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# Effect of 532 nm and 671 nm Diode Lasers Irradiation on Calcium Solubility in Human Dental Enamel: An *Ex-Vivo* Study

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Author NAA designed the study and wrote all the protocol in addition to the experimental procedure. Author EMA did the experimental work, collect the data and made the analysis. Both authors read and approved the final manuscript.*

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## ABSTRACT

**Aim:** This study aimed to measure the amount of calcium dissolved from dental enamel of extracted human teeth immersed in lactic acid solution after irradiation by 532 nm (100 mW) and 671 nm (100 mW) continuous wave diode lasers.
**Methodology:** One hundred and forty freshly extracted human premolar teeth were used. Samples were prepared by cutting each tooth into two cubic specimens of 4x4 mm enamel surface (one for irradiation and the other as control).The surfaces of the cube were coated by acid resistant varnish leaving the 4x4 enamel on one surface uncovered. Irradiation was done for each wavelength with 4, 8, 12 and 16 minute intervals. The control and the irradiated specimens were immersed in 3 ml lactic acid (pH 4.4) for 24 hours under a temperature of 37°C. The amount of calcium dissolved was measured by an atomic absorption spectrophotometer. Comparison between variables was done by ANOVA and Chi-square tests with the level of significance set at $p \leq 0.05$.

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**Results:** The findings of this study showed that the percentages of calcium dissolved in the laser groups were less. Eight minutes irradiation time revealed the highest percentages of teeth with less calcium content (for 671 nm diode laser–81.25% and for 532 nm–78.60 %).The mean of calcium dissolved in acid decreased with increasing the irradiation time with *P* values of 0.013 and 0.000 for 532 nm and 671 nm, respectively.

**Conclusion:** Low power diode laser S of wavelengths 532 nm and 671 nm showed a decrease in calcium solubility in comparison to the control group; so it is a promising result in caries prevention. By increasing irradiation time, to a certain value, resistance to acid dissolution also increases.

*Keywords: Caries prevention; diode laser; calcium dissolution; low power lasers.*

## 1. INTRODUCTION

Dental caries is a chronic and destructive disease to the teeth and according to the World Oral Health Report (2003) and remains a major public health problem in most developing and under developing countries [1]. Conventional methods for caries prevention, such as receiving fluoride, or new methods, like using lasers, are still challenging. Fluoride acts effectively against dental caries, but some individuals who receive fluoride still develop pit and fissure caries–such a finding indicates that fluoride is effective only in areas of smooth surfaces. Pit and fissure sealant has been developed to overcome this problem [2]. Pits and fissures are more liable to dental caries because they are normally too narrow for the removal of accumulated bacterial plaque by the bristles of a tooth brush, however complete or partial loss of sealant and secondary caries is common [3]. The mechanism of caries prevention suggested by fluoride or laser is to make enamel more resistant to acid dissolution by changing its structure. Laser enhances the uptake of fluoride and although there is no absolute prevention, it decreases the lesion depth and enhances the re-mineralization of enamel [4].

Also, as conventional methods used in caries prevention are costly, time consuming and need sustainability, the use of laser, especially cold lasers (soft of low level lasers), which is characterized by low cost, is portable, not of heavy weight, and provides easy application and handling, can be an alternative [3].

The laser-tissue interaction is controlled by irradiation parameters: wavelength, continuous or pulse emission, repetition rate, pulse duration, pulse energy, beam size and delivery method, and optical properties of the tissue. The optical properties are characterized by the refractive index (transmission), scattering coefficient (scattering) and the absorption coefficient (absorption). Diode lasers in the visible region are absorbed weakly in the enamel. Theories regarding the mechanism by which laser irradiation enhances enamel resistance to artificial caries range from a physical seal achieved by melting the surface through partial fusion and re-crystallization of enamel prisms to a change in enamel composition only [3-5]. The use of laser in the field of dentistry has been a subject of discussion ever since lasers were introduced and studies of using soft (low powers) lasers for caries prevention are scarce. These few studies focused on studying laser alone or in conjunction with fluoride. Interestingly there are no previous studies in literature for the use of these two wavelengths of diode laser (523 nm and 671 nm) for caries prevention.

This study aimed to evaluate the effectiveness of low power diode laser S with two different wavelengths (532 nm and 671 nm) in caries prevention (ex-vivo) by measuring the amount of calcium dissolved after irradiation and to assess the effect of irradiation time in the dissolution process.

## 2. MATERIALS AND METHODS

The materials used in the study were:

- Diode lasers (continuous wave) of two wavelengths: 532 nm and 671 nm, each with an output power of 100 mW, supplied from Roithner Laser Technik – Austria.
- Lactic acid (extra pure $C_3H_6O_3$) supplied from Loba – Chemie, India.
- Atomic spectrophotometer (model 410) supplied from Sherwood, UK.
- One hundred and forty premolars teeth extracted from young adult patients undergoing orthodontic treatment by fixed appliances.

The procedure was done as follows: after approval of the study by the ethical committee of the University of Medical Sciences and Technology, the experiment and the purposes of the study were explained to the patients having their teeth planned for extraction for orthodontic treatment to leave their teeth voluntarily with informed consent. The teeth specimens were cleaned well and dried using a clean towel. A 4 x 4 mm area of enamel surface from buccal and lingual sides were marked using a pencil (the area was marked approximately simulating the area where smooth surface caries occurs). Each tooth was bisected longitudinally from its center into two halves. From each half a 4 x 4 mm area was cut using a fine separating disc forming a cubic shape with six surfaces [6]. Five surfaces were coated with acid resistant varnish leaving the sixth surface of the enamel uncovered. The cubic samples from one side acted as a study for laser irradiation sample while the other side acted as a control without irradiation.

Each of the two specimens, one for laser irradiation and the other as control, were kept in a 10 ml plastic tube filled with deionized water till the experiment procedure. The tubes containing the specimens were numbered for easy identification for each group according to the two wavelengths of diode laser used (532 nm and 671 nm). Each specimen was adjusted so as the uncoated area faced the laser beam (diameter of 4 mm) directly. For each laser type there were four groups according to different exposure times (four, eight, twelve and sixteen minutes) with a laser wavelength of 532 nm – 15 specimens for each time interval with a total of 60, while laser wavelength of 671 nm–20 specimens for each time interval with a total of 80. For each study group there was an equivalent number of control specimens without irradiation. Time was adjusted using a stopwatch and laser safety measures were taken. Specimens from laser and control groups were stored in clean, empty and dry tubes. For each tube containing either irradiated or controlled specimens, three ml of 4.4 pH lactic acid was added. The tubes were stored in a water bath for 24 hours at 37°C and 100% humidity. The amount of calcium ion ($Ca^{+2}$) dissolved in each solution (in µg/ml) was determined by an atomic absorption spectrophotometry technique. Data was analyzed using the SPSS software (version 17; SPSS Inc., Chicago, IL). Data was presented in the form of tables. ANOVA test was used to compare the means of calcium dissolved according to different irradiation times; Chi-square test was used for non parametric data and T test for parametric data. Statistically significant differences were assessed, with the level of significance set at $P$ value ≤ 0.05.

## 3. RESULTS

(Table 1) lists the results of using diode laser at wavelengths of 532 nm and 671 nm which revealed that increasing the irradiation time from 4 to 8 minutes, raised the percentage of teeth with less calcium dissolution to 78.6% in the laser group compared with 21.4% in the control group ($P$ = 0.043) for 532 nm while in case of using the diode laser with 671 nm wavelength, irradiation for 8 minutes led to the highest percentage of teeth with less calcium dissolved compared with the control group ($P$ = 0.000). For the two wavelengths, when the irradiation time was increased to 12 and 16 minutes, the percentage decreased compared to 8 minutes but still more than the control group. The mean of calcium dissolved in µg/m in case of 532 nm, decreased when irradiation time increased resulted in less calcium dissolution ($P$ = 0.013), while in case of 671 nm the mean of calcium dissolution, revealed that 8 minutes irradiation showed less calcium dissolution compared with other times, and increasing the time led to decrease the calcium dissolution to certain amount and became stable after that ($P$ = 0.000) (Table 2). The pattern of calcium dissolution, according to the number of teeth, was almost the same in case of the two laser wavelengths (532 nm and 671 nm). The mean difference of calcium dissolved by the two wavelengths showed no statistical difference as listed in (Table 3).

## 4. DISCUSSION

The results obtained in this study are promising. It was justified *in vitro*, as the oral environment can be simulated; the enamel mineral is present in form of biological apatite. Demineralization of the enamel leads to loss of minerals primarily calcium. The phosphate groups of hydroxyl apatite can be protonated in an acid environment causing them to dissolve. This phenomenon was exploited in an in vitro experiment in order to simulate the caries process. Buffer solutions with a pH of less than 5.5 are generally used. The loss of calcium is often dependent on the pH of acid solution used, temperature, humidity, and length of application. In this study, lactic acid (pH 4.4) was used to demineralize enamel which is thought to be a condition under which dental caries is formed in the oral cavity (enamel dissolves and caries starts when the pH of the oral cavity drops] to less than 5.5). The temperature and humidity were kept similar to that of the oral cavity. Therefore this technique could provide a similar environment for the detection of calcium loss as in the oral cavity, but a draw back and limitation is that oral cavity containing saliva that buffers the pH, also there is continuous process of demineralization and re mineralization based on the presence of dissolved minerals and changes in the pH. Also the presence of bacterial flora and immune system of the host against caries process may be a drawback.

Studies concerning the use of low power lasers for caries prevention are scarce; the use of high power laser, still not commonly used, has however been proven by many studies to be effective [5-7]. Interest in diode lasers has grown steadily since its invention due to its inherent advantages and its range of applications with a wide range of wavelengths. Several other laser types have shown good results in caries prevention [8-10]. However, there were few studies on dental tissue interactions using high power diode lasers [11] or low power diode lasers [12-14]. The mechanism by which laser is thought to produce acid resistance is still controversial. The most prominent hypothesis, by which hard laser cause an effect in decreasing acid solubility, is based on thermal changes in dental enamel. After heating enamel to a temperature between 300°C and 400°C, in an experiment concerned with demineralization, revealed a relative solubility minimum along with slight lesion depths [15]. For instance, the water permeability of dental enamel was seen to be lower after heating. More hydroxide and pyrophosphate, but less carbonate, is also generally found in comparison with unheated enamel [15].

Low power visible and near-infrared lasers appear to be an appealing alternative for other lasers or other mechanisms of caries prevention. The results of this study are in agreement with the theoretical explanation of the change in organic substances; based on the assumption that the demineralization of dental enamel depends, among other factors, on organic

**Table 1. Percentage of teeth with decreased calcium dissolution among those irradiated by diode laser (at 532 and 671 nm wavelengths) and teeth in control groups at different time intervals**

| Time in minutes | Number and % of teeth in the control groups | | Number and % of teeth in the Laser groups | | Total number and % | |
|---|---|---|---|---|---|---|
| | 532 nm | 671 nm | 532 nm | 671 nm | 532 nm | 671 nm |
| 4 | 7 (46.7%) | 6 (35.29%) | 8(53.3%) | 11 (64.71%) | 15(100%) | 17 (100%) |
| 8 | 3(21.4%) | 3 (18.75%) | 11(78.6%) | 13 (81.25%) | 14(100%) | 16 (10%) |
| 12 | 5(38.5%) | 5 (27.77%) | 8(61.5%) | 13 (72.22%) | 13(100%) | 18 (100%) |
| 16 | 5(38.5%) | 4 (28.41%) | 8(61.5%) | 12 (70.58%) | 13(100%) | 16 (100%) |

*P value for 532 nm = 0.043) (P value for 671 nm = 0.000*)

**Table 2. Comparison between mean of calcium dissolution (µg/ml) among teeth lased by diode laser (532 nm & 671 nm wavelengths) for different irradiation times**

| Time (minutes) | Number of teeth | | Mean (µg/ml) | | Standard deviation | |
|---|---|---|---|---|---|---|
| | 532 nm | 671 nm | 532 nm | 671 nm | 532 nm | 671 nm |
| 4 | 15 | 17 | 28.40 | 24.15 | 7.453 | 2.815 |
| 8 | 14 | 16 | 23.40 | 20.27 | 7.049 | 5.946 |
| 12 | 13 | 18 | 25.64 | 21.17 | 5.500 | 3.480 |
| 16 | 13 | 16 | 21.13 | 21.12 | 3.378 | 3.278 |

*(ANOVA test) P value for 532 nm groups = 0.013 & P value for 671 nm group = 0.000*

**Table 3. Comparison between the mean (µg/ml) of calcium dissolution after irradiation by diode lasers of 532 nm and 671 nm wavelengths**

| Diode laser wavelength (nm) | Mean (µg/ml) | Standard deviation | P value |
|---|---|---|---|
| 532 | 24.63 | 6.512 | 0.658 |
| 671 | 25.18 | 6.785 | |

components. The organic matrix is located in the inter-prismatic and intra-prismatic spaces and thus exactly in the diffusion path of the demineralization process. Laser irradiation destroys the organic components and the decomposition products block the diffusion path of the ions [15]. We think that there is a change in the organic components of enamel, loss of carbonate (the weakest part that initiates caries process), creation of micro-pores and re-precipitation of dissolved minerals. This theory needs confirmation. The use of low power diode lasers with these two wavelengths is promising in that they can provide a new approach towards caries prevention as they inherit a low cost when compared to other types of lasers. Also, they are not heavy in weight and are portable, safe to the pulp, show no rise in temperature and no heat transmission, are easy in handling and can be used via optical fiber.

Increasing irradiation time to a certain value increased the effect as more energy is deposited when increasing time and consequently the chemical composition of the exposed enamel will be affected, especially when the energy absorbed is high enough to drive organic substances and carbonate out of the enamel crystals resulting in more acid-resistant apatite crystals. The results showed a high caries reduction effect in 8 minutes time, then it decreased to reach a level of saturation, to a point where increasing the time of irradiation did not make a change; a similar result was obtained by Apel, et al [6].

## 5. CONCLUSION

- Diode lasers with wavelengths of 671 nm and 532 nm showed better results compared with the control, where 671 nm showed less calcium dissolved of 81.25% compared with the control, while 532 nm showed 78.6%.
- There is no statistical significant difference between the mean in µg/ml of calcium dissolved in case of 671 nm and 532 nm wavelengths; the *P* value was 0.658.
- Low power visible diode lasers have a considerable effect on prevention of calcium dissolution by lactic acid *ex-vivo*, hence resistance to an artificial caries-like process.

It is recommended to use SEM for some of the specimens so as to see the difference between the surfaces of lased and the control groups after acid dissolution.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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