

EFFECT OF DIODE LASER IRRADIATION IN COMBINATION WITH DIFFERENT RE-MINERALIZING AGENTS ON ENAMEL MICRO-HARDNESS (IN-VITRO STUDY)

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ABSTRACT

Objective: This study was carried to evaluate the influence of Fluor protectors (1.5% amonium fluoride varnish) and white varnish (5% sodium fluoride varnish) after irradiation with diode laser on enamel micro-hardness.

Materials and methods: Fifteen maxillary premolar teeth will be selected and divided into six groups (N=15): G1: control group; samples received no treatment, G 2: (AF); 1.5% AF gel was applied for 4 minutes, G 3: (NF): 5% sodium fluoride gel was applied for 1 minute, G 4: (DL): specimens were subjected to diode laser irradiation for fifteen seconds, G 5: (AFL): specimens were irradiated by the diode laser and then treated with AF varnish as in G 2, G 6: (NFL): specimens were irradiated by the diode laser and then NF varnish was applied as in G 3.

Results: Results show statistically significant difference between remineralization protocols $P < 0.001$.

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

KEYWORDS: Remineralization, diode laser, enamel micro-hardness, fluoride varnish.

INTRODUCTION

Although developments in caries prevention materials and techniques, dental caries is still one of the most common infectious dental diseases all over

the world. Recent methods for caries prevention including fluoride application could not efficiently prevent the caries incidence and progression specially in occlusal caries (Tham et al. 2019).

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Thus, modern methods and materials for caries prevention become mandatory. Topical application of different fluoride forms such as solutions, varnishes, and gel [sodium fluoride (NaF) and ammonium fluoride (AF)] are considered the most current caries preventive methods.

Major mechanism of action of fluoride therapy is to prevent teeth demineralization and promote the procedure of remineralization. This is done through enhancing the penetration of the hydroxyapatite crystals and replace them with the hydroxyl ion then formation of fluoro-apatite crystals which is more resistant to acid dissolution (Esfahani et al. 2015). Ammonium fluoride varnish has been reported as an important factor in tooth demineralization inhibition and more effective in preventing dental caries (Rob et al. 2017). Moreover; the combination between fluoride and other preventive measures is suggested to be better effective for caries prevention. One of these novel methods is the combination of fluoride with the laser irradiation of the enamel surface prior to the fluoride therapy.

Laser irradiation of enamel surface are considered as an effective caries preventive method; however, the combination of laser with fluoride reported better results in this aspect. Some studies reported that the Diode laser (DL) increases the efficiency of topical fluoride application (Nastaran et al. 2018) and (Al-Maliky et al. 2023). Others found that laser irradiation did not report significant improvement than fluoride application solely (Bahrololoomi et al. 2015). In addition to that, it is not yet clear which combination of laser and fluoride would be considered as an effective preventive protocol.

Therefore, it is important to use recent preventive measures to prevent the need for extensive restorations in future. This study was carried out to evaluate the effect of two fluoride varnishes (1.5% ammonium fluoride varnish and 5% sodium fluoride varnish) applied to the enamel surface after diode laser irradiation on enamel micro-hardness.

MATERIALS AND METHOD

Fifteen sound intact human premolar 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, after that, each tooth was sectioned longitudinally into six blocks using a sectioning machine (MECATOME T 201 A PRESI, Germany) A total of 90 samples were painted with nail polish from all sides, leaving 2×3 mm of exposed enamel surface uncovered (Berdan et al. 2015). One block from each tooth was allocated randomly into one of the six groups (N=15): Group 1(CO): samples were kept in distilled water without treatment. Group 2 (AF): samples were treated with 1.5% AF gel (POLIMO, Turkey) for 4 minutes (according to the instructions of the manufacturer). Then, they were rinsed with water then air-dried for 5 seconds. Group 3 (NF): samples were treated with 5% sodium fluoride gel (which was prepared immediately before its use according to the manufacturer's instructions) for 1 minute and then rinsed and air-dried for 5 secs. Group 4 (DL): the specimens were irradiated by diode laser (Sirolase advance, Sirona Dental company, Germany) of wave length 970nm and power 2W were used in continuous mode to the prepared teeth enamel surface for 15 seconds (Khallaf et al. 2019). It was conducted to an optic conductor fibers as transmission elements. The laser beam was directly applied to the enamel surface before fluoride application. The tip of the optic fibers was placed with no-contact with a standard distance of 2mm using putty molds to assure an equal distance between the tip of the optic fibers and the enamel surface (Saadony et al. 2019). Group 5 (AFL): samples were irradiated by diode laser by the same way then they were treated with AF varnish as mentioned before. Group 6 (NFL): samples were irradiated by diode laser and then treated with NF varnish as mentioned before.

After 24 hours, enamel surface micro-hardness of all specimens in each group were measured by a technician who was blind to the study groups. Data were analyzed by one-way ANOVA and Tukey post-hoc tests. The significance level was set at $p < 0.05$.

RESULTS

Statistical analysis

Data were represented as mean and standard deviation (SD) when appropriate. Data showed normal distribution when checked using Kolmogorov-Smirnov and Shapiro-Wilk test. One-way ANOVA was used to test the effect of different remineralization protocols on Vickers hardness number of enamel, 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 using one-way ANOVA have shown statistically significant difference between remineralization protocols $P < 0.001$. Tukey post-hoc test revealed that there was statistically significant difference between all remineralization protocols except between CO and DL groups.

Results showing that specimens in group 5 which were irradiated with diode laser then treated with ammonium fluoride varnish showed the highest mean micro-hardness values followed by group: 6. However, specimens in group 4 which were irradiated with diode laser without fluoride varnish and group: 1 (control group) showed the lowest micro-hardness.

TABLE (1) Materials and devices used in this study:

Material	Fluoroprotector S Varnish	White Varnish	Sirolase Advance
Composition	Fluoride Varnish (Ethanol, Water polymer, additive, saccharin, Mint flavour, 1.5% ammonium fluoride (corresponds to 7700 ppm fluoride))	Fluoride Varnish 5% sodium fluoride and an innovative tri-calcium phosphate ingredient. Alcohol based solution of modified rosin. Sweetened with xylitol the unit dose package containing 0.5 ml contain 25 mg sodium fluoride equivalent to 11.3 mg fluoride ion	Laser Device wave length 970nm and power 2W 220 Hm optic conductor fibers as transmission elements.
Application	Apply thin layer with a viva brush G and let the varnish dry for one mn.	Thin varnish coating was applied the varnish and nonvarnish slides were placed in separate vial of de ionized water	Power 2W were used in continuous mode to assigned prepared teeth enamel surface for 15 seconds. The laser beam was directly applied to the enamel surface directly before the fluoride application. The tip of the optic fibers was placed at no contact with a standard distance of 2mm using putty impression material to insure an equal distance between the tip of the optic fibers and the enamel surface.
Manufacture	Ivoclar Vivadent	3M Company	Sirona Dental Company, GmbH Fabrikstr. 31.64625 Bensheim.Germany)
Batch number	639520	53970566	MFD A01

Descriptive statistics:

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
CO	6	209.8333	3.12517	1.27584	206.5537	213.1130	206.00	214.00
AF	6	314.3333	3.98330	1.62617	310.1531	318.5135	311.00	320.00
NF	6	301.1667	5.41910	2.21234	295.4797	306.8537	295.00	310.00
DL	6	203.6667	4.22690	1.72562	199.2308	208.1025	200.00	210.00
AFL	6	370.1667	10.41953	4.25376	359.2320	381.1013	356.00	383.00
NFL	6	341.1667	8.49510	3.46811	332.2516	350.0817	328.00	354.00
Total	36	290.0556	63.94593	10.65766	268.4194	311.6917	200.00	383.00

ANOVA

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	141849.889	5	28369.978	671.214	P < 0.001
Within Groups	1268.000	30	42.267		
Total	143117.889	35			

Grouping Information Using the Tukey Method and 95% Confidence

Groups	N	Mean	Grouping
AFL	6	370.17	A
NFL	6	341.17	B
AF	6	314.33	C
NF	6	301.17	D
CO	6	209.83	E
DL	6	203.67	E

Means that do not share a letter are significantly different.

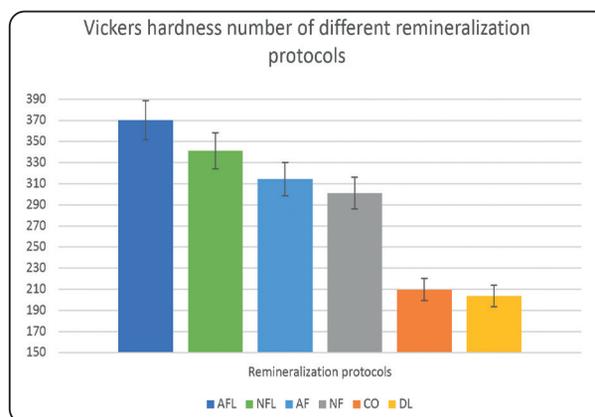


Fig. (1)

DISCUSSION

This in-vitro study was conducted to measure the enamel micro-hardness to evaluate the effect of the combination of fluoride application with laser irradiation on enamel surface.

The results suggest that mean vicker’s hardness number was 209.83 for intact enamel, these findings were in agreement with some studies (Moghadam, Seraj et al. 2018) who found that the vicker’s hardness number has a range of 202 to 297 for sound enamel without receiving any treatment. Measurements after fluoride treatment showed

significant differences in vicker’s hardness number between groups (Gutiérrez-Salazar and Reyes-Gasga 2003). The fluoride groups “A, B, C and D” mean values was significantly higher than groups “E and F” that not receiving fluoride therapy. Different in-vitro studies have shown that fluoride varnish significantly increases enamel micro-hardness when compared to the control group (Cardoso et al., 2014, Mohd Said et al., 2017). Other in-vivo studies found that application of fluoride varnish can significantly improve white spot lesions when applied once / month for six months after orthodontic treatment (Du et al., 2012, He et al., 2016).

In this study, fluoride varnish after laser activation showed the significantly highest micro-hardness. 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.

Studies on the influence of diode laser irradiation on enamel micro-hardness show controversy. Florin et al. found that there is an increase in enamel micro-hardness with fluoride varnish application after laser activation. Majori et al. reported that there is no significant change in enamel micro-hardness following laser activation of sodium fluoride varnish. Moreover, there was one study suggested that laser irradiation may decrease the enamel micro-hardness after remineralization (Tagomori et al. 1995).

On the other hand, another study showed that fluoride varnish when used in combination with diode laser was not significant when compared with fluoride application only (Kutuk, Ergin et al. 2019). The investigators in this previous study exposed the study samples to a pH-cycling process after fluoride treatment, this might be responsible for the difference.

Regardless whether they support the combination of topical fluoride application with laser activation or not, there were differences between studies regarding the materials and methods which makes it difficult to reach firm conclusion (Vlacic et al., 2007, Vitale et al., 2011).

Differences among studies include whether the study used animal or human teeth, primary or permanent teeth, also the active ingredients of fluoride varnishes, application time, the laser irradiation mode regarding wavelength, power, continuous or pulse mode and the different methods of measurements including micro-hardness, fluoride uptake, surface roughness and microscopic analysis of the enamel surface.

CONCLUSION

The micro-hardness of enamel irradiated with diode laser was statistically significantly greater than the groups which were not irradiated when used in combination with fluoride varnish as a remineralizing agent.

Therefore, using diode laser application is recommended as a caries preventive method to increase the enamel surface hardness and resistance to acidic dissolution.

RECOMMENDATION

Although that the use of micro-hardness measuring to study the enamel remineralization is considered a reliable method, it is recommended to use another method of assessment as an adjunctive test in order to measure the compositional changes in the enamel depth (**Lippert and Lynch 2014**).

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