

## Effect of 940 nm Diode Laser as Adjunct Treatment to Mechanical Instrumentation on Root Surface Gingival Fibroblast Adhesion in Periodontally Compromised Extracted Teeth : An in vitro Study

shabnam Aghayan<sup>1</sup>, Hooman Ebrahimi<sup>2</sup>, Ehsan Seyed Jafari<sup>3</sup>, Setareh Sadat Hashemi<sup>4</sup>, Maryam Sheidaeean<sup>5</sup>

<sup>1</sup>Assistant Professor, Department of Periodontology, Faculty of Dentistry, Tehran medical sciences, Islamic Azad University, Tehran, Iran

<sup>2</sup>Assistant Professor, Department of oral medicine, Faculty of Dentistry, Tehran medical sciences, Islamic Azad University, Tehran, Iran

<sup>3</sup>Associate Professor, Department of Biotechnology, University of Tehran, Tehran, Iran

<sup>4</sup>Member of Craniomaxillofacial Research Center, Faculty of Dentistry, Tehran medical sciences, Islamic Azad University, Tehran, Iran

<sup>5</sup>Dentist

*Received 4 December 2019 and Accepted 4 June 2020*

### Abstract

**Introduction:** Mechanical debridement of diseased root surfaces produces a smear layer that encompass microorganisms and residual cementum which may interfere with periodontal healing and regeneration of connective tissue attachment. Accordingly, this study aimed to determine impact of 940nm diode laser on adhesion of fibroblasts to root surface of extracted teeth from patients with chronic periodontitis. **Methods:** Twenty extracted single-rooted teeth with hopeless prognosis were collected and debrided with hand cures. Afterward, two specimens were obtained from each tooth by splitting them with a sterile diamond disk. Samples were submerged in fibroblast suspension and randomly divided into two groups. Group A comprised of 20 specimens subjected to scaling and root planing only and group B included 20 specimens which received SRP and 940 nm diode laser irradiation. The adhesion of fibroblasts was investigated by MTT and cell morphology was assessed with scanning electron microscopy (SEM). **Results:** The extent of adhesion was higher in group B compared with group A, though this difference was not statistically significant. In the laser group, fibroblast cells showed more elongated morphology and a smaller number of rounded forms was

found. But no significant difference was observed between the two groups. **Conclusion:** A diode laser with a wavelength of 940 nm has a negligible effect on adhesion of fibroblasts to the root surface of teeth extracted because of chronic periodontitis.

**Keywords:** Chronic periodontitis, Diode laser, laser(s), Fibroblast(s), Root planing.

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Aghayan SH, Ebrahimi E, Seyed Jafari E, Hashemi SS, Sheidaeean M. Effect of 940 nm Diode Laser as Adjunct Treatment to Mechanical Instrumentation on Root Surface Gingival Fibroblast Adhesion in Periodontally Compromised Extracted Teeth : An in vitro Study. J Dent Mater Tech 2020; 9(3): 123-129.

### Introduction

Pathogenesis of periodontal disease and approaches to treat it has endured fundamental alterations in the past 20 years (1). The present model for periodontal disease states that complex interactions between biofilm and the inflammatory immune response is in charge of nearly 80% of periodontal tissue destruction (2). Nevertheless, pathogenic biofilm is a crucial prerequisite for periodontitis. Removal of biofilm and all factors that facilitate its accumulation is primary goal in a non-

surgical therapy (1). Although mechanical debridement of diseased root surfaces has confirmed to be effective, it does not reestablish the original histological architecture of periodontium and also produces an amorphous layer of inorganic and organic debris including microorganisms, bacterial products and residual cementum called “smear layer”(3). Smear layer might act as a physical barrier between periodontal tissue and root surface preventing formation of new attachment (4) Hence, additional methods such as employment of chemotherapeutic agents or lasers have demonstrated further benefits over mechanical periodontal therapy alone (4). Soft tissue lasers with their ability for decontamination of root surfaces and promotion of tissue healing are a decent choice to use in a periodontally compromised dentition (5). In particular, diode laser has gained considerable attention in treatment of periodontal diseases (6). Diode laser does not interact with dental hard tissues, making it suitable for soft tissue procedures including cutting, coagulation, soft tissue curettage or pocket debridement (7).

Low-level laser therapy using diode laser with a wavelength of 600-1000 nm, has been claimed to have biostimulatory effect on various cells and tissue types through its capability to affect mitochondrial respiratory chain increasing adenosine triphosphate generation. This would enhance fibroblast proliferation, angiogenesis, growth factor release and collagen synthesis (8, 9). Indeed, periodontal wound healing is a series of interactions among periodontal tissue cells including gingival fibroblasts, osteoblasts, cementoblasts and periodontal ligament fibroblasts (10). As the most abundant structural cells in periodontium, gingival and periodontal ligament fibroblasts play a significant role in preservation of periodontal health and function (11).

Though low-level laser therapy has been considered as an adjunct periodontal treatment model, there are few reports on its effects on gingival fibroblast (12). This study aimed to evaluate effect of 940 nm diode laser as an adjunct to mechanical scaling and root planing on gingival fibroblast attachment to periodontally compromised teeth root surface extracted from patients with chronic periodontitis.

## **Materials and Methods**

### *General Design*

This in vitro cell culture study was conducted in compliance with Helsinki Declaration of 1975, as revised in 2013. Twenty single-rooted periodontally compromised human extracted teeth were included in this study. None of these teeth had caries, restorations, fractures or endodontic treatment. Teeth were extracted

due to hopeless periodontal prognosis in patients diagnosed with generalized chronic periodontitis who presented at Department of Periodontology, Faculty of Dentistry, Islamic Azad University of Tehran, and were collected after acquiring written informed consent from patients.

Exclusion criteria included presence of caries, restorative materials or hypoplastic lesions. Similarly, patients with a history of systemic conditions, antibiotic therapy three months before the study and patients who had periodontal debridement in the last six months were excluded from the study.

### *Sample Preparation*

After extraction, teeth were cleaned of blood, saliva, and soft tissue debris by a light scrubbing move with a sterile brush and then scaling and root planing (SRP) was performed by one examiner via a sickle scaler (Towner Jacques, U15/30, Double End Hu-Friedy Mfg Co. Inc. USA). Teeth were then preserved in sodium azide solution (0.2%; pH: 7.05) at 4°C until the time of treatment (13). Forty specimens (Two samples from each tooth) with size of 4 (Height) × 5 (Width) × 1 (Depth) mm were obtained from mesial and distal sides of the selected teeth by a sterile diamond disk (45µm, Diatech, Goltene AG, Altstatten, Switzerland) at low speed. The first section was selected 2 mm apically to the cemento-enamel junction and the second section was 2-3 mm coronal from the root apex(14) Samples were transported to the laboratory in a sterile flask and once more sterilized with CH<sub>2</sub>O<sub>5</sub> 20% solution.

### *Human Gingival Fibroblast Culture*

Dulbecco's modified Eagle's medium impregnated with 10% fetal bovine serum, 100 µg/ml streptomycin, 100 U/ml penicillin, 2.5 µg/ml amphotericin B and two mmol/l Glutamine was employed as cell culture medium (15-17). The human gingival fibroblast (HGF) cells (Purchased from the cell bank, Pasteur Institute, Tehran, Iran) were cultured at 37°C in an incubator with 5% carbon dioxide. Then, Fibroblast cells were harvested using sterile trypsin - EDTA solution, re-suspended in experimental cell culture medium and diluted to 1 × 10<sup>5</sup> cells/ml. Finally, 5 ml of fibroblast cell suspension was poured into polystyrene tissue culture containing root samples, incubated for three days and transferred to dental faculty to be irradiated with a diode laser.

### *Experimental Design*

Forty collected specimens were randomly allocated to two groups: Group A, used as control group in which 20 specimens subjected to scaling and root planing (SRP)

was performed by one examiner via a sickle scaler until all visible calculus was removed; and group B in which 20 specimens submitted to SRP and irradiated with 940nm diode laser.

#### *Laser treatment*

Diode laser (Epic10, Biolase, Irvine, CA, USA) was employed at calibrated power of 0.2 W, emitting a pulsed

light at 940 nm wavelength with low-level handpiece (diameter: 10 mm power density: 0.8 W/cm<sup>2</sup>, energy : 2J ,energy density: 2.5 J/cm<sup>2</sup>, exposure time:10 seconds) (Figure 1). Tip of the probe barely touched surface of polystyrene (5mm away from solution) and the angle of incidence was kept at a constant 90°. The light was focused and applied separately for each specimen (18).



Figure 1. The employed diode laser (Epic 10, Biolase, Irvine, CA, USA) at a calibrated power of 0.2 W, emitting a pulsed light at a wavelength of 940 nm, energy of 2J and energy density of 2.5 J/cm<sup>2</sup>.

#### *Scanning Electron Microscopic (SEM) Examination*

Each specimen was fixed in freshly prepared 4% glutaraldehyde in DPBS (Dulbecco's phosphate buffered saline solution (pH: 7.2) at room temperature for 2 hours and washed three times with DPBS for 10 min each. Then, specimens were dehydrated in a sorted sequence of aqueous ethanol (50, 70, 80, 95 and 100%) for 10 min in each concentration. After last step in 100% ethanol, dehydration was accomplished by a 30 min immersion in hexamethyldisilazane. Specimens were then air dried, mounted in scanning electron microscope (SEM) (Hitachi S-3400 N) operated at an accelerated voltage of 15-20 kV. All specimens were viewed at magnification values ranging from  $\times 2000$  to  $\times 3500$ . Briefly, three photomicrographs were taken from each root specimen and were considered to be representative of total surface area. The cell morphology was assessed with scanning electron microscopy. 3-(4,5-Dimethyl-Thiazol-2-yl)-2,5-Diphenyl-Tetrazolium Bromide Experiments (MTT)

3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay was performed for assessment of human gingival fibroblast adhesion as previously

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described (13). This test is centered on reduction of soluble yellow MTT tetrazolium salt to a blue insoluble formazan product by a mitochondrial succinic dehydrogenase. The rate of formation of formazan in cells represents number of living cells. Therefore, number of living cells can be obtained by MTT testing. After attachment of cells to the root surfaces, specimens were transferred to 24-well plates (TPP, Trasadingen, Switzerland). Later, plates were incubated with 200 ml medium containing MTT at a concentration of 0.50 mg/ml at 37°C for 4 hours. Each well then was washed with 200 ml of PBS and 200 ml of dimethyl sulfoxide was added to each one. To solubilize the formazan, test plate was agitated by microplate shaker for 30 minutes. Content of each well was transferred to 96-well plates for measurements. Finally, automatic microplate reader (Anthos 2020, Biochrom Ltd., UK) measured optical density at a wavelength of 590 nm.

#### *Statistical analysis*

Mean of three examinations per sample was entered in a spreadsheet in Microsoft Excel 2016 (Microsoft Corporation, California USA). The descriptive statistics (mean, standard deviation [SD], minimum and

maximum) were calculated for elongated, round and total number of fibroblasts in each of the groups. The difference between groups was calculated with Independent samples t-test . The level of significance was adjusted at 0.05 for all tests and data analysis was performed in SPSS 22.0 software (SPSS Inc., IBM Company, Somers, NY, USA).

## Results

According to One-Sample Kolmogorov-Smirnov Test, distribution of data in both groups and about MTT and quantity was not statistically significantly different with normal curve ( $P=0.104$ ). Based on the MTT assay, adhesion of fibroblasts was (Mean $\pm$  SD)  $0.16 \pm 0.03$  in diode laser group and  $0.15 \pm 0.03$  in the SRP group which the difference was not statistically significant ( $P =0.597$ ).

Thus, irradiation of root surfaces with 940nm diode laser did not show a significant effect on attachment of human gingival fibroblasts (Table I). Moreover, quantitative adhesion level was not statistically significant between groups according to the number of fibroblasts ( $P =0.722$ ), which was  $4789.30 \pm 1105.48$  in diode laser group and  $4374.35 \pm 1109.30$  in SRP group (Table II).

SEM analysis revealed a more elongated cell morphology and a smaller number of rounded fibroblasts in diode laser group compared with SRP group, though the difference was not statistically significant ( $P =0.597$ ) (Figure 2). Additionally, in diode laser group more elongated cells were observed than round cells, although this difference was not statistically significant ( $P =0.243$ ) (Table III).

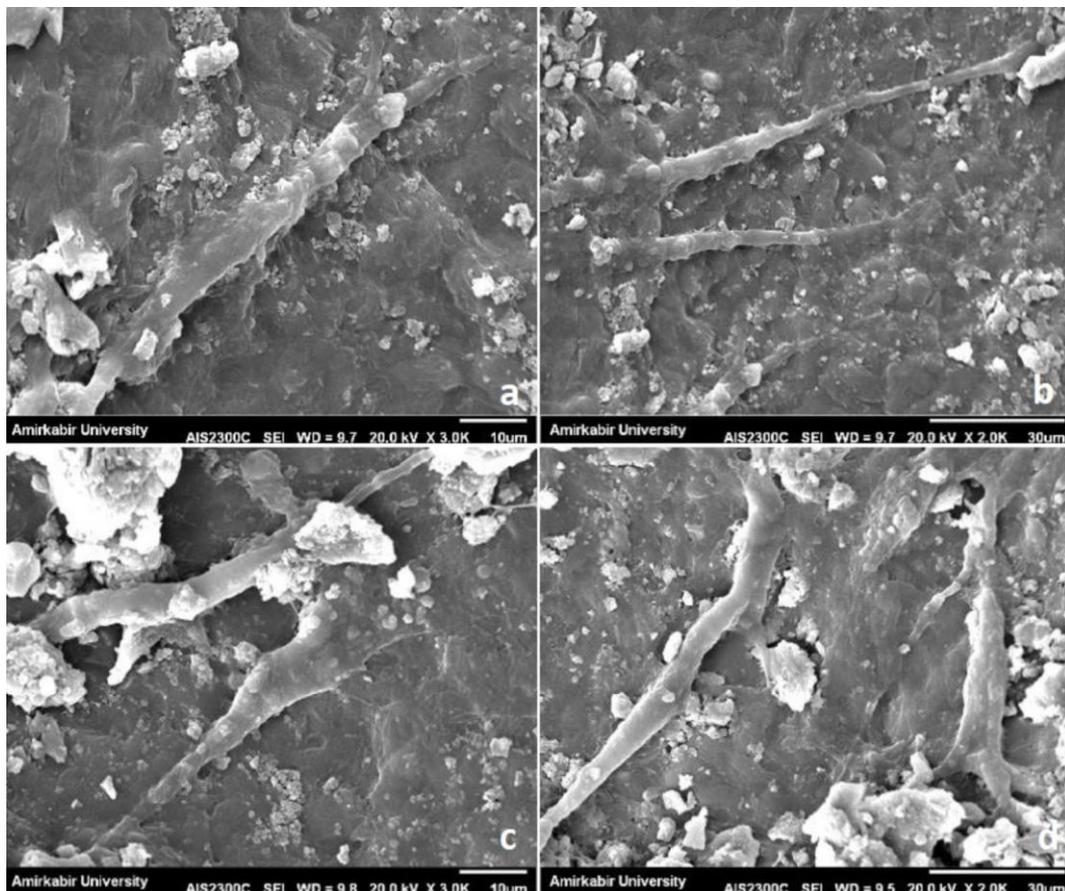


Figure 2. SEM microphotograph of the specimens. (a), elongated fibroblast in the diode laser group ( $\times 3000$ ). (b), elongated fibroblast in the diode laser group ( $\times 2000$ ). (c), elongated fibroblast in the SRP group ( $\times 3000$ ). (d), elongated fibroblast in the SRP group ( $\times 2000$ ).

Table I. Comparison Mean±SD of adhesion level of fibroblasts according to MTT assay

groups	Mean	SD	Minimum	Maximum	P value=0.597
<b>SRP only (n=20)</b>	0.145	0.033	0.074	0.208	
<b>Diode laser (n=20)</b>	0.158	0.033	0.085	0.205	

Table II. Comparison Mean±SD of adhesion level of fibroblasts based on cell number (Quantitative adhesion).

groups	Mean	SD	Minimum	Maximum	P value=0.722
<b>SRP only (n=20)</b>	4374.330	1109.311	1987.66	6454.33	
<b>Diode laser (n=20)</b>	4789.330	1105.567	2354.33	6354.33	

Table III. Comparison Mean±SD of fibroblast cells with elongated or rounded configuration, as revealed by SEM m

Groups	Flat	Round	P-value
<b>SRP only (n=20)</b>	3124.53 ± 792.35	1249.81 ± 316.94	0.121
<b>Diode laser (n=20)</b>	4105.11 ± 947.55	684.18 ± 157.85	0.243
<b>P-value</b>	0.597	0.579	

icrophotograph at a magnification of ×2000 to ×3500.

### Discussion

On the contrary to positive effects of laser treatment, numerous questions remain unanswered such as fibroblast attachment to treated root surfaces. The current study aimed to investigate fibroblast adhesion to root surfaces treated by a 940nm diode laser.

In the present study, quantitative adhesion level of fibroblasts in diode laser group was higher compared to SRP group, but no statistically significant difference was found (P > 0.05). In a study by Negi et al. (14), level of adhesion was significantly higher in laser-treated groups compared to SRP-only and the control group, which is inconsistent with our findings. Flat fibroblast with their tightly attached lamellipodia characterizes a normal cell whereas round fibroblasts with fewer attachment projections were immature fibroblasts. Hence, difference of wavelength lead to significant different results.

Basso et al. (5) mentioned that exposure of fibroblasts with energy density of 0.5 and 3 J/cm<sup>2</sup> and power of 0.025W caused a significant increase in cell metabolism compared to non-irradiated group. Both energy doses promoted a significant increase in cell number as well as cell migration. In the present study, quantitative adhesion level of fibroblasts in diode laser group was higher compared to SRP group, but no statistically significant difference was found. The contrasts in the results could be due to different wavelengths, times of irradiation and different power applied.

In a study by Choi et al. (10), there was a significant difference in cell proliferation in laser-treated group after 24 and 48 hours, but no significant difference was found with control group after 72 hours. The authors stated that GaAlAs semiconductor diode laser promoted proliferation and differentiation of human PDL fibroblasts. Due to inherent characteristics of 810 nm

laser that is acceleration in wound healing and enhance tissue regeneration, results were in contrast to the current study. This could be explained because of different wavelengths used.

In a study by Kreisler et al. (12), analysis of 150 specimens revealed no significant differences between groups. However, cell numbers were slightly higher on laser specimens. Authors concluded that diode laser at parameters used did not have a substantially positive effect on new attachment of PDL cells on tooth specimens. Results of their study was along with the results of current study. It should be mentioned that not only laser wavelength and/or group could have an effect on cells and tissue phenomenon, but also the treatment setting is an important issue. The power higher than 1W might cause risk of carbonization on root surface (12).

In a study by Hakki et al(15), both laser set-ups provided a biocompatible surface for survival of fibroblasts. Surface which was treated with short pulse Er,Cr:YSGG seemed to provide better condition for fibroblast attachment than specimens treated by long pulse laser or hand instruments. It should be noticed that because of ablation effect of erbium family lasers, it is imperative to use the set-up that do not have an alteration effect of root surfaces.

Due to several different parameters such as wavelength of laser system, power output, mode of operation, time of exposure, distance of the tip from the specimen, working angle and lack of abundant studies on 940nm diode laser, it is challenging to compare results from different investigations.

The application of diode laser with a wavelength of 940 nm and parameters used in this study, did not affect fibroblasts cultured on the root surfaces. Quantitative cell adhesion was marginally higher in laser-treated group than in SRP group. The difference was not significant and probably not of any clinical importance.

The findings of this research indicate that diode laser as an adjunct treatment to mechanical debridement has a questionable role in improving fibroblast adhesion and new connective tissue attachment formation. Additionally, in vitro studies are indispensable to approve application of diode laser and to recommend ideal laser properties for use in pocket debridement.

## Conclusions

Within the limitations of this study, it was found that diode laser with a wavelength of 940 nm has an insignificant effect on degree of adhesion of fibroblasts to root surface in teeth extracted because of chronic

periodontitis. However, there are still uncertainties about laser biologic mechanism, which requires further studies with larger sample size and more extended period.

## Conflict of Interest

No potential conflict of interest relevant to this article was reported.

## Acknowledgment

There is no acknowledgment for this study.

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#### **Corresponding Author**

Shabnam Aghayan

Assistant Professor, Department of Periodontology, Faculty of Dentistry, Tehran medical sciences, Islamic Azad University, Tehran, Iran

Tell: 02188566509

Email: shabnamaghayan@yahoo.com