Original Article

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Effect of Diode Laser and Fluoride Application on Micro-hardness of Demineralized Enamel (An In Vitro Study)

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Abstract:

Objectives: This study aims to evaluate the effectiveness of topical fluoride application and diode laser-irradiation on the Micro-hardness of demineralized enamel. Materials and Methods: Sixty enamel samples were prepared; Vicker's microhardness was measured at baseline, after demineralization and after treatments. After demineralization the teeth were randomly assigned into four groups (n = 15): group A, no treatment (control), group B fluoride only; group C, combined therapy of fluoride and diode laser 2 watts and group D, combined therapy of fluoride and diode laser 3 watts. A one-way ANOVA and Tukey post-hoc test procedure were performed for statistical analysis. The level of significance was set at a = 0.05 for all tests. Results: No significant differences were seen in the mean VHN between groups at baseline and demineralized stages. The mean VHN of group C&D after treatment was significantly higher than for groups A and B ($P < 0.05$). The mean VHN of group B after treatment was significantly higher than group A ($P < 0.05$).

Conclusion: Diode laser irradiation of the enamel surface increases enamel micro-hardness when used in combination with fluoride application.

Keyword: Demineralized Enamel; Fluoride; Diode laser; Microhardness.

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Introduction:

Dental caries is one of the main causes for dentition loss and oral health deterioration. Fluorides application recognized as caries preventive agents which inhibit demineralization and enhance remineralization of hard dental tissues (1,2,3). The fluoride anti-cariogenicity mechanisms include inhibition of demineralization and enhancement of remineralization(4). Fluoride interacted with dental hard tissues to produce a more stable acid-resistant hydroxyapatite (HAP) lattice(4). The reaction products after fluoride application includes calcium fluoride (CaF_2), calcium hydroxide ($Ca(OH)_2$) and fluorapatite (FAP, $Ca_5(PO_4)_3F$). These compounds protect enamel surface and serve as fluoride reservoirs.

The use of fluorides is one of the most efficient methods of caries prevention. Twenty years ago, the effects of topical fluoride seemed to have been highly effective in preventing caries incidence (5). Lasers have been investigated as an alternative to fluoride to enhance the quality of tooth enamel and increase its resistance to demineralization. Additionally, rather than ablating the enamel surface, lasers boost enamel resistance by altering the morphology, chemical structure, or solubility of enamel (6). This study aims to evaluate the effectiveness of topical fluoride application and fluoride application followed by 2 or 3 watt diode laser-irradiation on the Micro-hardness of de-mineralized enamel.

Material and method:

Thirty sound intact upper human premolars teeth which were extracted for orthodontic and periodontal reasons were selected for this study, which were be visually examined to be free from any fracture, cracks, decay, attrition or any other defects. After they were disinfected in 1% thymol solution the root of each tooth was removed using a low-speed diamond disc and the crown was sliced into two parts mesio-distally. Each part was embedded in epoxy resin (Alteco, Quick Epoxy Adhesive, Indonesia). Sixty samples were prepared. To create a flat enamel surface, the slabs were serially polished using 300, 600, and 1200 grit silicon carbide sheets and an alumina slurry of 0.2 and 0.05 µm. A total of 60 samples were painted with nail polish from all sides, leaving 2×3 mm of exposed enamel surface uncovered. Baseline Vickers

micro-hardness was established for each enamel surface. the experimental area for the surface where demineralized by stored in an acidic solution containing 0.2 wt% 450 kDa MW polyacrylic acid (Polysciences Inc.USA) and 0.1 M lactic acid (Sigma-Aldrich, USA) solution partially saturated with hydroxyl-apatite (Bio Rad, Hercules, CA, USA) and adjusted to a pH of 5 for 2 weeks (White 1987).(7) The teeth were then cleaned with distilled water. Then the samples were divided into four groups (n=15)

Group A: (control group): The teeth did not receive any kind of treatment.

Group B: (Fluoride only) In this group, topical fluoride Gel (2% sodium fluoride NaF) (Top fluoride gel by Alwady for trading, made in A.R.E) was applied for 4 hr as it was recommended by the manufacturer that the fluoride should remain on the teeth for 4–6 hr, and then cleaned with distilled water (10).

Group C: Combined therapy of fluoride and 2-watt diode laser.

Group D: Combined therapy of fluoride and 3-watt diode laser.

Irradiation was performed using epic X diode laser (Biolase Compass med USA) **Fig (1)** in continuous mode at 980 nm with an exposure time of 15 s. The irradiation distance was set at 5 mm and the optic fiber tip diameter was 300 µm. A holder was used to ensure that the laser hand piece tip on the enamel surface was equidistant. All customary safety precautions were followed during laser application.

Fig (1) epic X
Diode laser system

The micro-hardness was measured at baseline, after demineralization and after treatments by Micro Vickers Hardness Tester (HMV-G Series: SHIMADZU **Fig (2)** Micro Vickers Hardness Tester) and the results was recorded. Analysis of variance (ANOVA) was used to compare micro-hardness variations following treatment between the four groups. Statistical analyses were performed using SPSS version 16 (IBM, Armonk, NY, USA).

Fig (2) Micro Vickers Hardness Tester

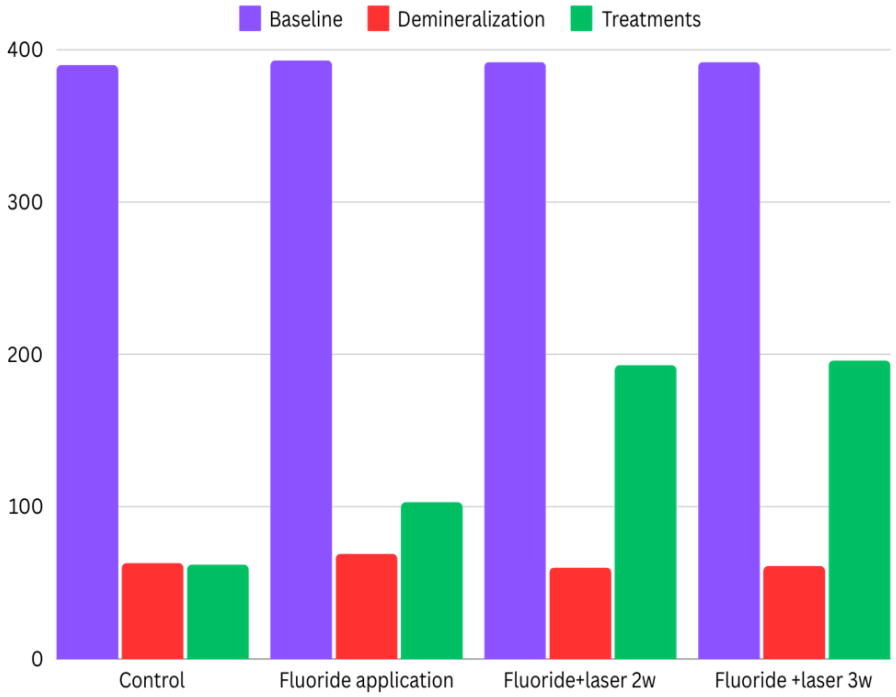
Results:

One way ANOVA was used to test the effect of different enamel treatments on Vickers micro-hardness number table**1**& Fig **(3)**, followed by tukey post-hoc test with $P < 0.05$ considered to be significantly different. Statistical Analysis was performed using IBM SPSS (version 26, Armonk, USA). Analysis of the results by using one-way ANOVA has shown statistically significant difference between fluoride group and fluoride and laser group $P < 0.001$. Tukey post-hoc test revealed that there was statistically significant difference between all groups. Results showing that specimens in group C & D which were irradiated with diode laser (2,3watt) There was no significant difference between group C&D.

But after they were treated with fluoride gel showed the highest mean micro-hardness values followed by group: B (fluoride only) However, specimens in group A (control)) showed the lowest micro-hardness.

Table (1) The means and standard deviations of VHN for each group (n = 15) at four stages.

	Baseline	Demineralized Enamel	Treatment	AVHN
Group A (Control)	390.26 ± 20.38	63.33 ± 15.36	------	5.41 ± 13.53
Group B Fluoride only	393.21 ± 18.29	69.98 ± 43.63	103.56 ± 11.38	33.57 ± 40.76
Group C Fluoride +Diode Laser 2W	392.97 ± 12.53	60.01 ± 12.96	193.56 ± 11.38	53.97 ± 18.91
Group D Fluoride +Diode Laser 3W	392.97 ± 12.53	61.01 ± 12.96	196.56 ± 11.39	54.93 ± 18.96



(Fig.3) Bar chart represents the mean of VHN for each group at demineralized stages. No significant differences were seen in the mean VHN between groups at baseline and demineralized stages. * The mean VHN of group C&D after treatment was significantly higher than for groups A and B($P < 0.05$). ** The mean VHN of group B after treatment was significantly higher than group A ($P < 0.05$).

Discussion:

The effects of topical fluoride application in combination with laser irradiation 2 & 3W on dental enamel surfaces were significantly higher than control and fluoride groups. Fluoride group was significantly higher than control group. Fluoride is a well-known, thoroughly researched, and effective method for preventing caries. The ability of fluoride to inhibit the onset and progression of tooth decay is behind its successful caries prevention (8). Laser types produce structural and ultrastructural changes in the tooth enamel (9). There are various theories regarding how reactivity occurs in enamel treated with diode lasers (10). Another theory suggests a relationship between diminished porosity and melting, fusion, and re-crystallization of enamel particles, forming bars on the tooth exterior. Therefore, the optimal procedure is to use a diode laser before the appearance of a lesion. Laser irradiation of enamel can affect caries through surface melting and re-crystallization of enamel hydroxyapatite crystals (11), changes in enamel solubility, porousness and calcium fluoride deposition. This may also have promoted resistance. The treatment of samples with a 2 or 3 W diode laser combined with NaF gel improved fluoride uptake by the tooth enamel. This difference was statistically significant because diode laser irradiation resulted in melting and resolidification of the enamel surface of teeth, making the enamel more resistant to acids using this mechanism. Therefore, it plays an important role in the inhibition of tooth decay. After evaluating the results of fluoride application in our study, a significant increase in enamel microhardness observed fluoride groups (12). These results are similar to a study by Nalbantgil D et al., which concluded that microhardness after application of fluoride varnish was greater than that of the control group (13). Sh et al. (2013) the study showed that fluoride enhances the remineralization process through the cumulative growth of demineralized enamel crystals (14). Dionysopoulos D concluded in their study that the topical fluoride treatments (0.05 % NaF daily, 0.2 % NaF weekly, and 5 % NaF final topical fluoridation) significantly increased the surface microhardness of tooth enamel after bleaching (15). Gatti et al. also concluded that toothpaste with high fluoride concentrations (1100 ppm) and fluoride varnish in combination with toothpaste reduced tooth enamel demineralization compared to the negative control group. Frequent use of fluoride toothpaste showed a

greater effect on the microhardness of fluoride absorption on the tooth enamel surface compared to the combination with fluoride varnish (16). Otherwise Biria et al. that fluoride gel (Sina) was not significantly different than the control group with regard to fluoride intake (17). Lippert and Lynch 2014 et al the diode laser irradiation might lead to more fluoride uptake and remineralization at the deepest areas of the incipient lesions (18). Maha et al 2023 Diode laser irradiation of the enamel surface increases enamel micro-hardness when used in combination with fluoride varnish. This is in contrast to another study which reported that there was no significant change when using combination of topical fluoride together with laser (Andreana et al. 2019). These differences may be due to difference in types of fluoride varnish and mode of laser irradiation.(19)

Conclusion:

Within the limitation of this study, the use of a diode laser after a fluoride gel applied to the enamel surface increases enamel micro-hardness and has a greater effect on the resistance of the enamel to caries.

Research Ethical Approval: The study was approved by the Research Ethics Committee (REC) of the Dentistry Faculty, Ahram Canadian University, Egypt. Research number (IRB00012891#112)

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