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Effect of CO_2 Laser-Assisted Titanium Tetra-fluoride on Demineralization of Enamel Around Orthodontic Brackets

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Abstract

Introduction: One of the clinical problems following orthodontic treatment is white spot lesions around orthodontic brackets due to enamel demineralization. Confronting enamel demineralization during fixed treatments has long been a challenge for orthodontists. The aim of this in vitro study was to evaluate the effect of CO_2 laser and Titanium Tetra-fluoride (TiF4) application on the prevention of enamel demineralization around orthodontic brackets.

Methods: Eighty permanent premolars were selected and bonded with brackets. They were randomly divided into four groups (n = 20): topical titanium tetra-fluoride gel 4% (TiF4), CO_2 laser (10.6 µm wavelength for 10 seconds, peak power = 291 W), fluoride + laser (F + L) and control (C). All specimens were demineralized for 10 days in a 0.2 M acetate buffer solution. The mean lesion depths were determined by using polarized light microscopy.

Results: The mean depth of lesion was the highest in the C group and then decreased in the TiF4, CO_2 laser, and F + L groups, respectively. The difference between all groups was significant ($P < 0.05$), except for the CO_2 laser and F + L groups.

Conclusion: The lowest amount of demineralization around the orthodontic brackets was observed in the L + F group, followed by the CO_2 laser, TiF4, and control groups, respectively.

Keywords: Laser; Titanium tetrafluoride; Tooth demineralization; Orthodontic brackets.

Introduction

Patients treated with fixed appliances are at higher risk of dental caries or “white spot” formation. One reason for this is the difficulty in removing plaque and debris around the orthodontic brackets, either manually (by brushing) or chemically (by rinsing). Øgaard et al have reported that these lesions can develop within 4 weeks, which is the usual time frame between orthodontic visits. Confronting enamel demineralization during fixed treatments has long been a challenge for orthodontists.[1]

Fluoride is the most well-known single factor in significantly reducing caries worldwide. The topically applied fluoride ion controls caries by inhibiting demineralization and facilitating the remineralization of damaged enamel.[2] However, although topical fluoride administration during treatment reduces caries problems statistically, patients’ compliance with fluoride usage at home is often poor.[3] Geiger et al have observed that 52.5% of the patients did not use the prescribed fluoride solutions properly at home.[4]

Titanium tetra-fluoride (TiF4) has been proposed to be a comparable or even a better substitute for NaF, and as a result, it has gained increasing attention as an anti-caries agent during the past decades.[5-7] Animal studies have suggested that TiF4 provides a favorable level of protection against caries and it is at least as good as NaF.[8,9] The protective capacity of TiF4 is obtained from both the fluoride and titanium rich layer that covers the enamel surface after application, reducing the enamel’s solubility levels and preventing cariogenic activities.[7,10,11]

Another hope for the prevention of enamel demineralization is a laser. As early as 1965, investigators showed that some degree of protection against enamel demineralization could be achieved by laser irradiation.[12] Many theories are available on the mechanisms of enhancing the resistance of tooth enamel to acidic exposures by laser radiation. Such theories contain a wide spectrum, from surface melting using partial fusion and recrystallization of enamel prisms to alternations in the organic matrix of the enamel.[13,14] Previous studies have

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demonstrated an improvement in enamel caries resistance after CO_2 laser irradiation.[15,16] In addition, the CO_2 laser has been shown to increase fluoride uptake by the enamel and reduce its acid solubility when used in combination with topical fluoride.[15,16] A combination of fluoride and laser can also decrease the frequency of fluoride application needed for optimal prevention. Therefore, the combined use of laser and fluoride, especially TiF_4, could be the method of choice for patients with fixed orthodontic appliances.[17]

Few studies have investigated the synergic effects of lasers and TiF_4 on enamel demineralization around orthodontic brackets. The aim of this study was to evaluate the effect of the combined treatment method with the CO_2 laser and titanium tetra fluoride gel on the demineralization control of enamel around orthodontic brackets.

Material and Methods

Sample Selection and Preparation

This study was performed after obtaining the approval of the ethics committee (#8794112). Eighty caries-free human premolars were used. Then, all the teeth were cleaned and polished by using non-fluoridated pumice and prophylactic rubber cups on a low-speed handpiece. Afterward, a solution containing 0.1% thymol was used to store the teeth in a refrigerator for not more than 4 weeks until they were used in this study. Teeth with caries, developmental enamel defects, enamel cracks, visible white spots or other stains, and previous treatments were excluded.

Prior to etching, a self-adhesive tape with a cutout window, in the size of a bracket base, was used for all the teeth to avoid etching and sealing of enamel areas that will not be sealed by the bracket.

The buccal surfaces were etched with 37% phosphoric acid (Kerr, Bolzano, Italy) for 15 seconds and washed rigorously by using water spray for half a minute, followed by drying with an oil-free airstream for 20 seconds, which resulted in enamel a chalky surface. Thereafter, one bracket was bonded to the buccal surface of each tooth using Transbond XT (3M/Unitek, Monrovia, California) in line with the producer's instruction. An LED light curing device (Blue Phase; Ivoclar Vivadent, SCHAAN, Lichtenstein) with an approximate power of 600 mW/cm^2 was used for a total of 20 seconds on each tooth to cure the bonding agent (5 seconds from each side of the bracket). In the next step, a thin coating of acid resistance nail varnish was used to paint all the teeth, which covered all surfaces except the buccal surface.

Treatment of the Samples

The teeth were randomly allocated into four groups of 20 teeth each. The control group (C) received no treatment. The CO_2 laser (Ultra Dream Pulse V, DS_40U, Daeshin Enterprise, Seoul, South Korea), which can emit energy on a 10.6 µm wavelength for 10 s, was used to treat the second group (L). The parameters were set according to the guideline published by the manufacturer: peak power = 291 W; pulse duration = 100 µs, and interval time = 20 ms.[18] The size of the beam spot was set at 0.2 mm. Before performing the irradiation, the distance was set at 10 mm in the focal point of the laser beam. The irradiation was carried out in swabbing motion, and this area was precisely irradiated. Water was not used as a coolant during the process.

The samples in the third group (F) were immersed for 4 minutes in 4% TiF_4 gel (pH 3.2) which was prepared from titanium (IV) fluoride (Alfa Aesar, MA, USA), carboxymethyl cellulose (CP Kelco Chemicals Co., Taixing, China) and distilled water. The TiF_4 gel was then washed off. In the fourth group (L + F), the samples were first irradiated with the CO_2 laser for 10 seconds and then treated with TiF_4 gel for 4 minutes exactly as explained for groups L and F.

Induction of Artificial Caries Lesions

All samples were subjected to the dynamic demineralization and remineralization cycling model as suggested by Featherstone et al[19] and the solutions proposed by Argenta et al,[20] while brackets were in place. The pH cycling challenge involved the immersion of the specimens in a demineralization solution (2.0 mmol/L of calcium, 2.0 mmol/L of phosphate, and 75 mmol/L of acetate at pH 4.5) for 6 hours, and then it was washed in deionized water, dried by using jets of air, and immersed in a remineralization solution (1.5 mmol/L of calcium, 0.9 mmol/L of phosphate, 150 mmol/L of potassium chloride, and 20 mmol/L of Tris buffer at pH 7.0) for 18 hours at 37 °C. This process was repeated for two weeks every day, and the solutions were changed every 5 days. After exposure to the artificial caries solution for 2 weeks, visual inspection of the teeth revealed the presence or absence of demineralization by observing frosty white enamel on the dried teeth.

Caries Lesion Depth Measurement

A casting resin was applied to the top of each tooth that was diagnosed with demineralized enamel and allowed to polymerize. This was done in order to protect the surface of the specimen during the cutting procedure. Brackets were removed with a Lift off De-bracketing Instrument (3M/Unitek), and the teeth were sectioned buccolingually 1mm far from the cervical of the bracket attachment site with a CNC cutting section machine (Axes Full Automatic, NemoFanavaran Pars, Mashhad, Iran) along the long axis of the tooth to obtain specimens that were 200 microns thick. The thickness of sections was then reduced to 80-90 microns with a Polisher Machine (Full Automatic Polish, NemoFanavaran Pars, Mashhad, Iran). The specimens were then mounted on glass slides with 80% glycerol. Afterward, the sections were immersed in water (refractive index 1.33) to evaluate them under polarized light microscopy using an Olympus polarized optical microscope (Olympus Optical CO. Ltd. BX60F5, Tokyo, Japan). Before photographing sections using maximum illumination, areas of demineralization were centered in the field of view. Evaluation of photomicrographs was performed at 40 × magnification. Figures 1 and 2 show the demineralization in the magnified tooth section(s).

An operator, who was blind to the study group to which each specimen belonged, measured the maximum

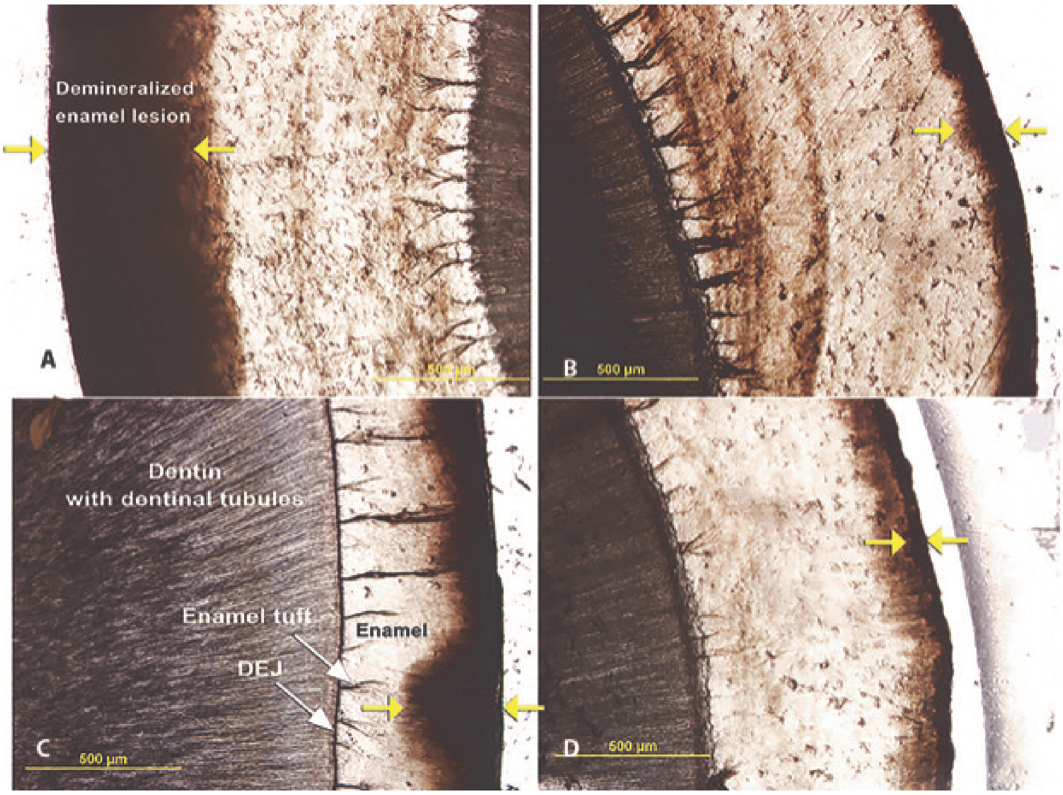

Figure 1. Polarized Light Photomicrograph of the Demineralized Enamel Lesion in A: Control group, B: Laser Group, C: Flouride Group, D: Laser + Flouride Group

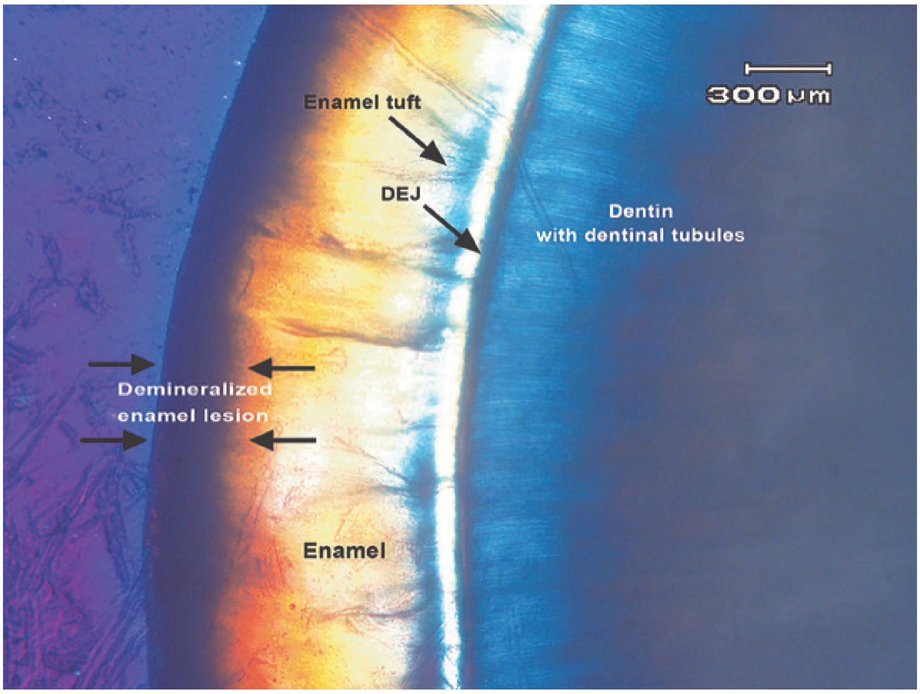

Figure 2. Polarized Light Photomicrograph of the Demineralized Enamel Lesion

depth of white spot lesions using commercial software (DP Controller, Olympus Corporation). In each section, the software should be calibrated prior to taking measurements with regard to the image magnification. The software projected an adjustable scale line on the computer screen which could be moved into a desirable location. After the operator adjusted the scale line to the desired location of each white spot lesion, the software calculated the depth of the lesion automatically. This process was repeated three times within a one-week interval by the same trained operator and calibrated by an oral pathologist. The mean of the three measurements made on each section was considered as the depth of demineralization lesion for that specimen. For all groups, mean depth measurement was performed by using lesion depth made for each tooth in that group. Differences in mean lesion depth between the four groups were applied for statistical analysis.

Statistical Analysis

Data were analyzed by SPSS software using one-way analysis of variance (ANOVA) and Tukey post-hoc. Lesion depth for each study group was summarized as mean and standard deviation (SD). Statistical significance was considered when P value < 0.05 and was calculated two-sided.

Results

The mean and SD of decalcification depths in all groups are provided in Table 1. The mean depth of lesion was the highest in the C group and then decreased in TiF4, CO_2 laser and F + L groups, respectively. The difference between all groups was significant (P < 0.05), except the CO_2 laser and F + L groups. However, the caries lesion depth was smaller in the L + F group than in the L group. Therefore, the F + L group showed the best results. Multiple comparisons between groups are shown in Table 2.

Discussion

This experiment sought to compare the capability or efficacy of combined treatment with the CO2 laser and TiF4 gel in preventing the demineralization of enamel in human teeth.

The performance of such treatment was compared with treatment procedures that have long been studied in the literature involving TiF4 gel and CO2 laser irradiation. Our finding demonstrated that the F + L and L groups had significantly decreased enamel demineralization. While the F + L and L groups were not statistically different, the mean depth of caries lesion in the F + L group was 11.25 µm smaller than the laser group. Based on these results, it can be concluded that CO2 laser irradiation greatly increases the overall performance of TiF4 fluoride gel, and this fact justifies their combined usage.

The findings of this study demonstrated that the treatment of the enamel surface with the CO2 laser decreases enamel demineralization compared to the control group. Studies have shown that wavelengths of 9 to 11 µm of CO2 lasers are effectively absorbed by dental hydroxyapatite, causing the loss of the carbonate mineral, which in turn reduces the acid reaction.[21] Featherstone et al in a clinical study that used the CO2 laser reported significant outputs.[22] There are many controversial studies regarding laser therapy due to differences in the type of applied lasers, irradiation parameters, administered demineralization, and evaluation technique.[23] It can

Table 1. Mean Value of the Caries Lesion Depth (µm) and Standard Deviation of Each Group.

Groups	Mean (SD)	Min	Max
Control (C)	406.60 (±94.56)	302	590
Laser (L)	152.00 (±42.31)	101	214
Fluoride (F)	193.50 (±46.19)	121	277
Laser + Fluoride (L + F)	140.75 (±39.38)	95	201

Table 2. Multiple Comparisons of Groups by Tukey Post Hoc Test

Group	Group	Mean Difference	Standard Error	Significance	95% Confidence Interval	
					Lower	Upper
F	L	41.500*	14.007	0.031	2.61	80.39
	L + F	52.750*	13.573	0.002	15.03	90.47
	C	-213.100*	23.534	0.000	-.279.78	-.146.42
L	F	-41.500*	14.007	0.031	-80.39	-2.61
	L + F	11.250	12.925	0.948	-24.63	47.13
	C	-254.600*	23.166	0.000	-320.48	-188.72
L + F	F	-52.750*	13.573	0.002	-90.47	-15.03
	L	-11.250	12.925	0.948	-47.13	24.63
	C	-265.850*	22.907	0.000	-331.18	-200.52
C	F	213.100*	23.534	0.000	146.42	279.78
	L	254.600*	23.166	0.000	188.72	320.48
	L + F	265.850*	22.907	0.000	200.52	331.18

be argued that changing the irradiation parameter of a laser towards the optimum condition is associated with a significant effect on caries prevention. According to Hsu et al, in a pilot study, if the energy density of CO2 laser irradiation on enamel is more than 3 J/cm^2, crater formation, surface melting and surface flaking will be observed.[24] In addition, it is reported that if the temperature reaches 400 °C, the majority of enamel structures become positively birefringent.[25,26] In the present study, laser parameters were selected in such a way that they can minimize the adverse effects of the CO2 laser on teeth, that is, preventing pulp temperature increase, crater formation, and enamel flaking.[24]

Fluoride is the most potent cariostatic agent. Clinical studies have shown the caries preventive effect of topical fluoride (20 to 40%),[27] but fluoride cannot completely stop the development of tooth decay. The result of this study showed that the application of TiF4 topical gel could decrease enamel demineralization around orthodontic brackets significantly compared to the control group. Even though these results are considered positive, they are inferior compared to other study groups as the decline was lesser than that in both the laser and fluoride + laser groups. Contrary to these findings, a recent study observed similar acid resistance in groups treated with APF topical fluoride with and without CO2 laser irradiation.[28] This means TiF4, by itself, can provide a higher resistance to demineralization compared to APF. The difference could be attributed to the mechanism of action of TiF4. TiF4 applied to the tooth structure reacts with oxygen to prevent the penetration of acids produced by bacteria by forming a layer of titanium dioxide as a physical barrier.[29,30] However, bacterial acid exposure is observed in some areas because the titanium oxide layer is heterogeneous and the gaps in the titanium dioxide layer are evident in the morphology of the enamel.[31,32]

In this study, the combined administration of laser and fluoride resulted in the highest level of effectiveness. Consistent with the findings of the present study, some studies reported a synergistic effect of the combined use of laser irradiation and fluoride on acid resistance.[14,33,34,35,36] Studies have shown that associating CO2 and other lasers like Nd:YAG laser irradiation with a topical application of fluoride results in enhanced resistance to caries lesions.[17,37,38] In this research, the association of fluoride with the CO2 laser could increase the demineralization resistance of tooth enamel, which is also confirmed by the results of other studies that examined this issue without the use of orthodontic brackets.[39] Nevertheless, there are controversies, as such synergistic effects are not reported by some authors.[15,39,40] For instance, such effects are not reported by Seino et al for CO2 laser irradiation and topical APF fluoride application.[28] In addition, de Souza-e-Silva et al corroborated such findings since they reported no synergism after comparing the effects of CO2 laser associated with/without fluoride.[15]

Different explanations have been proposed for the mechanisms of laser-fluoride combination efficacy in reducing enamel demineralization. One concept is based on the importance of trapping fluoride ions into microscopic spaces in the enamel, which are produced during laser irradiation. These small spaces are also important in preventing enamel dissolution. The morphological changes of the enamel surface after topical fluoride administration followed by CO2 laser irradiation can be used to justify the enhanced resistance to demineralization compared to the sole fluoride gel. Hydroxyapatite transforms into fluorapatite during laser irradiation which causes the HA crystals to melt and fuse again. A series of studies mentioned the ability of irradiated enamel to retain fluoride for longer durations.[37,39,40] Structural alteration in the enamel surface might be the main mechanism by which laser irradiation increases enamel acid resistance when used alone, but this mechanism can explain the results seen in the L + F group to some degree. In our study, because the laser used prior to fluoride application was in direct contact with the enamel surface, there should be much structural change in the surface of enamel in the L + F group, which could cause better integration between fluoride and the enamel structure. The superior demineralization resistance seen in the L + F group is therefore because of the fluoride action accelerated by laser structural and thermal effects. It is suggested that future studies should compare the microscopic structure of the titanium oxide layer with and without CO2 laser irradiation to confirm this. The theory that the CO2 laser has a synergistic effect with TiF4 needs to be further investigated.

Conclusion

According to the results of this study, the lowest amount of demineralization around the orthodontic brackets was observed in the L + F group, followed by the CO2 laser, TiF4, and control groups, respectively. Therefore, the most appropriate option to prevent demineralization around orthodontic brackets is to use a combination of laser and fluoride.

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Authors' Contribution

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Investigation: Reza Zandian, Reza Fekrazad.
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Visualization: Reza Zandian, Reza Fekrazad.
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Writing–review & editing: Vahid Moshkelgosha, Reza Fekrazad.

Competing Interests

There is no conflict of interest to declare.

Ethical Approval

This study was performed after obtaining the approval of the ethics committee (#8794112).

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