Original Article

Energy Dispersive (Edx) Analysis of Structural Changes on Human Dental Enamel Trated with Co2 (10.60nm) and Diode (980nm) Lasers

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Abstract

Objective: This in-vitro study was to investigate enamel surface compositional changes using CO_2 (10.600nm) and infrared diode laser (980nm) and their ability to increase its resistance to demineralization. **Methods:** Seventy premolar teeth, extracted for orthodontic reasons. The samples were randomly divided into three groups. group 1control ten samples group group 2 $C0_2$ laser, group 3 diode laser group thirty samples for each was divided into three sub group according to parameter 2,4, 6 watt for one minute after irradiation the samples were immersed in acetate buffer (ph 4.5) and incubated at 37^{OC} for 24 hours and then acid resistance were evaluated by determining calcium ion concentration by energy dispersive x-ray spectroscopy (EDX). Data were collected, tabulated and analyzed. The results showing 2w**:** There was no statistically significant difference between (Co_2) and (Diode) where (p=0.139). The highest mean value was found in (Diode) while the least mean value was found in (Co2).4w**:** There was no statistically significant difference between (Co2) and (Diode) where (p=0.432). The highest mean value was found in (Diode) while the least mean value was found in (Co2) 6w**:** There was a statistically significant difference between (Co2) and (Diode) where (p=0.011). The highest mean value was found in (Diode) while the least mean value was found in (Co2). **The study concluded:** Laser application is a promising technique for tissue remineralization and to increase the enamel resistance against the bacterial acid attack.

Key words: laser, Enamel, morphology, $C0_2$ diode.

INTRODUCTION

Dental caries is a common problem in most developed and developing country (1). Tooth decay occurs as a result of an imbalance between demineralization and remineralization. Thus, the development of more effective methods to prevent dental caries is extremely important to control the disease **(Rodrigues et al., 2004)** (2). The disease of caries originates in the dental enamel, a composite of 85% mineral, 12% water, and 3% protein and lipid by volume. The mineral component is hydroxyapatite **(White et al., 2005)** (3).

There is a positive correlation between carbonate solubility and enamel solubility once apatite diminishes in crystal stability **(Zero et al., 1999)** (4). In populations especially affected by caries, there are common sub clinical signs, like white spot lesions. These lesions are the earliest sign of a carious lesion, and they appear as chalky white spots on the surface of the tooth, indicating an area of enamel demineralization. **(Featherstone et al., 2003)** (5). prevention of dental caries, which is a complex multifactorial disease, requires dental clinics with appropriate preventive modalities and proper" oral hygiene education **(Hicks et al., 2004)**(6).

Preventive modalities include use of fluoride, reduction of dietary cariogenic refined carbohydrates, removal of plaque, and use of oral hygiene techniques and antimicrobials **(Westerman et al., 2004)** (7). A relatively simple and noninvasive caries preventive regimen is treating primary and permanent tooth enamel with laser irradiation, either alone or in combination with topical fluoride treatment. This treatment results in reduced enamel solubility and dissolution **(Sant Anna et al., 2007)** (8).

It has been consistently demonstrated that lasers under certain conditions can reduce the rate of subsurface demineralization in the enamel, by altering the crystallinity, acid solubility, and permeability of enamel **(Liu et al.,**

2007) [9]. Studies over the past 30 years have indicated that lasers such as the carbon dioxide (CO_2), Nd: YAG, argon, and diode laser can be used to thermally modify the chemical composition of dental enamel to render it more resistant to acid dissolution and potentially more resistant to dental caries **(Ana et al., 2012)** [10].In 1964, the CO_2 laser was developed, The active medium of CO_2 laser is a gas, and it emits infrared light at different wavelengths. The 10,600mn CO_2 laser is the most commercially available, and it is used as both a pulsed- and a continuous-wave. The primary advantage of CO_2 laser surgery over the scalpel is its strong hemostatic and bactericidal effects **(Rodrigues et al., 2004)** [11].

Short- pulsed CO_2 laser in the prevention of demineralization by cariogenic challenge has shown favorable results. Limited studies have evaluated the influence of CO_2 laser irradiation on erosion prevention. [12]
However, the CO_2 laser is also highly absorbed by the principal mineral components of hard tissue, especially the phosphate ions (- PO4) in carbonated hydroxyapatite. Several studies, both in vitro and in vivo have shown increases in enamel acid resistance **(Aoki et al., 2004)** [12]. Diode laser is a semiconductor laser that produce coherent radiation in the visible or infrared (IR) spectrum. Laser diodes are used in optical fiber systems, reports in the literature suggest that their use with or without topical fluoride, can lead to increases in the teeth's resistance against dental caries **(Sant'Anna et al., 2009)** [13].

The chemical characteristics of the sample following laser irradiation are important, Energy dispensing X-ray fluorescence spectrometry (EDX) allows for qualitative and semi-quantitative elemental analyses of inorganic enamel components such as calcium (Ca) and phosphorus (P), in very small areas **(Silva et al., 2000)** [14]. This is a versatile and nondestructive spectroscopic technique that allows for the accurate determination of the global chemical composition of a solid sample, with simple or no sample preparation procedures, rapid analysis, good reproducibility, and low cost **(Berlin et al., 1975)** [15]. The aim of

This in-vitro study was to investigate enamel surface compositional changes using CO_2 (10.600nm) and infrared diode laser (980nm) and their ability to increase its resistance to demineralization.

MATERIALS AND METHODS

Tooth selection
This study was approved by the research Ethical Committee (REC)-NILES-EC-CU23 – 1-4. Seventy premolar teeth, extracted for orthodontic reasons from college of oral and dental medicine Cairo University will be used. The teeth were selected according to the following criteria: intact enamel surfaces without developmental defects, restorations or fluorosis, not subjected to any pretreatment chemical agents, such as hydrogen peroxide, no cracks and no caries.

Sample preparation
All selected teeth were cleaned with a brush and deionized water; subsequently, then dried with compressed, oil-free air.

Treatment of the enamel surface
The samples were randomly divided into three groups.

In each group

1. Ten tooth was not irradiated (control group).
2. Thirty tooth were irradiated with CO_2 laser (10.600nm).
3. Thirty tooth were irradiated with diode laser (980nm).
4. All teeth will be irradiated for one minute.

Each group was divided to three subgroup according to parameter

Group 1 CO2 group subgroup a was use 2 watt, sub group b was use 4 watt, sub group c was used 6 watts,

Ten teeth for each

Group 2 diode laser was divided to three sub group sub group a was use 2 watt, sub group b was use 4 watt, sub group c was used 6 watts,

After irradiation
The samples were immersed in acetate buffer (ph 4.5) and incubated at 37^{OC} for 24 hours and then acid resistance were evaluated by determining calcium ion concentration by energy dispersive x-ray spectroscopy (EDX).

Finally
Data were collected, tabulated and analyzed.

Results of EDX Analysis

EDX analysis was used to investigate the effect of CO_2 laser (2w, 4w, 6w) (10.600nm) and infrared diode laser (2w, 4w, 6w) (980nm) on calcium content of enamel surface.

1. Control Group

Table (1) CO_2 concentration by weight of the buccal surface of the control group

Element	Weight %	Atomic	Net int.	Error %
OK	46.56	66.58	188.85	10.95
PK.	17.42	12.86	571.98	3.45-
CaK	36.02	20.56	811.79	2.17

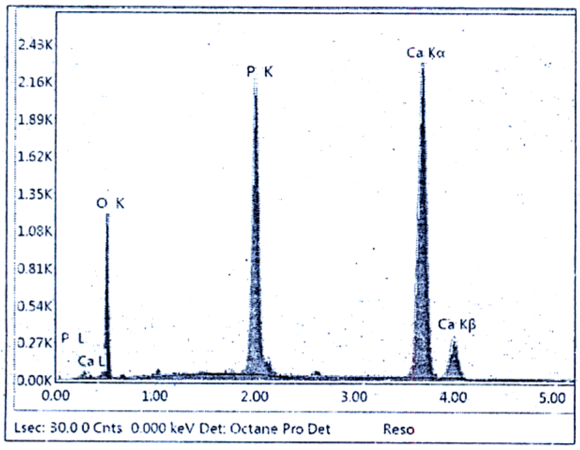


Figure (1) Control group

2. CO_2 Laser Group

As a function of laser beam power, samples subjected to 6 watt CO_2 laser beam showed highest Ca content percentage followed by 4 watt and 2 watt respectively.

a. Laser power 2 Watt

Table (2) Calcium concentration by weight of enamel irradiated by CO 2 (2W)

Element	Weight %	Atomic	Net int.	Error %
OK	46.49	66.51	179.71	11
PK.	17.46	12.9	547.1	3.58
CaK	36.04	20.58	774.74	2.22

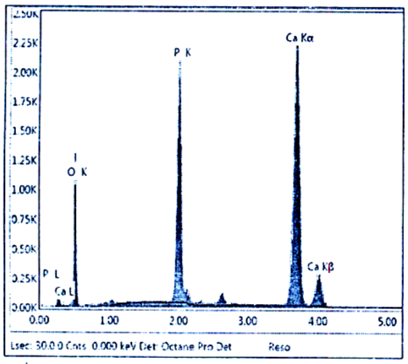


Figure (2) CO_2 (2Watt)

b. Laser power 4 Watt

Table (3) Calcium concentration by weight of enamel irradiated by CO_2 (4W)

Element	Weight %	Atomic	Net int.	Error %
OK	42.3	62.67	127.28	11.37
pk.	1837	14.06	485.36	3.63
CaK	39.34	23.27	709.79	2.27

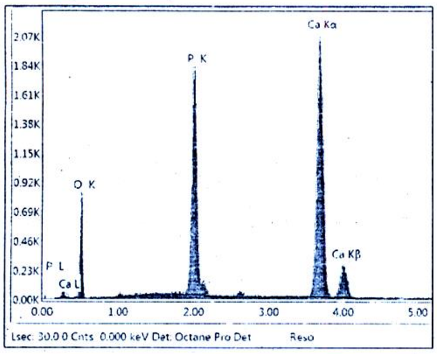
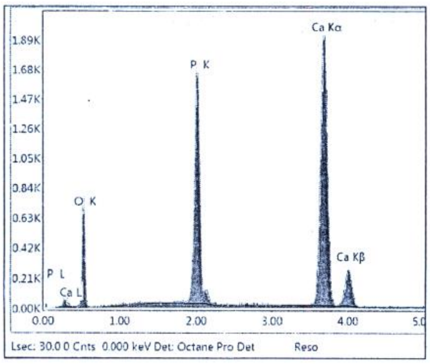


Figure (3) CO_2 (4Watt)



c. Laser power 6 Watt

Table (4) Calcium concentration by weight of enamel irradiated by CO_2 (6W)

Element	Weight %	Atomic	Net int.	Error %
OK	41.36	61.85	111.26	11.53
pk.	17.93	13.85	433.65	3.69
CaK	40.71	24.3	673.18	2.28

Figure (4) CO_2 (6Watt)

3. Diode Laser Group

As a function of laser beam power, samples subjected to 6 watt diode laser beam showed highest Ca content percentage followed by 4 watt and 2 watt

Table (5) Calcium concentration by weight of enamel irradiated by diode (2w)

Element	Weight %	Atomic	Net int.	Error %
OK	38.66	59.19	105.46	11.65
pk.	18.49	14.62	475.68	3.64
CaK	42.85	26.18	751.45	2.26

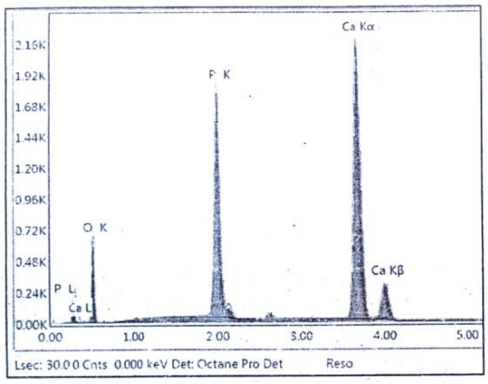


Figure (5) Diode laser (2watt)

b. Laser power 4 watt

Table (6) Calcium concentration by weight of enamel irradiated by diode (4W)

Element	Weight %	Atomic	Net int.	Error %
OK	38.27	58.82	98.8	11.72
pk.	18.35	14.57	450.8	.3.67
CaK	43.38	26.61	726.98	2.26

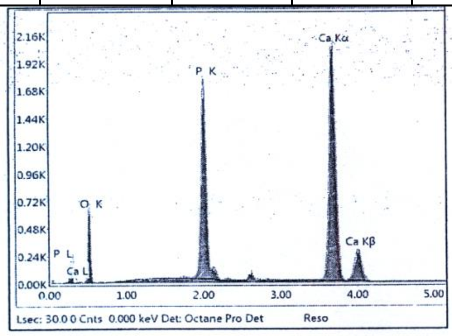


Figure (6) Diode laser (4watt)

c. Laser Power 6 Watt

Table (7) Calcium concentration by weight of enamel irradiated by diode (67)

Element	Weight %	Atomic	Net int.	Error %
OK	27.76	47.28	49.36	12.75
pk.	18.76	18.48	389.45	3/77
CaK	53.47	36.3	735.87	2.28

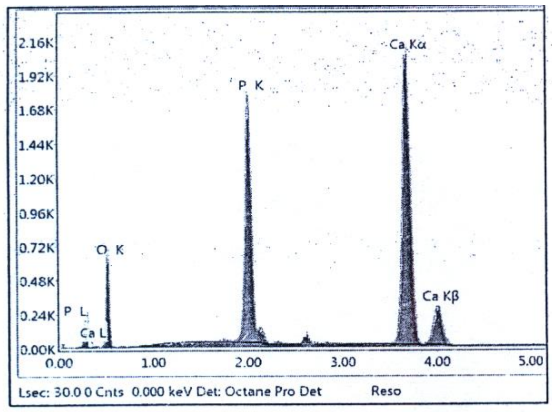


Figure (7) Diode laser (6watt)

The mean and standard deviation values were calculated for each group in each test. Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests and showed parametric (normal) distribution.

One way ANOVA followed by Turkey post hoc test was used to compare between more than two groups in non-related samples. Independent sample t-test was used to compare between two groups in non-related samples

The significance level was set at P ≤ 0.05. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

Calcium results

1. **Relation between different experimental and control group**
   There was a statistically significant difference between (Control) and (Diode 6w) where (*p*<0.001).
   No statistically significant difference was found between(Control) and any other experimental group.

2. **Relation between different subgroups in each group**
   a. Co2
   There was no statistically significant difference between (2w), (4w) and (6w) where (*p*=0.572).
   The highest mean value was found in (6w) while the least mean value was found in (2w).

   b. Diode
   There was a statistically significant difference between (2w), (4w) and (6w) where (*p*=0.019).
   A statistically significant difference was found between (6w) and each of (2w) and (4w) where (*p*=0.029) and (*p*=0.030) respectively.
   No statistically significant difference was found between (2w) and (4w) where (*p*=0.999).
   The highest mean value was found in (6w) while the least mean value was found in (2w).

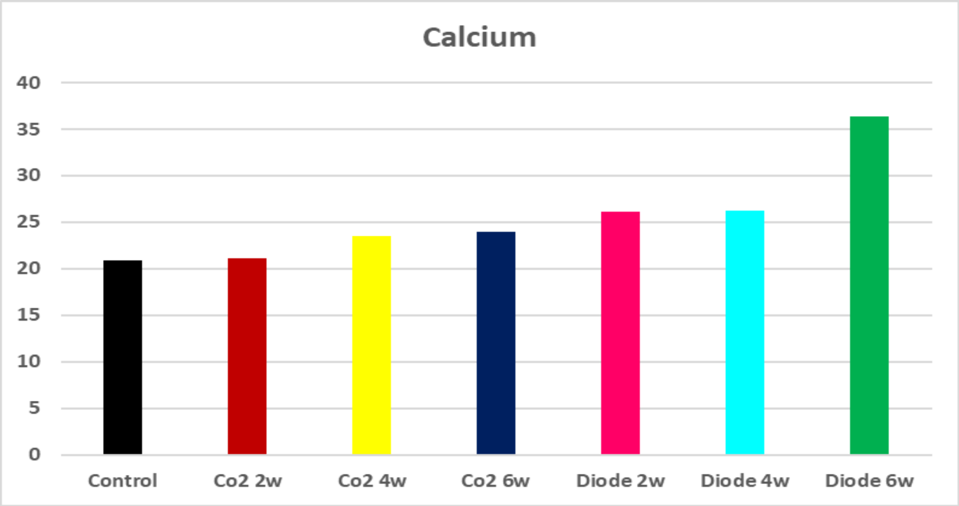
3. **Relation between different groups in each Subgroup**
   a. 2w
   There was no statistically significant difference between (Co2) and (Diode) where (*p*=0.139).
   The highest mean value was found in (Diode) while the least mean value was found in (Co2).

   b. 4w
   There was no statistically significant difference between (Co2) and (Diode) where (*p*=0.432).
   The highest mean value was found in (Diode) while the least mean value was found in (Co2).

   c. 6w
   There was a statistically significant difference between (Co2) and (Diode) where (*p*=0.011).
   The highest mean value was found in (Diode) while the least mean value was found in (Co2).

Table (8) The mean, standard deviation (SD) values of calcium of different groups.

Variables	**Calcium**	
	Mean	**SD**
Control	20.85	3.61
Co2 2w	21.09	2.49
Co2 4w	23.56	3.91
Co2 6w	23.93	3.66
Diode 2w	26.16	4.05
Diode 4w	26.20	3.52
Diode 6w	36.40	3.05
p-value	**0.002***	



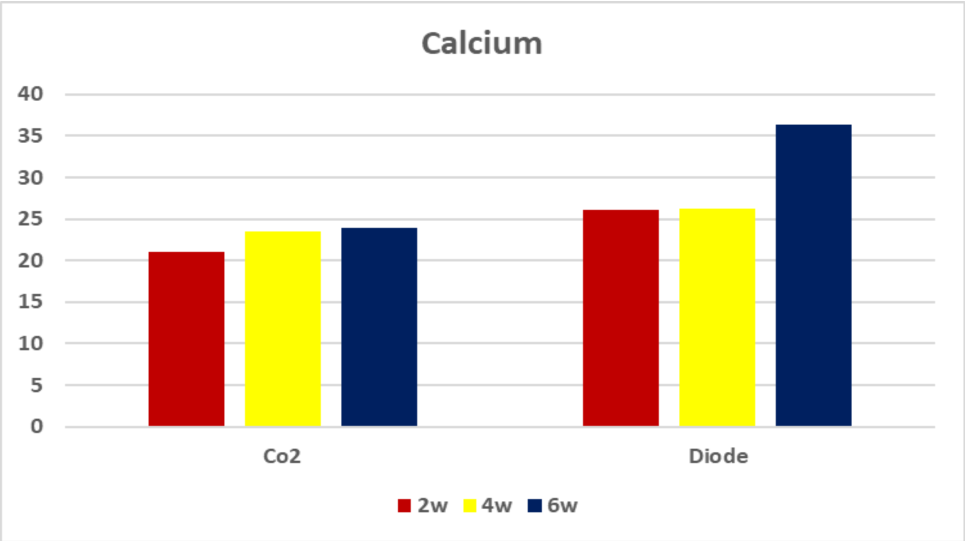
*; significant (p<0.05) ns; non-significant (p>0.05)

Figure (8) Bar chart representing calciu

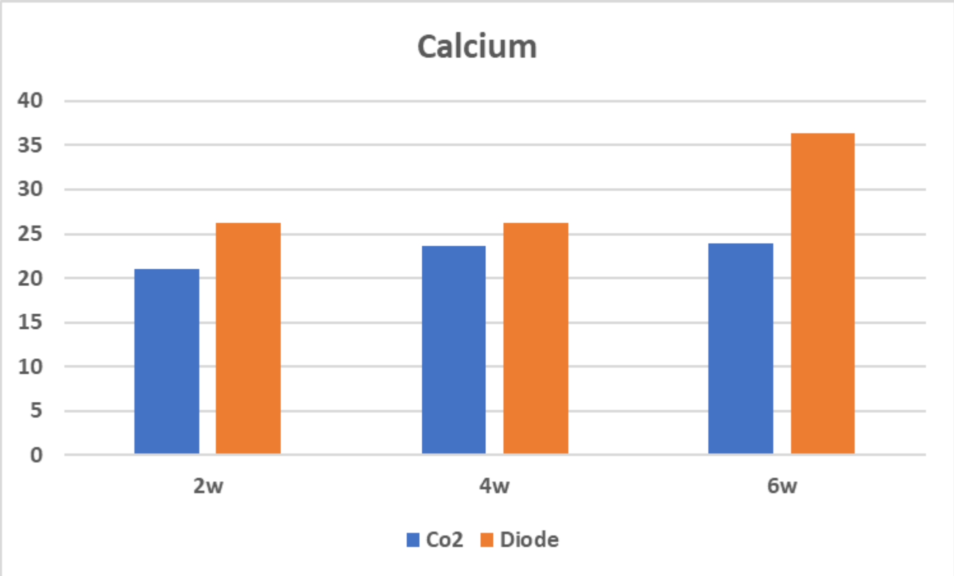
Table (9) The mean, standard deviation (SD) values of calcium of different groups.

Variables	**Calcium**						
	2w		**4w**		**6w**		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	***p-value***
Co2	21.09	2.49	23.56	3.91	23.93	3.66	**0.572ns**
Diode	26.16	4.05	26.20	3.52	36.40	3.05	**0.019***
p-value	**0.139ns**		**0.432ns**		**0.011***		

*; significant (p<0.05) ns; non-significant (p>0.05)



(A)



(B)
Figure (9) Bar chart representing calcium

Phosphorus results

1. **Relation between different experimental and control group**
   There was no statistically significant difference between (Control) and any of experimental groups where (*p*=0.887).

2. **Relation between different subgroups in each group**
   a. Co2
   There was no statistically significant difference between (2w), (4w) and (6w) where (*p*=0.973). The highest mean value was found in (6w) while the least mean value was found in (2w).

   b. Diode
   There was no statistically significant difference between (2w), (4w) and (6w) where (*p*=0.698). The highest mean value was found in (6w) while the least mean value was found in (2w).

3. **Relation between different groups in each Subgroup**
   a. 2w
   There was no statistically significant difference between (Co2) and (Diode) where (*p*=0.674). The highest mean value was found in (Diode) while the least mean value was found in (Co2).

   b. 4w
   There was no statistically significant difference between (Co2) and (Diode) where (*p*=0.613). The highest mean value was found in (Diode) while the least mean value was found in (Co2).

   c. 6w
   There was no statistically significant difference between (Co2) and (Diode) where (*p*=0.396). The highest mean value was found in (Diode) while the least mean value was found in (Co2).

Table (10) The mean, standard deviation (SD) values of Phosphorus of different groups.

Variables	Phosphorus	
	Mean	SD
Control	13.39	2.89
Co2 2w	13.22	3.74
Co2 4w	13.37	3.82
Co2 6w	13.89	3.40
Diode 2w	14.46	2.90
Diode 4w	14.84	2.66
Diode 6w	16.39	3.05
p-value	**0.887ns**	

*; significant (p<0.05)   ns; non-significant (p>0.05)

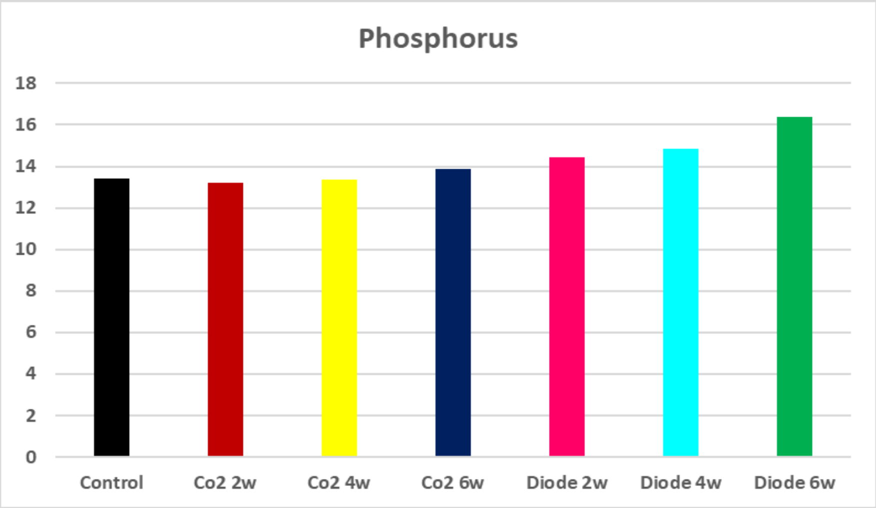
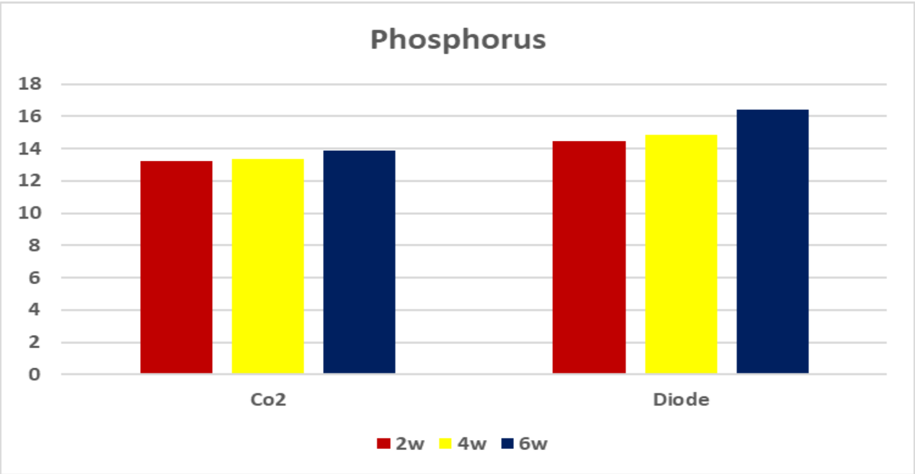


Figure (10) Bar chart representing Phosphorus

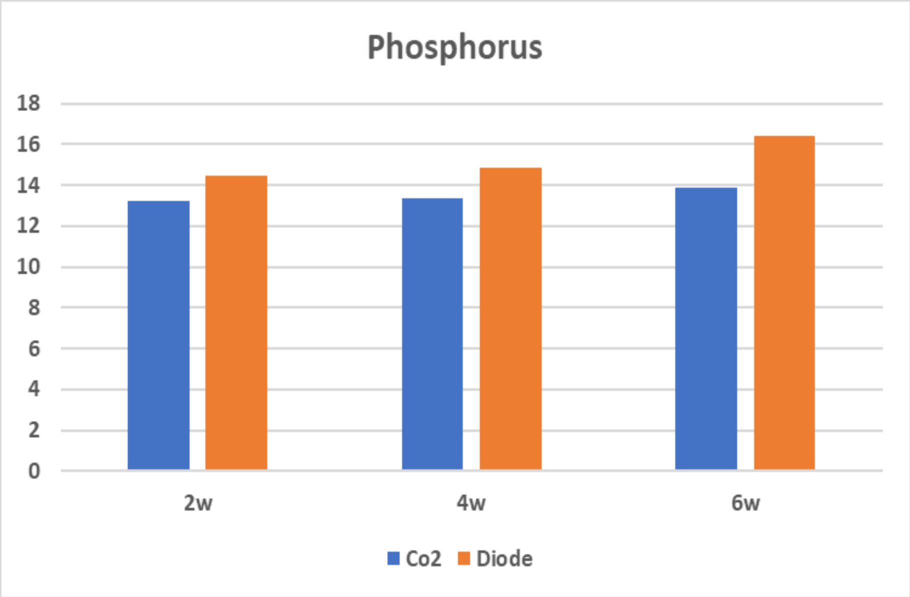
Table (11) The mean, standard deviation (SD) values of Phosphorus of different groups.

Variables	Phosphorus						
	2w		4w		6w		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	***p-value***
Co2	13.22	3.74	13.37	3.82	13.89	3.40	0.973ns
Diode	14.46	2.90	14.84	2.66	16.39	3.05	0.698ns
p-value	0.674ns		0.613ns		0.396ns		

*; significant (p<0.05)   ns; non-significant (p>0.05)



(A)



(B)

Figure (11) Bar chart representing Phosphorus

Discussion

The current study was performed in vitro to investigate the effect of CO_2 laser (2w, 4w, 6w) (10.600nm) and infrared diode laser (2w, 4w, 6w) (980nm) on the calcium content of the enamel surface.

In the current study, human premolar teeth freshly extracted for orthodontic reason were used. It seemed - valuable to monitor the structural configuration of the intact sound enamel before and after laser application to avoid any misleading in the results due to presence of a variable as cavity preparation and its effect on the enamel structure. Calcium content especially on the enamel surface plays a critical role in minimizing the surface susceptibility to caries. Moreover, the hardness of enamel is a function of the degree of mineralization. Porosity of partially demineralized. Enamel indicates that components of enamel dissolved thus the surface hardness was compromised. Conversely, in remineralization the micro spaces are filled with calcium that reflects the increase of surface micro hardness **(Feagin et al., 1969)**. [17]

The mechanism of caries prevention suggested by laser is to make enamel more resistant to acid dissolution by changing its structure. The application of laser beam enhances the uptake of fluoride, decreases the lesion depth and enhances the re-mineralization of enamel **(Almuslet et al., 2015)** [18].

It was valuable to assess the effect of two types of laser beam on the enamel surface mineral content (Diode and C O_2). A major advantage of diode laser is its small size and portability. As reviewed by many literature CO_2 laser wavelengths has the maximum absorption rate in hydroxyapatite, which is 1,000 times the absorption rate of diode laser. However it was reported that CO_2 laser therapy may have adverse effects on the enamel causing pitting zones ,cracks, fracture lines and thermal damage to dentin or pulp **(Bahrololoomi et al., 2015)** [19].

The chemical characteristics of the sample following laser irradiation are important, and quantitative elemental analysis of inorganic enamel components using EDX provides information on the sample stoichiometry and Ca content percentages **(Table 1)**. It has the advantage of being rapid analysis, good reproducibility, and low cost **(Rodriguez et al., 2011).** [20]

Different powers of the two types were tested (2, 4 and 6 Watt). The power density includes the nature of the spot size, the amplitude of the wave, and the specific wavelength involved. Laser interactions with tissue are dramatically affected by the difference in laser parameters as wave length, power density and repetition rate **(Shengqiang Zhang, 2008).**[21]

The results of EDX analysis showed significant increase in Ca content percentage in surface enamel than normal after the application of the two types of laser. The group of diode laser **(Fig. 5,6,7)** showed better performance and higher Ca content % than CO_2 laser group **(fig. 2,3,4).**

This result was agreed by **Almuslet et al., (2015)** [18:i] who speculated that laser cause enamel remineralization due to the heating effect. The water permeability of dental enamel was seen to be lower after heating. More hydroxide and pyrophosphate, but less carbonate (the weakest part that initiates caries process), is also generally found in

comparison with unheated enamel (denoting less susceptibility to dissolution). The energy absorbed is high enough to drive organic substances arid carbonate out of the enamel and more Ca content in resulting in more acid-resistant apatite crystals (Re-precipitation of the dissolved minerals).
(Bahrololoomi et al., 2015)[19] Suggested that application of laser beam can cause fusion of homogeneous and inhomogeneous crystals of apatite with different shape and larger size than untreated enamel, and a loss of prismatic structure were found, thus the percent of Ca content per unit area is significantly increased.

Rodrigues et al., **(2011)**[20] suggested that this effect is caused by the denaturation of the organic matrix that leads to the obstruction of the diffusion pathways within the enamel by the melted hydroxyapatite crystals **(Shengqiang Zhang, 2008)**[20] agreed that diode laser is better in caries prevention and enamel remineralization than CO_2 laser. This might be due to the high absorption of the beam energy by the tissues that cause rapid expansion and explosion of heated water and subsequent ablation of the surface enamel tissue that has higher Ca content percentages than the inner enamel. He suggested that CO_2 laser produce better performance in enamel cutting and etching procedures. The results showed the highest Ca content on the enamel surface when using laser beam with power 6 watt. That was supported by **(Bahrololoomi et al., 2015)**[17] who suggested that increasing the laser power can produce higher heat generation, further loss of carbonate, formation of more stable and more resistant substances such as tetra-calcium diphosphate monoxide with more Ca ions which lead to obstruction of interprismatic spaces and low permeability.

Increasing the laser power up to 6 watt might lead to excessive heat generation that can cause ablation of the surface enamel, micro cracks and fractures **(Bahrololoomi et al., 2015)** [17].

Conclusion

Laser application is a promising technique for tissue remineralization and to increase the enamel resistance against the bacterial acid attack. Diode laser application can produce higher remineralization performance with minimal heat generation when compared to CO_2 laser. Excessive increase of laser beam power has destructive effects on the enamel structure like cracks and fracture lines.

References

1. Soltanimehr E, Bahran pour E,youserfva Z, Efficacy of diode and CO2 lasers along with calcium and fluoride containing compound for reminrilsation of primary teeth J BMC oral health 2019 19: 121.
2. Rodrigues LK, Nobre dos Santos M, Pereira D et al .Carbon dioxide laser in dental caries prevention. J Dent, 2004, 32:531-540.
3. White T, Ferraris C, Kirn, J. and Madhavi, S. Apatite an adaptive framework structure. Rev. Mineral Geochem.2005, 57,307-401.
4. Zero, D.T. Dental caries process. Dent. .Clin. North Am.1999; 43:635-663.
5. Featherstone, J.D. The caries balance: contributing factors and early detection. J. Calif. Dent. Assoc.2003, 31: 129-133.
6. Hicks J, Garcia-Godoy F. and Flaitz C. Biological factors in dental caries: role of saliva and dental plaque in the dynamic process of demineralization and remineralization (part 3). J. Clin. Pediatr. Dent. 2004, 28: 203-214.
7. Westerman G.H, Hicks M.J, Flaitz C. and Powell G.L. In vitro enamel caries formation: argon laser, lightemitting diode and APF treatment effect. Am. J. Dent.2004; 17: 383-387.
8. de Sant'Anna G.R, Paleari G.S.L, Duarte D.A, Brugnera A, Jr., and Pacheco-Soares C. Surface morphology of sound deciduous tooth enamel after application of a photoabsorbing cream and infrared low-level laser irradiation: an in vitro scanning electron microscopy study. Photomed. Laser Surg. 2007; 25:500-507.
9. Liu Y. and Hsu C.Y. Laser-induced compositional changes on enamel: aFT-Raman study. J. Dent.2007; 35: 226-230.
10. Ana PA, Tabchoury CP, Cury JA et al. Effect of Er, Cr: YSGG laser and professional fluoride application on enamel demineralization and on fluoride retention. Caries Res.2012, 46:441-445.
11. Rodrigues L.K.A, dos Santos M.N, Pereira D, Assaf, A.V. & Pardi, V. Carbon dioxide laser in dental caries prevention. Journal of Dentistry, 2004; 32, 7:531-40.
12. Sliva C, Mantilla, T, Tavares J, Frest P, Rechanan P., The effect of CO2 9300 nm short pulsed irradiation in enamel eroosion reduction with and without flouride application .J laser in medicasl sciences 2020 ; 35: 1213-1222.
13. Aoki A, Sasaki K.M, Watanabe H. & Ishikawa I. Lasers in nonsurgical periodontal therapy. Periodontology, 2000, 36:59-97.
14. Sant'Anna, Edson Aparecido Pereira dos Santos, Lui's Eduardo Silva Soares, Ana Maria do Espi'rito Santo, Dental Enamel Irradiated with Infrared Diode Laser and Photo-Absorbing Cream: Part 2—EDX Study 2009; 27:771-782.
15. Silva V.V, Lameiras F.S. and Domingues R.Z. Evaluation of stoichiometry of hydroxyapatite powders prepared by co-precipitation method. Key Engineering Materials.2000, 189:79-84.
16. Bertin. X-ray physics. In: Principles and practice of X-ray spectrometric analysis. 1975; 1078 pp.
17. W. Formation of hydroxyapatite under various conditions of remineralization of dental enamel. Archs oral Biol, 1969; (14): 1407-1417.
18. Nafie A. Almusletl, Elhadi M. Awooda. Effect of 532 nm and 671 nm Diode Lasers Irradiation on Calcium Solubility in Human Dental Enamel: An Ex-Vivo Study. Journal of Scientific Research & Reports, 2015; 5(4): 320-325.
19. Zahra Bahrololoomil, Faezeh Fotuhi Ardakani, Milad Sorouri. In Vitro Comparison of the Effects of Diode Laser and CO2 Laser on Topical Fluoride Uptake in Primary Teeth. Shahid Sadoughi University of Medical Sciences, 2015, 12:585-591.
20. Rodriguez-Vilchis L.E, Contreras-Bulnes R, Olea-Mejia O.F, Sanchez-Flores, I. & Centeno-Pedraza, C. Morphological and structural changes on human dental enamel after EnYAG Shengqiang Zhang. CO2 Laser Induced Structural Enamel Dissertation. University,