guidelines for conduct of research on experimental animals, by the Faculty of Dentistry, Alexandria University (IRB NO: 00010556 – IORG 0008839).

Thirty adult male albino rats (200–250 g) were obtained from the animal house of the Institute of Medical Research, Alexandria University. The animals were housed under standard conditions, and fed on a standard diet and free access to water.

The animals were sedated by placing the rat in a container with cotton soaked in ethylic ether and then received an intramuscular injection of xylazine chloride (0.2 cc/100 g body weight) to attain muscular relaxation and they were anesthetized intramuscularly with Ketamine chloride (0.2 cc/100 g body weight). A combination of scalpel blade #15 and appropriately sized elevator placed between the central

incisors were used to negotiate the luxation of the left central incisor, using controlled force. The dental papilla and the enamel organ of the extracted teeth was removed through a retrograde approach using a pre-curved size 10, 15 and 20 Hedstrom file, followed by saline irrigation, and drying using paper points. The canal was then filled with calcium hydroxide paste (33).

Animals (n=30) were randomly assigned into two equal groups, 15 rats each according to the treatment such that: G1 (control n=15) = the extracted incisors were placed on a piece of dry gauze for 30 minutes before replantation and replanted with no laser treatment.

G2 (laser n=15) = the tooth was stored for 30 minutes on dry gauze, the tooth is then irradiated with LLLT (the root surface is subjected to the laser with scanning and sweeping movements along the long axis of the root; Moreover, the alveolus interior is treated with laser. After replantation, laser treatment was done on the palatal and lateral surface of the replanted tooth (34). A continuous-wave diode laser (Dr Smile) with an output power of 50 mW and a wavelength of 680 nm was used. The spot size was 0.02 cm<sup>2</sup> and the optical power density was 2.5 W/cm<sup>2</sup>. The rat's eyes were protected to prevent any vision impairment.

The teeth were replanted into their respective sockets and held in place until the hemorrhaging has stopped, the teeth were splinted by etching using phosphoric acid for 1 min followed by washing with distilled water and drying with cotton, bonding and application of flowable composite resin, which is then polymerized using a light cure. Thereafter, the rat was administered intramuscular injection of antibiotic using ceftriaxone (20,000 IU) at the day of the procedure. G2(laser n=15) after replantation the rats were administered LLLT every 48 hours for 15 days (34).

Animals of each group were blindly subdivided into 3 subgroups 5 rats each according to time of scarification such one of the subgroups is sacrificed 15, 30 and 45 days from the replantation procedure (34).

The rats were euthanized by an overdose of diethyl ether and the anterior portion of the maxilla dissected out.

### Histological analysis

The obtained specimen was placed in 10% neutral buffered formaldehyde and demineralized by trichloroacetic acid, then dehydrated in ascending concentrations of alcohol, cleared in xylene, infiltrated, and embedded in paraffin wax. 5 micrometer serial sections were cut transversely at the mid-root region and stained with H&E stain. The histological analysis was performed and captured under light microscopy (Optica B-380 Brightfield) in 100, 400X magnifications. The examiners were blinded to the treatment and were asked to note down the histological observations (35).

### Histomorphometrical analysis

Histomorphometric analysis was performed by means of a computer program Imagelab® 2.3. From each specimen, 5 serial transverse sections were obtained at the middle third of the root, as this is the area least affected by the surgical procedures; in addition, shows cemental and periodontal ligament attachment at this area of the root (36). From each section an image was taken and imported into the program, were the estimated total root surface area and the actual obtained area (root surface with resorption) were traced and areas obtained by the program; from which root resorption percentage were calculated using the formula: total area of tooth resorption times to 100 and divided by the total tooth

area. The same procedure was repeated for each of the 5 sections of the same specimen and the mean was obtained. These previous steps were repeated in each of the 5 specimens in each group. The results were expressed as percentage of root resorption in means and standard deviations, tabulated and analyzed statistically with ANOVA, followed by student's t-test after normality has been accepted, the significance level was set at  $\alpha=5\%$ .

**RESULTS**

**Histological analysis**

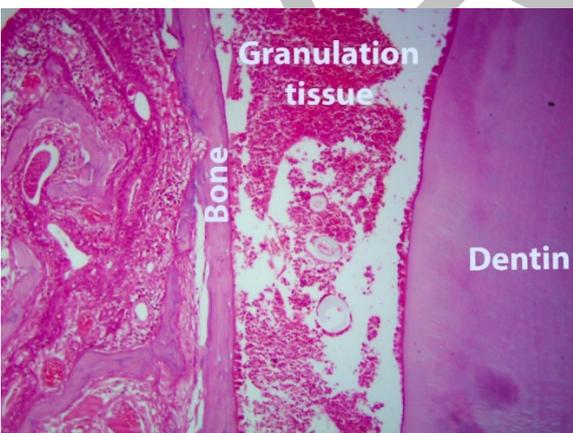
After 15 days of replantation, both groups showed loss of periodontal ligament attachment, the control group showed discrete areas of root resorption (figure1 and 2).

30 days from the replantation, it was evident larger areas of external root resorption in the control group, than that in the laser group. Areas of restoration of periodontal ligament attachment were observed in the laser group, with adequate vascularity (figure 3 and 4).

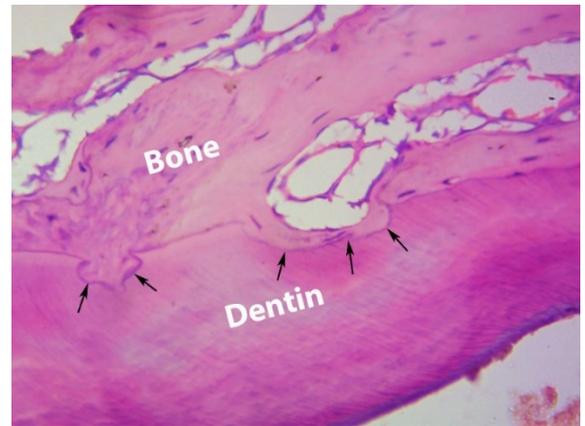
At the end of the experimental period both groups showed areas of external root resorption, but was more prominent in control group; the laser group showed areas of normal periodontal architecture (figure 5 and 6).



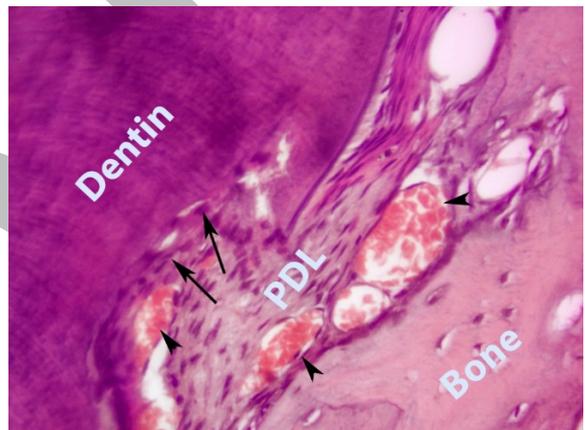
**Figure (1):** Light micrograph (L.M) {Control group 15 days} showing loss of periodontal ligament (PDL) attachment with area of root resorption (arrow). H&E (100x)



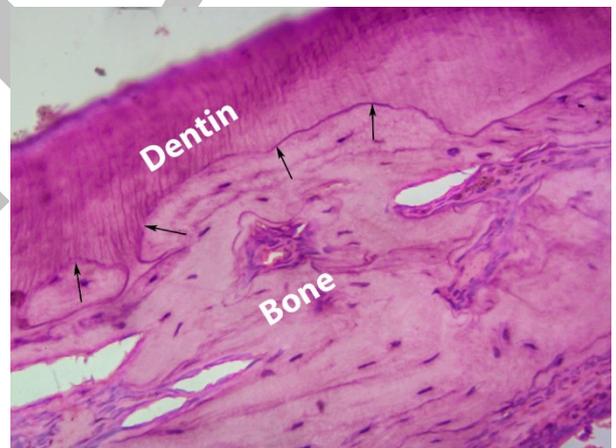
**Figure (2):** L.M. {laser group 15 days} showing loss of PDL with intervening granulation tissue. H&E (100x)



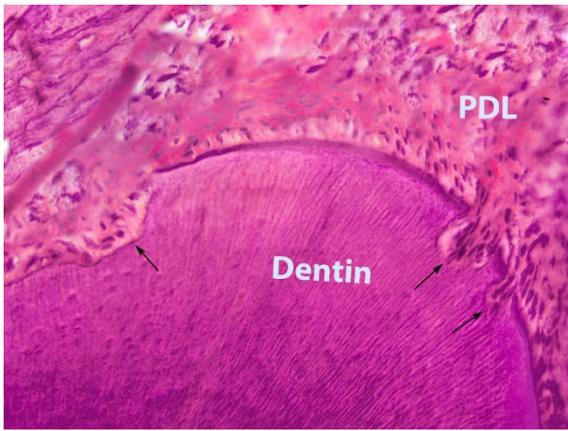
**Figure (3):** L.M. {Control group 30 days} showing areas of root resorption, with ankylosis, which is the direct attachment of the bone to the dentin (arrows). H&E (400x)



**Figure (4):** L.M. {laser group 30 days} showing areas of root resorption (arrows), with reestablishment of the PDL and increased vascularity (arrow heads). H&E (400x)



**Figure (5):** L.M. {Control group 45 days} showing extensive areas of root resorption with ankylosis (arrows). H&E (400x)



**Figure (6):** L.M. {laser group 45 days} showing areas of root resorption (arrows) with reattachment and restoration of normal architecture of the periodontal tissue. H&E (400x)

### Histomorphological analysis

The different values for root resorption in both experimental groups in the three observational periods were summarized in (table 1) in the form of means and standard deviation. An increase of root resorption areas was noted in both groups the longer the experimental periods. Analysis of the quantitative data revealed that there was a statistical significant differences between the control group and the laser group in all experimental periods ( $p < 0.01$ ) (table1).

### Statistical analysis

**Table (1):** Comparison between the two studied groups according to percentage of root resorption

	15 days	30 days	45 days
<b>Control group</b>	1.90 ± 0.37	8.71 ± 0.75	18.66 ± 1.45
<b>Laser group</b>	0.75 ± 0.18	5.15 ± 0.67	11.47 ± 1.22
<b>t</b>	7.411*	7.886*	8.502*
<b>p</b>	<0.01*	<0.01*	<0.01*

### t: Student t-test

p: p value for comparing between the two groups

\*: Statistically significant at  $p \leq 0.05$

## DISCUSSION

The study was conducted to access the effect of LLLT in modulation of the external root resorption in intentionally avulsed teeth of rat after being stored for 30 minutes post-extraction prior to reimplantation.

The Albino rats were chosen as a target species for the experiment mainly due the resemblances in dentoalveolar structure between the rats and humans in architecture (37). Secondly, rats are relatively inexpensive and readily available, which facilitates the use of large sample sizes and can be housed for long period of time. Thirdly, rats are easy to handle and can be obtained with different genomes and microbial status. Finally, the histological preparation of rat material is easier, than for example dog material (38).

The results of this experimental study revealed that groups irradiated showed significantly lower areas of root resorption and ankylosis, than the control groups, when analyzed quantitatively. When the specimens were assessed histologically, in addition to the lower areas of root resorption and ankylosis, it was noted that there was an increase in the blood supply in the groups treated with laser.

The observed results with the laser group, may be attributed to the modulation of the inflammatory process, which in turn is vital for diminution of the external inflammatory resorption. Vilela et al. (39) had shown similar observations, were they found that laser irradiation to the entrance of alveolus and root surface before replantation inhibited the osteoclastic action and enhanced the synthesis of formative cells such as: fibroblasts, cementoblasts, and osteoblasts.

Fonseca et.al. (40) had similar results, which showed that laser irradiation decreased the inflammatory root resorption and improved periodontal healing, they attributed this effect to the bio stimulatory property of LLLT by enhancement of positive effects on important biological activities, such as improved ATP synthesis, reduced edema and migration of inflammatory cells, decreased production of inflammatory cytokines and mediators, and increased fibroblast cell proliferation and collagen synthesis.

In the study group, the reattachment of PDL fibers were observed. This is agreement with studies done which concluded that LLLT may improve the wound-healing process of periodontal tissue since it can enhance the cell differentiation of fibroblasts (41, 42). LED or laser therapy can increase the expression of connective tissue growth factors, such as transforming growth factor and platelet-derived growth factor, leading to increased fibroblast proliferation, matrix synthesis and remodeling, and angiogenesis (43). These biological effects could be useful for preventing root resorption since they stimulate the proliferation of periodontal fibroblasts and capillaries that takes place during repair stages following active root resorption.

In the present study, there was an increase in the blood vessels in the groups treated with LLLT than in the control group, when examined histologically. This results corroborate with those of Fernanda-de, et al. (44), who tested the angiogenic effect of laser phototherapy in the periodontal tissue of replanted teeth, they concluded that LLLT caused a significant increase angiogenesis, when compared to non-irradiated groups.

Cellular signaling also has a major role in the angiogenic effect of laser phototherapy. Cury et al. (45) showed that both infrared and red lasers increased the expression of vascular endothelial growth factor (VEGF), hypoxia inducible factor (HIF-1 $\alpha$ ), and modulated matrix metalloproteinase (MMP-2) activity, which are three major mediators involved in angiogenesis. The VEGF plays a key role in angiogenesis by stimulating the proliferation and migration of endothelial cells and HIF-1 $\alpha$  regulates the cellular response to hypoxia by activating genes that are important to cellular adaptation under hypoxic conditions. The activity of MMP-2 is necessary for vascular basement membrane degradation and extracellular matrix remodeling, in order to allow the migration of endothelial cells during angiogenesis (45).

The findings of this present study differ from the results of Saito et al. (34), in which the treatment of the root surface and entrance of the alveolus with low power laser did not affect the healing process of tooth replantation in rats. They found no differences between the control group and irradiated group with regards to the occurrence of inflammatory and replacement resorption processes. Although the laser group demonstrated a more advanced stage in the healing process, this was without statistical

difference. Different results may be caused by the use of different methodologies, such as evaluation period, extra-alveolar time, and laser parameters.

## CONCLUSION

It was concluded from this study that LLLT can enhance the survival rate of replanted teeth. This can be attributed to reduction in both external root resorption and ankylosis, and enhancement of PDL attachment and angiogenesis.

## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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