



## Evaluation of CO<sub>2</sub> laser irradiation effect on enamel microhardness after incipient caries creation

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### ABSTRACT

*Objective: The important mission in modern science of caries prevention is identification and providing the necessary actions for caries prevention to patients with an elevated risk of caries. The aim of this study was evaluation of CO<sub>2</sub> laser irradiation effect on enamel microhardness after incipient caries creation. Material and methods: In this in vitro-experimental study, for evaluation of enamel microhardness 30 teeth after disinfection process were divided randomly into three groups A, B, C [n=10]: A] Control [normal saline] B] Immersed in cariogenic solution for 1 month C] Immersed in cariogenic solution for 1 month + CO<sub>2</sub> laser [10.6μm, 10Hz, 0.5W, 20s, beam diameter 0.2 mm]. Data analyzing was used by 16 SPSS software. Parametric one-way ANOVA and Tukey were used for surface microhardness at 0.05% significance level. Results: According to one-way ANOVA parametric test, there was a significant difference between three groups [p=0.047]. In the following, results of Tukey test showed that there was a significant statistical difference between the microhardness of control and other groups [P=0.038]. On the other hand, there wasn't statistical difference between A, C and B, C group means [P>0.05]. Conclusion: These study findings showed that CO<sub>2</sub> laser irradiation on enamel surface with incipient caries had no significant effect on surface microhardness enhancement.*

**Keywords:** Incipient caries, microhardness, CO<sub>2</sub> laser

### INTRODUCTION

The most important goal in the modern science of “tooth decay prevention” is to identify and provide the necessary care to those patients in the high risk of tooth decay in order to prevent this issue. Various techniques have been tested in recent years, but laser therapy has been the most promising one [1]. Appearance of the white spot indicates a sub-surface porosity in the enamel caused by the demineralization of the enamel [2]. As the studies indicate, the primary tooth decay complications are more common than cavity disorders and this can indicate a higher rate of tooth decay in future [3]. One of the major causes of formation of white spots on the surface of the tooth, tooth decay and gum inflammation is utilization of fixed tools for orthodonty which may influence the beauty and success of any types of treatment [4-6].

Recent researches have reported the formation of the white spots following utilization of fixed orthodonty devices among 73 to 95% of the cases [7]. Recently, several methods have been proposed to prevent demineralization and gum inflammation [2, 4]. The most important therapeutic measure taken against it is the appropriate mouth hygiene

and using tooth pastes which contain fluoride. Other methods include using mouthwash, varnish and adhesives [2, 8].

On the other hand, various types of lasers are used today to prevent and treat decays. Some of the most common types of laser used in this field with various powers include: Ruby, CO<sub>2</sub>, Neodymium: Yttrium-Aluminum-Garnet [YAG] and Argon. The application of these lasers to prevent tooth decay has been investigated substantially [9].

CO<sub>2</sub> laser is the first type of medical laser approved by the Food and Drug Administration [FDA] of the US [7]. As various researches indicate, CO<sub>2</sub> laser enhances the resistance of the enamel and dentin and reduces demineralization [10].

Correa-Afonso et al also said that CO<sub>2</sub> laser can enhance the resistance of dentin against acid [11]. Seino et al also showed that CO<sub>2</sub> laser is capable of preventing demineralization around the brackets of orthodonty[12]. The results of the studied conducted by Stangler et al indicated the higher hardness for enamel adjacent to orthodonty brackets after using CO<sub>2</sub> [2]. Less solubility of the enamel following the laser radiation due to chemical and physical changes is caused by the photo-chemical and photo-thermal effects of the laser [13]. The present research seeks to study the influence of CO<sub>2</sub> laser radiation on the hardness of the enamel after the initial decay.

## MATERIALS AND METHODS

30 healthy, recently-pulled, human, premolar teeth without any decay, crack, or previous repair which had a sound and healthy enamel surface were selected and randomly placed in three test groups.

### *Demineralization*

First, the teeth of each group [n= 10] was placed inside three separate and marked bottles and sterilized using the autoclave machine [ReyhanTeb, Iran] in a temperature of 121 °C and a pressure of 15 pounds for 15 minutes. The teeth were then randomly divided into 3 positive control groups [A, B, C]. Group A teeth were stored in normal saline for 30 days. Group B and C teeth were also placed inside a vial containing TSB [Tryptone Soy Broth] for demineralization. Streptococcus mutans bacteria with an opacity of 0.5 McFarlane was inoculated for 30 days and all the groups were placed inside an incubator [Shimaz, Iran] with a temperature of 37 °C. To control the process of preparing 0.5 McFarlane solution [equal to  $1.5 \times 10^8$  bacteria in each milliliter][14], a spectrophotometer device [A & E Lab. Guangzhou, P.R. China] with the wavelength of 625  $\mu\text{m}$  and an absorption range of 0.8 to 0.13 was utilized.

To prevent the bacteria from moving to the death phase from the phase of growth, some 30% of the TSB suspension was removed every other day and it was replaced by fresh culture medium. To check for other types of microbial contaminations in the test and control group environments, samples were regularly taken from the groups and cultivated linearly on the Blood Agar cultivation medium[15]. However, no microbial contamination was observed in the mediums. It is worth mentioning that all the phases were conducted under the supervision of the Microbiology Lab Specialist of Hamedan University of Medical Sciences.

### *Laser*

Having caused some degree of surface demineralization in groups B and C, the Buccal surface of group C teeth was radiated by CO<sub>2</sub> laser (DEKA Laser Technologies, Florence, Italy). The specifications of this laser are as follows: a wavelength of 10.6  $\mu\text{m}$ , a pulse duration of 3 seconds, a frequency of 10 Hz, a radiation diameter of 0.2 mm, a level of 1.5, and a power of 0.5 w. The laser was radiated in the sweeping manner from a distance of 5 mm.

### *Measuring the hardness*

Measuring the hardness of the enamel was accomplished using the MH 210 Hardness Gauge device [Mitech, Beijing, China] based on Vickers test. The hardness was tested three times on the surface of each tooth and then, the average hardness was set as the microhardness of the surface. The results were then analyzed using one way variance and Tukey test on SPSS version 16.

## RESULTS

Table (1) represents the average, standard deviation, the least and the most levels of micro-hardness among those three groups. The results of assessing microhardness data using Kolmogorov Smirnov Test indicated a normal distribution of data.

**Table 1. Average, standard deviation, the least and the most values of microhardness**

Groups	Average	Standard deviation	The least	The most
control	117.5	15.813	96	148
without laser radiation	98	9.006	90	122
with laser radiation	106	22.491	89	167

Thus, the parametric test of variance analysis was used to compare the microhardness values across all groups. According to the results of the one-sided variance test, the average of the three groups exhibited a significant difference [ $P = 0.047$ ].

Tukey test was used to compare the averages in pairs. The final results indicated a significant difference between the first and the second group. In other words, the first group exhibited more hardness compared to the second group [ $P = 0.038$ ]. On the other hand, no significant difference was observed between the averages of the first and the third, and the second and the third groups [ $P > 0.05$ ]. The results are presented in table (2).

**Table 2. The pairwise comparison of the groups' averages according to Tukey test**

Groups	Difference of the averages	Standard deviation error	Significance
control with laser radiation	19.5	7.47	0.038
without laser radiation	11	7.47	0.32
with/without laser radiation	-8.5	7.47	0.5

## DISCUSSION

In the present study, 30 premolar teeth without buccal decay selected and chosen for research. The premolar teeth are among the most common teeth which suffer from the whitening complication after orthodonty treatments. On the other hand, utilization of the teeth pulled for the purpose of orthodonty is more common than Bovine teeth and third molar teeth. For this purpose, Featherstone *et al* and Hsu *et al* used human, premolar, pulled teeth to investigate the role of laser in reducing tooth decay [13, 16].

In the studies conducted by Featherstone, Kantorowits, Hsu *et al*, the enamel was exposed to the decay process so that the influence of CO<sub>2</sub> and fluoride on preventing demineralization can be assessed. Farhadian *et al*. observed that within 4 weeks after placing the brackets of the orthodonty, the initial decay complications were observed [17, 18]. We used CO<sub>2</sub> laser with a wavelength of 10.6  $\mu\text{m}$  whose efficiency has been proved in the studies conducted by Featherstone, Kantorowits, Miresmaeili, Poosti *et al* [19-21]. In the study conducted by Seion *et al*, CO<sub>2</sub> laser with a wavelength of 10.6 has been considered to be more effective than Nd: YAG and even similar to the influence of fluoride [12]. In the study conducted by Correa-Afonso, the effectiveness of CO<sub>2</sub> laser in reducing the water and carbonate contained in the enamel after laser radiation has been proved. This is indicative of its significant role in reducing the decay [11].

The present research was conducted in the lab conditions and the most significant difference between it and other studies in this field is the radiation of CO<sub>2</sub> laser after creating an acceptable level of demineralization. The results point to a statistically significant difference in terms of the level of microhardness among the groups before and after demineralization. However, the microhardness among various groups studied was not statistically significant. In this respect, the results of this research were different from those of Poosti, Miresmaili and Seino [12, 19, 20].

The present research utilized a radiation power of 0.5 w which was less than the radiation power used in the study of Miresmaeili *et al* [19]. As it is believed that the energy caused by radiation on the exterior surface of the enamel can cause changes in the form of melting, adhesion, or re-crystallization without even being harmful to the lower layers and it stops the further development of decay [17], we may conclude that the reduction of porosity results in less surface changes and it can't change the surface microhardness significantly. This research was conducted under the laboratory conditions and, contrary to the study conducted by Miresmaeili *et al*, it did not benefit from the conditions of re-mineralization and fluoride adjacency. It is noteworthy to know that surface treatment with laser in the presence of fluoride can change the Hydroxyapatite to Fluoroapatite [19].

Contrary to the study of Poost *et al.*, this research did not use fluoride to investigate the influence of laser radiation in the process of enamel re-mineralization. They arrived at the conclusion that utilizing CO<sub>2</sub> before fluoride therapy can have a major role in reconstructing the enamel hardness after decay [16]. In line with this point, researches have shown that absorption of fluoride in the enamel under laser radiation takes place more effectively and this can improve resistance against acid compounds and microhardness properties [22].

Seino et al also studied the influence of radiating CO<sub>2</sub> laser with a wavelength of 10.6 μm and Nd: YAG laser with or without using fluoride local gel in preventing enamel decay around the brackets of orthodonty[12]. The difference between their studies is also the investigation of laser radiation in preventing decay. However, we set to investigate the therapeutic effect of CO<sub>2</sub> laser.

It is also necessary to note that despite the lack of any significant differences among the groups studied in our research, the average microhardness of the enamel enhanced after laser radiation. We may, thus, conclude that enhancing the radiation power and the number of samples can improve the results.

### CONCLUSION

As the results indicate, radiating CO<sub>2</sub> laser on the surface of the enamel suffering from primary decay has no significant influence on increasing the microhardness.

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