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In Vitro Evaluation Of Enamel Demineralization Around The Brackets After Er-Yag Laser Irradiation And Flouride Application

Braket Çevresindeki Mine Demineralizasyonunun Er- YAG Lazer ve Florid Uygulaması Sonrası İn-Vitro Olarak Değerlendirilmesi

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Öz

Amaç: Bu in-vitro çalışmanın amacı; birlikte ve ayrı ayrı florür ve farklı dozlarda Er:YAG lazer uygulamalarının, braket çevresindeki mine yüzeyinde oluşan demineralizasyona karşı etkilerini incelemektir. **Gereç ve Yöntem:** Bu çalışmada 80 üst daimi 1. premolar 8 grup olarak ayrılmıştır: G1, kontrol; G2, asidik fosfat florit (AFF); G3, 0.50 W Er:YAG lazer ; G4, 0.50 W Er:YAG lazer + AFF; G5, 0.75 W Er:YAG lazer; G6, 0.75 W Er:YAG lazer + AFF; G7, 1 W Er:YAG lazer; G8, 1 W Er:YAG lazer + AFF. Braketler premolarların bukkal yüzeylerine yapıştırılmıştır. Demineralizasyon değerleri dişin gingival ve braket arasındaki bölgede DIAGNOdent yardımıyla ölçülmüştür. Son ölçümde yüzey düzensizliği Atomik Kuvvet Mikroskobu (AKM) ile belirlenmiştir. Yapay çürük lezyonu oluşturmak için tüm gruplar 5 ve 9 günlük pH siklusuna tabi tutulmuştur. Veriler Kruskal-Wallis, Friedman and Duncan istatistik testleri kullanılarak analiz edilmiştir ($p<0.05$). **Bulgular:** G1 ve G7 gruplarında istatistiksel olarak önemli derecede demineralizasyon görülmüştür ($p<0.05$). Diğer grupların demineralizasyon değerlerinde istatistiksel olarak önemli değişiklikler görülmemiştir ($p>0.05$). AFM kayıtlarında en iyi yüzey görüntüsü grup 4'te saptanmıştır. **Sonuç:** Uygun dozlarda Er-YAG lazer uygulamalarının demineralizasyon üzerine pozitif etkileri bulunmaktadır.

Anahtar Kelimeler: Braket; Demineralizasyon; Er -YAG lazer; Florür

Abstract

Objective: The aim of this in vitro study was to investigate the effects of a fluoridated agent and Er:YAG irradiation with different doses, alone or in combination, on enamel resistance to demineralization. **Materials and Methods:** This study consisted of 80 premolars divided into eight groups: G1, untreated (control); G2, Acidic Phosphate Fluoride (APF) for 4 min; G3, 0.50 W Er:YAG laser; G4, 0.50 W Er:YAG laser + APF; G5, 0.75 W Er:YAG laser; G6, 0.75 W Er:YAG laser + APF; G7, 1 W Er:YAG laser; G8, 1 W Er:YAG laser + APF. Brackets were bonded to the buccal surfaces of premolars, and demineralization values were measured before and after treatment from the gingival aspects of the brackets, with DIAGNOdent. In last timepoint surface roughness was detected with Atomic Force Microscopy (AFM). All groups were subjected to 5 and 9 days of pH-cycling to produce artificial carious lesions. Data were tested using the Kruskal-Wallis, Friedman and Duncan tests ($p<0.05$). **Results:** G1 and G7 demonstrated significant deminerali-

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zation when compared to the initial measurements to 5th day measurement ($p<0.05$). The other groups did not reveal significant changes in the demineralization values ($p>0.05$). The best surface was observed in the AFM records of group 4.**Conclusion:** Optimum Er:YAG laser irradiation with settings has a positive effect on the decrease in demineralization.

Keywords: Bracket; Demineralization; Er YAG laser; Flouride

Introduction

It is known that dental caries are the result of a situation of unbalance between demineralization and remineralization (1). The maintenance of oral hygiene is often difficult with many orthodontic materials (elastics, springs, plastic sleeves), and this causes much easier plaque accumulation in these areas (2,3). The non-uniform surfaces of orthodontic appliances restrict the naturally occurring self- cleaning mechanisms of the oral tissues and saliva.

Clinical studies have shown that there is an increased incidence of carious lesions on the facial and lingual surfaces during treatment with fixed orthodontic appliances (1,4). Initial carious lesions in these regions are defined as white spot lesions (WSLs) in the literature. WSLs are areas of demineralized enamel that usually develop because of prolonged plaque accumulation. WSLs are the earliest indication of carious disease, and have the appearance of a chalky white spots on the surface of the tooth, showing an area of demineralization of enamel, which are widespread in populations with high levels of carious disease (5). An increase in and higher severity of white spots, after fixed orthodontic treatment have been found in treated teeth, around brackets and bands, than in untreated teeth (4,6).

Experiments have revealed that enamel demineralization has two initial stages: surface softening and surface lesion. Therefore, exposing the aspects of demineralization has become important. Many in vitro techniques for diagnosing and detection of enamel demineralization have been used in dentistry: clinical visualization, stereomicroscopy, electron microscopy and light-induced fluorescence (LIF) (7-9). In recent years, LIF has become popular because it is a non-invasive, easy and reliable method that does not require additional equipment (7,10,11).

Several preventive methods are used to reduce the amount of demineralization around the brackets. One of the most common of the widely used methods includes fluoride in toothpastes, gels, varnishes and mouth rinses (12). The mechanism of fluoride reduces decalcification and caries, and has also been shown to increase the resistance of enamel to acids (13).

In recent years, lasers have been widely used in dentistry for the detection of dental caries, root canal disinfection, treatment of dentin hypersensitivity, soft tissue surgery, pain reduction, etching of enamel for bonding, and inhibition of enamel demineralization, particularly when conventional treatments are not effective (14,15). Many researchers have demonstrated that treatment with different lasers, either alone or in combination with topical fluoride treatment, can reduce the rate of enamel demineralization (16-20).

It is known that demineralization causes irregularities in the enamel surface. Atomic force microscopy (AFM) can be used to compare the surface irregularities and get an idea of the demineralization level. AFM uses multiple mechanical scans in high resolution, and is often used for the analysis of surface irregularities (21,22).

The aim of this in vitro study was to investigate the effects of a fluoride agent and Er:YAG irradiation with different doses (alone or in combination) on enamel resistance to demineralization around orthodontic brackets with LIF, and observe these with AFM.

Material-Method

Eighty sound premolars, which were extracted for orthodontic reasons, were collected, and metal brackets (Master Series, American Orthodontics, Sheboygan, WI, USA) for upper first premolars were bonded to the buccal surfaces of the teeth with adhesive (Transbond XT, 3M Unitek, Monrovia, CA, USA). The study was carried out using the possibilities of Orthodontic Department of Gaziantep University Faculty of Dentistry and the personel resources of the researcher with the permission of head of the department. All of the teeth were divided into eight groups as follows (Table 1):

G1- Untreated (control)
G2- Only acidic phosphate fluoride (APF) applied for 4 min

G3- Only 0.50 W Er:YAG laser irradiated,
(0.50 W, 10 Hz, 50 mJ, Energy Density(ED):10 J/cm2)
G4- Er:YAG laser irradiated
(0.50 W, 10 Hz, 50 mJ, ED:10 J/cm2) + APF applied
G5- Er:YAG laser irradiated
(0.75 W, 10 Hz, 75 mJ, ED:15 J/cm2)
G6- Er:YAG laser irradiated
(0.75 W, 10 Hz, 75 mJ, ED:15 J/cm2) + APF applied
G7- Er:YAG laser irradiated
(1 W, 10 Hz, 100 mJ, ED:20 J/cm2)
G8- Er:YAG laser irradiated
(1 W, 10 Hz, 100 mJ, ED:20 J/cm2) + APF applied

The Er:YAG laser irradiation and fluoride applications were done by the same investigator (R.O.) to the enamel surface between the lower border of the brackets and the gums.

Laser Application

An Er:YAG laser (Fidelis Plus 3, Fotona, Ljubljana, Slovenia) with a contact handpiece with a diameter of 0.8 mm was used in this study. Each tooth surface was irradiated for 10 seconds at a 1 mm distance from the surface.

Fluoride Application

The groups including the fluoride application used 1.23% APF topically (Sultan, Topex, NJ). The fluoride gel was applied to the surface with disposable brush tips and left undisturbed for 4 minutes. After the exposure time, the fluoride was removed from the enamel surface with cotton rolls (23).

Timeframe Of Study Procedure

T1 (0th day): Measurement of demineralization values before the Er:YAG irradiation and fluoride application from the gingival aspects of the brackets with a fluorescence method, which used a tool for the detection of caries (DIAGNOdent, KaVo, Biberach, Germany) (8).

T2 (5th day): 5 days after the Er:YAG irradiation and/or fluoride application.

T3 (9th day): 9 days after the Er:YAG irradiation and/or fluoride application.

All demineralization measurements were done by the same operator. A single operator is used so that the same regions can be measured with the same precision.

Ph-Cycling Procedure

The pH-cycling consisted of two solutions which were used to produce artificial carious lesions. Each day, all of the teeth were stored for 6 hours in a demineralizing solution at room temperature, containing 2.0 mmol/L of calcium and 2.0 mmol/L of phosphate in a 75 mmol/L acetate buffer (pH 4.6). After the 6 hours of treatment, all specimens were transferred into a remineralizing solution for 17 hours, and were kept in this remineralizing solution containing

1.5 mmol/L of calcium, 0.9 mmol/L of phosphate, and 150 mmol/L of KCl in a 20 mmol/L cacodylic buffer (pH 7.0). Each group was immersed individually in 50 mL of solution. A two times thirty minutes wash in de -ionized and distilled water was done between the demineralizing and remineralizing phases, and at the end of the process. This procedure continued for 9 days and the demineralization records were taken on the 5th and 9th days (24).

Atomic Force Microscope (AFM) Procedure

Finally, the AFM study was conducted. AFM is a process to evaluate the surface roughness of the enamel, and all of the teeth were analyzed using this method at the end of the pH-cycle at the T3 timepoint.

All of the values were calculated as the mean ± standard deviation (SD) by SPSS software version 10.0 (SPSS Inc., Chicago, IL, USA). The obtained data were analyzed by using the Kruskal-Wallis, Friedman and Duncan tests for pairwise comparisons among groups ($p<0.05$).

Results

Differences among the groups in T1, T2 and T3 The demineralization scores did not show any significant differences among the groups in the base measurements at T1 ($p>0.05$) (Table 2). There were significant differences among the groups at T2 ($p>0.05$) (Table 3). The T2 measurements showed that the APF group (G2) had the lowest demineralization scores. The highest demineralization was recorded in the Er:YAG irradiated group (1 Watt) (G7) (Table 3). There were significant differences among the groups in the 9th day measurements at T3 ($p<0.05$). The last measurement revealed that the lowest demineralization scores were recorded on the APF + Er:YAG laser (0.50 Watt) irradiated group (G4). The highest demineralization was observed (again) in the Er:YAG laser (1 Watt) irradiated group (G7) (Table 4).

Differences between the groups T1-T2, T1-T3 and T2-T3

T1-T2 (0th day to 5th day); The control group (G1) and Er:YAG laser irradiated (1 W) group (G7) showed statistically significant higher demineralization than other groups (G3, G4, G5, G6 and G8) in this time period (T1-T2) ($p<0.05$) (Table 5).

T1-T3 (0th day to 9th day); The results showed no statistically significant differences between groups' demineralization score changes at this timepoint (T1-T3) ($p>0.05$) (Table 5).

T2-T3 (5th day to 9th day); The results showed no differences between the groups at this time period (T1-T3) ($p>0.05$) (Table 5).

AFM Results

The lowest surface roughness was obtained in group 4 (Er:YAG laser irradiated (0.50 W, 10 Hz, 50 mJ) + APF) with the AFM method. Group 8 (Er:YAG laser irradiated (1 W, 10 Hz, 100 mJ) + APF) showed the highest surface roughness with the AFM method (Table 6) (Figure 1).

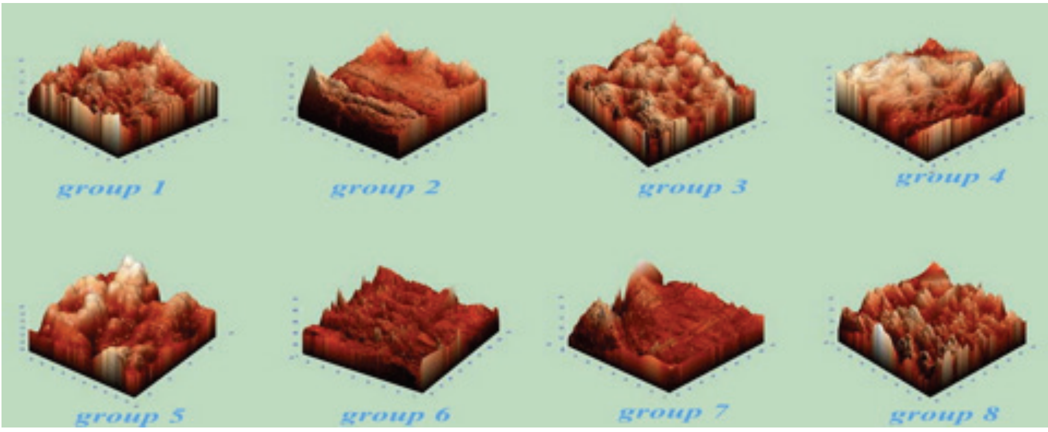

Figure 1. Atomic force microscope (AFM) images of groups at T3 timepoint

Table 1 APF and laser application procedures

Groups	APF Application	Laser Application
Group 1	-	-
Group 2	+	-
Group 3	-	0,50 Watt
Group 4	+	0,50 Watt
Group 5	-	0,75 Watt
Group 6	+	0,75 Watt
Group 7	-	1,00 Watt
Group 8	+	1,00 Watt

Table 2 Comparison of the mean demineralization scores in 8 groups measured with LIF in T1 (0 th day).

Groups	T1 (0^{th} day) Mean +/-S.d	p value
Group 1	3.6 +/-0.70	0.203
Group 2	3.3 +/-1.57	
Group 3	4.7 +/-1.49	
Group 4	3.6 +/-0.70	
Group 5	4.3 +/-1.64	
Group 6	4.5 +/-1.58	
Group 7	3.5 +/-0.71	
Group 8	4.2 +/-1.81	

Discussion

It has been shown that nearly 50% of orthodontic patients exhibit clinically visible WSLs during treatment, which lasts approximately 2 years. Therefore, our research focused on the effects of preventive attempts on demineralization. Diagnosing the demineralization of teeth becomes important, and DIAGNOdent is a useful, reliable and non-invasive method to detect carious lesions. Bechtold et al. used the LIF method to analyze the effectiveness of enamel sealants around orthodontic brackets (25).

Table 3 Comparison of the mean demineralization scores in 8 groups measured with LIF in T2 (5th day).

Groups	T2 (5thday) Mean +/- S.d	Duncan Test	P value
Group 1	4.2+/-1.03	b	0.011*
Group 2	2.8+/-0.63	ab	
Group 3	3.8+/-1.03	c	
Group 4	3.0+/-0.47	a	
Group 5	4.2+/-1.81	c	
Group 6	3.7+/-1.34	ab	
Group 7	5.1+/-2.13	c	
Group 8	3.9+/-1.10	c	

-Groups in the same column with different letters are statistically significantly different

*p<0.05 is statistically significant.

Table 4 Comparison of the mean deminera- lization scores in 8 groups measured with LIF in T3 (9th day).

Groups	T2 (9thday) Mean +/- S.d	Duncan Test	P value
Group 1	3.7+/-0.82	ab	0.044*
Group 2	3.0+/-0.67	ab	
Group 3	3.7+/-0.95	ab	
Group 4	2.9+/-0.57	a	
Group 5	3.8+/-1.99	ab	
Group 6	4.1+/-0.99	b	
Group 7	4.6+/-1.84	b	
Group 8	4.1+/-1.37	b	

-Groups in the same column with different letters are statistically significantly different

*p<0.05 is statistically significant.

Table 5 Comparison of the mean demineralization score changes in 8 groups measured with LIF between T1-T2 (0th day to 5th day), T1-T3 (0th day to 9th day) and T2-T3 (5th day to 9th day).

Groups	T2 (0thday) Mean +/- S.d	T2 (5thday) Mean +/- S.d	T2 (9thday) Mean +/- S.d	Mean changes T1-T2 (*p=0.013)	Mean changes T1-T3 (p=0.062)	Mean changes T2-T3 (p=0.161)
Group 1	3.6 +/-0.70	4.2+/-1.03	3.7+/-0.82	0.60 a	0.10 c	-0.50 d
Group 2	3.3 +/-1.57	2.8+/-0.63	3.0+/-0.67	-0.50 b	-0.30 c	0.20 d
Group 3	4.7 +/-1.49	3.8+/-1.03	3.7+/-0.95	-0.90 b	-1.00 c	-0.10 d
Group 4	3.6 +/-0.70	3.0+/-0.47	2.9+/-0.57	-0.60 b	-0.70 c	-0.10 d
Group 5	4.3 +/-1.64	4.2+/-1.81	3.8+/-1.99	-0.10 b	-0.50 c	-0.40 d
Group 6	4.5 +/-1.58	3.7+/-1.34	4.1+/-0.99	-0.80 b	-0.40 c	-0.40 d
Group 7	3.5 +/-0.71	5.1+/-2.13	4.6+/-1.84	1.60 a	1.10 c	-0.50 d
Group 8	3.5 +/-0.71	3.9+/-1.10	4.1+/-1.37	-0.30 b	-0.10 c	-0.20 d

-Groups in the same column with different letters are statistically significantly different

*p<0.05 is statistically significant.

Table 6 The mean AFM values in 8 groups in T3

Groups	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
The mean AFM values in 8 groups in T3	162.3	139.9	141.3	114.1	208.6	130.7	207.1	291.9

-Groups in the same column with different letters are statistically significantly different

*p<0.05 is statistically significant.

The base measurement score of the demineralization was between 3.60 and 4.70. All of the groups showed similar demineralization scores at T1. These results led us to conclude that the initial status of the groups showed similar responses to the demineralization process.

Five days later, the lowest demineralization scores were observed in the APF (only) group. Significant differences were achieved by comparing the base measurement with the 5th day scores. Correspondingly, the control group and Er:YAG laser (1 W) group were significantly different than the other groups, performing the highest in the demineralization scores. The lowest demineralization score was observed in the Er:YAG laser irradiated (0.50 W) group (Table 5). Interestingly, the lowest and the highest scores both belonged to laser irradiated groups. High laser doses may be cause microfissured morphology and mineral loss on enamel surface. Due to this, demineralization scores may be increased.

Our study showed that the Er:YAG laser, lower than 1 W average power, has reduced the demineralization scores. It is known that applying the laser can reduce the rate of enamel demineralization, and this can prevent dental caries. The Er:YAG laser is an effective instrument for the ablation of dental hard tissue due to its wavelength of light emission, which coincides with the absorption peak of water and hydroxyapatite. This thermo-mechanical interaction allows the removal of enamel and dentin effectively (26).

In the current study, the results of the Er:YAG laser with 1 W average power increased the demineralization scores dramatically. The Er:YAG laser irradiation with 1 W average power may injure the dental hard tissue and, therefore, cause high demineralization scores. The highest demineralization scores were observed in the group which used 1 W average power (Table 5). Many researchers also have concerns about enamel irradiation with Er:YAG lasers, which may cause some changes that create carious lesions. Rodrigez-Vilchis et al. showed this by using SEM to observe the changes in the enamel. They observed craters and cracks on the enamel surface, but these structures were not defined by the authors as greater than expected (27).

Rios et al. studied the asscociation between APF application and laser irradiation and found that this method is an alternative preventive measure against dental erosion (20). This increments were parallel to our study, but in this literature researchers was used Nd:YAG laser with high doses.

Many research studies have suggested the use of fluoride to reduce the incidence of decalcification (28). The fluoride deposits in hydroxyapatite are from fluorapatite, which activates the remineralization process. Our results support these findings and, additionally, that the application of only fluoride showed significantly better results than in the control group. Interestingly, applying fluoride not only decreased the decalcification, it reduced the negative effect of the Er:YAG laser. Although applying 1 W average power showed the highest harmful scores, the same value with fluoride did not show high demineralization scores. These results revealed that the Er:YAG laser irradiation plus APF increased the remineralization process. Contradictory to this study findings, the results of another laser study, Er:YAG laser irradiation did not increase acid resistance of the occlusal enamel surface as expected (27). This dissimilarity may be attributed by the application region of the laser irradiation, the present study we applied the laser irradiation buccal enamel of first premolars but in this study they were used the occlusal surfaces of third molars.

This result led us to think that the average power over 0.50 W may have a negative effect on the enamel of the teeth. Therefore, the low average power of the irradiation of the laser and application with APF has a significantly positive effect on the demineralization process.

The 9th day demineralization scores showed different results from the previous data. The best score was observed in the 0.50 W laser

irradiation with APF group. A comparison of the groups revealed that the change between time points did not show a statistically significant difference. Another study for detecting initial lesions with DIAGNOdent revealed that technique was effective in detecting the first demineralisation on enamel; however, the method did not show any effect in monitoring lesion progression after three cycles of in vitro demineralisation (29).

Because of these results, the initial scores of demineralization are important. The final values are dependent on the previous values. Using laser irradiation or applying fluoride were both settled at the 9th day.
In our study, we used AFM to observe the surface roughness of the enamel. According to our study, the greatest roughness was detected in the 1 W average power with APF group. The average power with 0.75 W group had similar results to the 1 W group. Finally, the laser irradiation under 1 W with the fluoride application showed lower scores then the laser only application groups.

Further laser and fluoride application studies are needed to evaluate the demineralization process with new diagnosing and detecting technologies such as; atomic absorption spectrometry and energy dispersive X-ray spectrometry.

Conclusions

Within the limits of this study, the following conclusions were drawn:

1. Er:YAG laser irradiation with optimum power settings has a positive effect on the decrease in demineralization (as in applying fluoride).
2. The overdose values of applying the laser contrarily increase the demineralization, while applying the overdose laser and fluoride inhibit the increase in the demineralization.
3. Adding the fluoride application to the laser irradiation may prevent the harmful effects of the laser irradiation.

Conflict of interest disclosure: The author declare no conflict of interest related to this study.

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