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Research Article

Evaluation of orthodontic bracket bonding strength on demineralized enamel: Effects of remineralization agents and SEM examination

Farklı yöntemlerle tedavi edilen erozyonlu mine yüzeyine uygulanan ortodontik braketlerin bağlanma dayanımlarının karşılaştırılması

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Abstract

Aim: To evaluate the shear bond strength (SBS) of orthodontic brackets bonded to demineralized enamel treated with four different remineralization agents and examine enamel surfaces using scanning electron microscopy (SEM).

Material and Methods: In this in vitro study, 120 premolar teeth were examined. The premolars were divided into six groups as; negative control (Group NC) with erosion remineralization cyclus were applied without any treatment agent, positive control (Group PC), treated with fluoride (Group F), with combined fluoride and diode laser (Group F+D), with CPP-ACP (Group M) and treated with nano-hydroxyapetite (Group B). Erosion remineralization cycle were applied to all samples except PC group. For each group, 20 samples were tested in SBS and examined using scanning electron microscopy (SEM).

Results: There was a statistically significant difference between groups in terms of the SBS values of the orthodontic brackets ($p<0.001$). There was no statistically significant difference between Group M and Group B ($p = 0.375$). There was a significant difference in all other binary comparisons ($p<0.001$). The sequence in terms of the SBS values of orthodontic brackets applied after erosion remineralization cycle of the groups were; Group NC (sound enamel)>Group B~Group M >Group F+D >Group F >Group PC (eroded enamel). SEM examinations corroborated the findings.

Conclusions: It was determined that remineralization materials applied in all study groups significantly increased the shear bond strength values of the eroded tooth surfaces. Group M and Group B were more efficient than other groups providing clinically acceptable SBS values for the bonding of orthodontic brackets to previously treated demineralized enamel surfaces.

Keywords: Enamel, erosion, remineralization, shear bond strength.

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Öz

Amaç: Çalışmanın amacı ortodontik braketlerin, dört farklı remineralizasyon ajanı ile işlenmiş demineralize mineye bağlanma dayanımının (Shear Bond Strength- SBS) değerlendirmesi ve taramalı elektron mikroskobu (SEM) kullanarak mine yüzeylerinin incelenmesidir.

Gereç ve Yöntemler: Bu in vitro çalışmada, 120 premolar diş incelenmiştir. Premolar dişler 20'şer dişin olduğu altı gruba ayrıldı: negatif kontrol (Grup NC)- herhangi bir tedavi ajanı uygulanmadan erozyon remineralizasyon döngüsü uygulanan grup, pozitif kontrol (Grup PC), florür ile tedavi edilen grup (Grup F), kombinasyon halinde florür ve diyot lazer ile tedavi edilen grup (Grup F+D), CPP-ACP ile tedavi edilen grup (Grup M) ve nano-hidroksiapatit ile tedavi edilen grup (Grup B). PC grubu hariç tüm örneklerde erozyon remineralizasyon döngüsü uygulandı. Her bir grup için 20 örnek, yapışma dayanımı SBS testine tabi tutuldu ve SEM kullanılarak incelendi.

Bulgular: Ortodontik braketlerin SBS değerleri açısından gruplar arasında istatistiksel olarak anlamlı fark tespit edildi ($p<0.001$). Grup M ve Grup B arasında istatistiksel olarak anlamlı bir fark bulunmamaktaydı ($p = 0.375$). Diğer tüm ikili karşılaştırmalarda önemli bir fark vardı ($p<0.001$). Grupların erozyon remineralizasyon döngüsü sonrasında uygulanan ortodontik braketlerin SBS değerleri sırası şu şekildeydi; Grup NC (sağlam mine)> Grup B ~ Grup M> Grup F+D> Grup F> Grup PC (erozyona uğramış mine). SEM incelemeleri SBS bulgularını destekler niteliteydi.

Sonuç: Çalışmamızdaki tüm gruplara uygulanan remineralizasyon materyallerinin, erozyona uğramış diş yüzeylerinin yapışma dayanım değerlerini önemli ölçüde arttırdığı belirlendi. Grup M ve Grup B, demineralize mine yüzeylerine ortodontik braketlerin yapıştırılması için klinik olarak kabul edilebilir SBS değerleri sağlama konusunda diğer gruplardan daha etkili bulundu.

Anahtar kelimeler: Mine, erozyon, remineralizasyon, bağlanma dayanımı.

Introduction

Dental erosion is the loss of hard tissue caused by a chemical factor without bacterial involvement.[1] Erosion has been a condition in dentistry that received little attention for years and was often overlooked in its early diagnosis. With changes in lifestyle, the consumption frequency, and quantity of acidic foods and beverages have increased in recent times. As a result, the etiology, prevalence, and approaches to the treatment of dental erosion have become increasingly important.[2]

In orthodontic treatment, brackets are attached to the enamel surface of teeth using various adhesives. Studies in this area have mostly focused on determining the optimal bracket-adhesive combination for bonding in orthodontic treatment. Although there have been studies on the retention of brackets applied to enamel surfaces whose morphological structure has changed due to erosion, there is no complete consensus on this matter. Therefore, it is important for clinical applications to determine to what extent various remineralization agents applied to eroded tooth enamel before orthodontic treatment prevent erosion and affect bracket retention.

The use of fluoride-containing preparations is recommended to protect teeth against acid attacks.[3,4] When fluoride is applied to the teeth, it forms weakly bound calcium fluoride (CaF_2) crystals on the tooth surface, protecting it against repeated demineralization and serving as a fluoride reservoir. At the same time, it facilitates the reattachment of minerals to the surface through the formation of fluoroapatite and fluorohydroxyapatite.[5] This attachment is further enhanced when the fluoride preparation is acidic, such as acidulated phosphate fluoride (APF). Due to its low pH, APF gel forms submicron CaF_2 and less soluble forms of calcium, reducing enamel permeability for a longer period and strengthening surface precipitation.[6] In addition to fluoride agents, laser applications, calcium and phosphate-containing toothpastes, casein glycopeptide, casein phosphopeptide, and preparations containing amorphous calcium phosphate have been investigated for remineralization purposes.[7,8]

Laser application has been reported to result in morphological changes such as surface melting and recrystallization, making the surface less susceptible to acid attacks.[9,10] Laser treatment is thought to decrease the Calcium/Phosphorus ratio on the tooth surface, change the calcium-phosphate ratio, create a thin layer of dissolution, and then re-precipitate and solidify inorganic material, making the teeth more resistant to decay.[11] It has also been reported that the critical pH (5.5), at which enamel dissolution begins, decreases to 4.8 after laser application.[12] Some studies have shown that the effectiveness of fluoride is enhanced when applied in combination with laser treatment.13,14 The mechanism of increasing fluoride retention by the laser is explained by the thermal effect of the laser beam, creating changes such as pits, microcavities, and roughness on the surface, increasing fluoride retention.

Another remineralization agent used to prevent dental erosion as an alternative to fluoride is casein phosphopeptide-amorphous calcium phosphate (CPP-ACP). Phosphopeptides in CPP-ACP's structure are protective factors found in milk. CPP is obtained by breaking down casein with trypsin enzyme using selective precipitation methods and can stabilize calcium phosphate as a CPP-amorphous calcium phosphate (ACP) complex.15

CPPs prevent ACP from precipitating in the solution by binding it in small clusters with phosphoserine extensions. This results in the formation of saturated, basic nanocomplexes with calcium phosphate. CPP-ACP can bind to tooth surfaces and dental plaque, functioning as a reservoir for calcium and phosphate.16 It is reported that when compared to fluoride toothpaste, CPP-ACP treatment reduced the lesion depth more on enamel surfaces.17

The remineralization effect of nano-hydroxyapatite added to the contents of toothpaste and mouthwashes has been reported.18 It is determined in a study that samples treated with toothpaste containing carbonate-hydroxyapatite nanocrystals, SEM images showed a thick, homogeneous apatitic structure which is formed by nanocrystals, completely covering interprismatic and prismatic enamel structures.19

The aim of our in vitro study is to evaluate which treatment method is more effective in terms of bonding strength of applied orthodontic brackets, based on the data obtained after the treatment of eroded tooth enamel surfaces with four different remineralization agents namely, Fluoride, Fluoride and Diode Laser combination, CPP-ACP and a toothpaste that includes nano-hydroxyapatite.

Material and Methods

The study is designed as an in vitro study. The sample size for each study group was determined statistically through a power analysis with Power: 0.95 and α: 0.05. As a result, the sample size was determined as n=20 for each group. Teeth included in the study are the lower and upper jaw first and second premolar teeth, which have completed root development and exhibit no signs of decay, hypomineralization, cracks, erosion, abrasion, restoration, core damage, etc. These teeth are non-amorphous and have not been subjected to any chemical agents.A total of 120 extracted human premolar teeth were collected, cleaned of soft tissue, stored in a 0.1% thymol solution, and utilized within a 2-month period. These teeth were obtained from patients with their informed consent, and the extractions were unrelated to the objectives of this study. The research project received approval from the scientific council of the Keçiören Education and Research Hospital Clinical Research Ethics Committee (decision no: 1369, decision date: 08.03.2017). The research project received approval from the local ethical commitee (decision no: 1369, decision date: 08.03.2017). The samples were divided into six groups as illustrated in Figure 1.

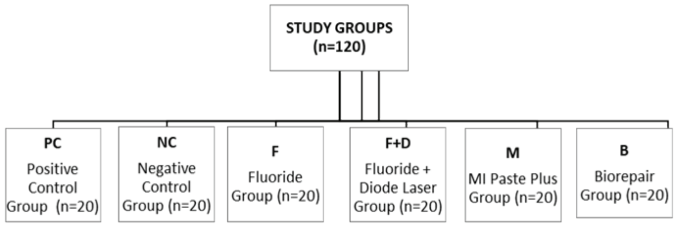

Figure 1. Study groups

Study Groups

a) Positive Control Group (PC): Throughout the experiment, apart from the removal of debris, the bracket bonding procedure with a 1% Citric acid solution (pH 2.4), and the erosion remineralization cycle, no additional surface treatment was conducted.

b) Negative Control Group (NC): During the experiment, apart from debris removal, the teeth were immersed in artificial saliva solution with a pH of 7, and no additional surface treatment was performed before the bracket bonding procedure.

c) Fluoride Group (F): In this group, in addition to erosion-remineralization cycle, fluoride gel (1.23% APF gel) was applied to the exposed enamel surfaces, including the bracket boundaries, using a cotton-tipped applicator and left for 2 minutes before bracket bonding.

d) Fluoride + Diode Laser Group (F+D): In this group, in addition to erosion-remineralization cycle, fluoride gel (1.23% APF gel) was applied to the enamel surface, followed by diode laser treatment following the manufacturer's recommendations (4 Watts, 60 Joules, 15 seconds). The diode laser beams, with pulse duration of 1 ms-1s and repetition rate of 0.5 Hz-0.5 kHz, were delivered to the sample surfaces via a fiber transport system before bracket bonding.

e) CPP-ACP Group (M): In this group, in addition to the erosion-remineralization cycle, CPP-ACP + 900 ppm fluoride (GC MI Paste Plus, RECALDENT™) was applied to the exposed enamel surfaces, including the bracket boundaries, using a cotton-tipped applicator and left for 2 minutes before bracket bonding.

f) Nano-Hydroxyapatite Group (B): In this group, in addition to erosion-remineralization cycle, a toothpaste containing nano-hydroxyapatite (BioRepair®, Coswell S.p.a, Bologna, Italy) was applied to the exposed enamel surfaces, including the bracket boundaries, using a cotton-tipped applicator and left for 2 minutes before bracket bonding.

Demineralization-Remineralization Cycle

During the erosion-remineralization cycle, for erosion, teeth were immersed in a 1% citric acid solution (pH 2.4) for 2 minutes, six times a day. For remineralization, the teeth were removed from citric acid solution, rinsed with distilled water, and then specific remineralization agents for each group, except for the control groups, were applied and afterwards the teeth were placed in an artificial saliva solution with a pH of 7, and this erosion-remineralization cycle was repeated for 10 days.

Bracket Bonding

Bracket bonding was performed using the same standards by a single operator. Firstly, a 37% orthophosphoric acid gel was applied to the buccal surface of each tooth for 20 seconds. Afterward, the teeth surfaces were cleaned and dried, and adhesive primer Transbond XT primer (3M Unitek, USA) was applied. Following this step, the adhesive bracket base was applied and attached to the tooth surface. In the study, brackets were attached to tooth enamel surfaces treated with different remineralization agents using light-cured Transbond XT resin (3M Unitek, USA).

Mini Master 0.018 slot stainless steel brackets (American Orthodontics, USA) were used as brackets. The base area of the brackets is 10 mm2. A Light Emitting Diode (LED) machine that emits blue light in the range of 430-480 nm was used as the light source (3M Elipar FreeLight 2, 3M ESPE, USA). Light was applied to the attached brackets for 20 seconds, with 10 seconds from the mesial and 10 seconds from the distal direction.

Thermal Cycling Process

Samples for evaluating bond strength were placed in a thermal cycling device (Dentester, Salubris Technica, Istanbul, Turkey) to simulate intraoral temperature changes after the brackets were bonded. The prepared samples were immersed 500 times in water baths at temperatures of 5°C and 55°C, respectively. Each time the samples were immersed in the bath, they remained inside for 20 seconds, and the transfer time between baths was set to 10 seconds by the device.

Shear Bond Strength Test

Shear bond strength (SBS) tests of the bonded brackets were conducted using a universal testing machine (Instron Universal Testing Machine, Elista, Istanbul, Turkey). Within the machine, there is a setup to hold the sample securely. The loading tip, which tapers to a sharp edge like a blade, was positioned parallel to the surface where the bracket was bonded. A shear force was applied to the tooth-bracket interface at a rate of 0.5 mm/minute until the bracket separated. The resulting data was recorded in Newtons using a computer connected to the machine. The results were later converted to Megapascals (Mpa) using the equation Mpa(N/mm2) = Force (Newton)/Bracket area (mm2).

Scanning Electron Microscopy Examination

Samples for Scanning Electron Microscopy (SEM) were embedded in special acrylics (SamplKvick Acrylic System, Buehler Lake Bluff, Illinois, USA). Images were acquired at different magnifications after being coated with a 100 Angstrom (A°) thick layer of platinum and were evaluated.

Statistical Analysis

Statistical analyses were conducted using the SPSS software (Statistical Package for Social Sciences, SPSS for Windows 17.0, IBM, USA). Descriptive statistics, including the mean, standard deviation, minimum, and maximum values of the force data in Mpa obtained from six groups, were calculated. Group comparisons were performed using Analysis of Variance (ANOVA) and Tukey post hoc tests, and $p<0.05$ was considered statistically significant.

Results

The descriptive statistics and statistical comparisons of the SBS values for six groups are presented in Table 1 and Table 2. The lowest SBS values were obtained in the PC group (6.56 ± 0.74 MPa), whereas the highest values were obtained in the NC group, (14.32 ± 1.66 MPa) .

Table 1. The descriptive statistics of the SBS values.

Group	SBS values (mean±SD Mpa)	SBS values (minimum,Mpa)	SBS values (maximum,Mpa)
NC	14.32±1.66	10.62	16.85
PC	6.56±0.74	5.13	7.90
B	11.67±1.56	8.59	14.48
M	10.85±1.10	9.48	13.77
F+D	9.49±1.00	7.73	11.71
F	7.95±1.59	5.58	11.40

SBS: Shear Bond Strength, NC: Negative control group, PC: Positive control group, B: Biorepair group, M: MI Paste Plus group, F+D: Fluoride + Diode laser group, F: Fluoride group, SD: standard deviation.

Table 2. Comparison of the means of SBS values among groups. Tukey multiple comparison test.

Groups	Significance
NC/ B	<0.001
NC / M	<0.001
NC / F+D	<0.001
NC / F	<0.001
PC / B	<0.001
PC / M	<0.001
PC / F+D	<0.001
PC / F	0.016
B / M	0.375
B / F+D	<0.001
B / F	<0.001
M / F+D	0.018
M / F	<0.001
F+D / F	0.005

SBS: Shear Bond Strength, NC: Negative control group, PC: Positive control group, B: Biorepair group, M: MI Paste Plus group, F+D: Fluoride + Diode laser group, F: Fluoride group. The mean difference is significant at the 0.05 level.

According to the ANOVA, there were significant differences between the SBS values of the groups ($p<0.001$). Comparison of the means of SBS values among groups with Tukey multiple comparison test showed statistically significant differences between all groups, except for groups B and M ($p=0.375$).

SEM images of intact, demineralized, and treated enamel surfaces are presented in Figure 2. In SEM images taken from the sound enamel surface, it is observed that the surface is smooth and due to the regular arrangement of enamel prisms, it has a homogeneous appearance. Typical enamel structures such as grooves and perikymata lines are distinct on the sound enamel surface. Additionally, small indentations or pits, indicative of cumulative mechanical effects on teeth, were observed (Figure 2a). SEM images of the demineralized enamel (Group PC) presented a porous structure, with the prisms increasing in size and distributing irregularly (Figure 2b). In F and F+D groups, demineralized areas were still observed, however, structures resembling CaF_2, similar to those seen in sound enamel morphology, were also observed (Figure 2c and 2d). On the surfaces of enamel samples treated with CPP-ACPF paste, remineralization was smoother and more homogeneous (Figure 2e). SEM image of Group B showed acicular nano-hydroxyapatite crystals deposited on the enamel surface, resulting in reductions in voids and erosion areas (Figure 2f).

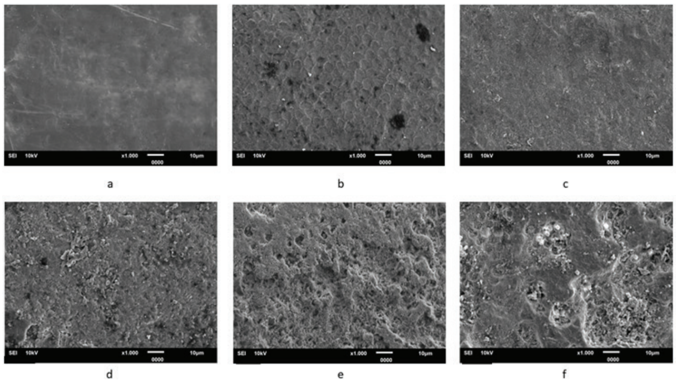

Figure 2. Scanning Electron Microscopy (SEM) images of the groups tested. Scanning Electron Microscopy (SEM) images of the groups tested with 1000x magnification. **2a:** sound enamel surface (Group NC), **2b:** demineralized enamel (Group PC), **2c:** Group F**, 2d:** Group F+D, **2e:** Group M, **2f:** Group B.

Discussion

In recent years, changes in lifestyles and conditions, along with an increase in the consumption of acidic foods and beverages, have led to an increased incidence of erosion in teeth. In our in-vitro study we used citric acid solution to simulate enamel erosion, samples were immersed in lemon 1% citric acid solution with a pH of 2.4 for 2 minutes, six times a day and then in artificial saliva solution with a pH of 7. It has been shown that when the teeth which were exposed to an erosive solution were stored in saliva, there were less erosion. Artificial saliva is based on the electrolyte composition of natural saliva. It does not contain saliva proteins that bind calcium and is highly saturated. Some proteins in natural saliva, such as statherin, tend to inhibit calcium phosphate precipitation. Artificial saliva, which lacks these proteins, causes excessive calcium phosphate precipitation.[20] Therefore, in vitro remineralization provided is higher than that occurring in vivo with artificial saliva.[20,21] In our study, 10 ml of artificial saliva was used for each sample, and the samples were kept in artificial saliva during each cycle.

Shear Bond Strength Testing

In fixed orthodontic treatment, it is crucial for brackets not to dislodge and to withstand chewing forces. In a study conducted by Reynolds in 1975, it was suggested that the minimum bond strength should be in the range of 5.9-7.8 MPa.[22]

Our results showed that all the applied remineralization treatment agents significantly increased the SBS values and the SBS values of demineralized specimens were lower than those of the other groups. The highest mean SBS value was obtained in the NC group (14.32±1.66 MPa). This was followed by Group B (11.67±1.56 MPa), Group M (10.85±1.10 MPa), Group F+D (9.49±1.00 MPa), Group F (7.95±1.59 MPa) and Group PK (6.56±0.74 MPa). There were no statistically significant differences between Group B and Group M (p=0.375).

There are several studies in the literature regarding fluoride use for demineralized teeth on SBS.23–25 This is because many patients use fluoride-containing toothpaste to clean their teeth before bonding brackets. The results of these studies showed that, fluoride increases the SBS values compared to the control groups which were the demineralized specimens. In our study, we found higher SBS values on erosive enamel surfaces treated with fluoride compared to our PC group, which was only subjected to demineralization.

Studies in the literature regarding Fluoride + Diode laser applications have mostly focused on measuring the level of microhardness or the decrease in Ca+2 and Ca+2/P ratios on the erosion surface. It is reported in the literatüre that when flüoride is used in combination with laser.26,27

The results of our study indicate that the Fluoride + Diode laser irradiation of the demineralized specimens significantly increased the SBS values compared to fluoride gel application alone.

CPPs have been shown to stabilize ACP, localize ACP in dental plaque, and have anti-cariogenic effects in animal and in situ human dental caries models.28,29 The increasing number of patients receiving CPP-ACP as a prophylactic agent before orthodontic treatment has led to an increase in studies on the effects of CPP-ACP on the bond strength of orthodontic brackets. The first ACP-containing orthodontic composite adhesive available on the market received FDA approval in 2002, and the results of a study conducted by Dunn30 were published. In this study, the bond strengths of orthodontic brackets attached to enamel surfaces using conventional resin-based orthodontic adhesive and ACP-containing orthodontic adhesive were compared. The results showed that the bond strength of orthodontic brackets attached with ACP-containing adhesive to teeth was significantly weaker than that of conventional resin-based orthodontic adhesive. Kecik et al.31, reported that the shear bond strength was positively affected when enamel surfaces were treated with 1.23% APF, CPP-ACP, or their combination. All bond strength values obtained in the study were well above the range of values recommended by Reynolds, providing preliminary data about the effect of CPP-ACP on the SBS of brackets. In the study by Al-Kawari et al.32, CPP-ACP+F (MI Paste Plus) was applied to enamel surfaces after acid erosion, and it was found that it significantly increased bond strength when compared to the control group before acid erosion and the results after application. Uysal et al.23 compared the effects of fluoride and CPP-ACP on the SBS of orthodontic brackets bonded to demineralized enamel and found no significant differences between the control and CPP-ACP groups. In our study, we used MI Paste Plus, one of the current products, as one of the remineralization agents we applied after erosion. We found that the MI Paste Plus group in our study significantly increased SBS values but not to the level of sound enamel, which was the negative control group.

Nano-hydroxyapatite (HA) crystals, one of the most popular remineralization agents in recent years, have the ability to penetrate the enamel surface, reform stronger crystals, and distribute freely available ions, thus exerting an effect on the tooth surface.33 Synthetic apatite or hydroxyapatite has been proven to be beneficial in enamel remineralization.34 HA crystals exhibit high biomimetic properties due to their composition, structure, morphology, mass, and surface physical-chemical properties. Cossellu et al.33, analysed the effects of six different prophylactic agents on the bond strength of orthodontic brackets. They compared commonly used prophylactic techniques (fluoride, CPP-ACP, ozone) with a new material, toothpaste containing nanohydroxyapatite (BioRepair®). Considering the limitations of this in vitro study, the results showed that the use of fluoride varnish before acid etching and bonding negatively affected the SBS of brackets, but no differences were observed in teeth treated with BioRepair®, ozone, and CPP-ACP.

In our study, BioRepair® (Coswell S.p.A., Bologna, Italy), which is a fluoride-free toothpaste made from hydroxyapatite nanoparticles. Showed the highest SBS values. Although this value was not statistically significantly higher than the MI Paste Plus group, it was significantly higher than all other treatment groups.

SEM Examination

The findings obtained from the SEM images support the SBS results. In the SEM images of F and F+D groups, demineralized areas were still somewhat visible in a honeycomb-like structure, and a mild irregular erosion pattern was observed. At the same time, structures resembling CaF2, similar to those seen in enamel morphology, were also observed. In the F+D group, the structures resembling CaF2 were observed in various regions of the enamel surface to a greater extent compared to SEM images of F group. It is reported in the literature that SEM images of fluoride treated groups, although less in amount, presented exposion of enamel prisms, similar to the demineralized control group. and also a more robust surface appearance35 that are consistent with our findings.

Magalhaes and colleagues36 examined the effect of using sodium fluoride and titanium tetrafluoride (TiF4) preparations in conjunction with Nd:YAG laser on enamel erosion, using SEM. They reported that in the group where TiF4 and Nd:YAG laser were used together, there were fewer pores and microcracks on the enamel surface compared to other groups. They concluded that TiF4 varnish protected against enamel erosion, without the influence of laser irradiation. Alsherif et al26 suggested in their in vitro study that the group that was treated with combined use of nanosilver fluoride and diode laser, displayed a homogenous subsurface enamel, that is similar to normal enamel mineralization. The SEM findings of our study was similar to the findings of Alsherif et al.26

Our results revealed that on the surfaces of enamel samples treated with CPP-ACPF paste (MI Paste Plus), remineralization was smoother and more homogeneous compared to the F and F+D groups. This difference can be attributed to the inclusion of casein, which contributes to the smoother and more uniform remineralization. It was shown that the SEM images of specimens that treated with CPP-ACP, the remineralized enamel surface was more resistant to acid attacks compared to normal enamel surfaces. CPP-ACP acts as a reservoir for calcium and phosphate by breaking down in the presence of low pH and helps reduce demineralization and Hemingway et al.37 showed that citric acid modified with casein, significantly prevented erosion, and SEM images revealed the presence of an approximately 5µm thick amorphous layer covering the surface. Oshiro et al.29 examined the results of applying CPP-ACP-containing paste by comparing SEM images and reported that in the control group images, significant demineralization was observed in the enamel surface layer, whereas in the group where CPP-ACP paste was applied, only very mild porosity was present in the enamel, indicating that demineralization was prevented.

The SEM findings of our study also revealed the presence of a remineralization layer, and it was observed that the remineralization was smoother and more homogeneous in F+D group.

The SEM images of our results demonstrated that the enamel surface treated with a toothpaste containing nano-hydroxyapatite after demineralization (Group B) showed acicular nano-hydroxyapatite crystals that deposited on the enamel surface, leading to a reduction in voids and erosion areas. The results of a study comparing the remineralization effect of a 10% nano-hydroxyapatite solution with a 2% sodium fluoride solution on initial enamel lesions were showed that nano-hydroxyapatite particles adhered to the pores resulting from demineralization in SEM images. These adhered nanocrystals grew at the sites of deposition and formed microclusters, creating a uniform apatitic layer on the demineralized enamel surface.38 Jeong et al.39 reported that SEM examinations performed after toothpaste application revealed that hydroxyapatite particles interacted with the enamel surface. that increased the concentration of Ca and P ions, resulting in the repair of the demineralized surface. In another study assessing the remineralization efficacy of nano-hydroxyapatite + fluoride, bioactive glass, and strontium acetate-fluoride-containing toothpaste, the results showed that in the group where nano-hydroxyapatite + fluoride toothpaste was applied, demineralization on the surface disappeared, and the surface was covered with a protective layer.40

In our study, it was observed that the enamel surface treated with toothpaste containing nano-hydroxyapatite was covered with a newly formed apatitic layer that extended over both the prismatic and interprismatic enamel structures. However, it was noted that the remineralization layer did not have a homogeneous surface. This lack of homogeneity was believed to be due to the absence of fluoride in our remineralization agent, which we used.

The main limitation of our study was calculation of the SBS values were in vitro, and more clinical research is needed to confirm the findings in clinical settings.

Conclusions

- Enamel demineralization significantly reduces the SBS of orthodontic brackets.
- SBS values in all groups met the bonding strength values required for clinical success, as determined by Reynolds (5.9-7.8 MPa).
- Fluoride, combined fluoride and diode laser, CPP-ACP and nano-hydroxyapatite applications, used after demneralization, increased SBS of orthodontic brackets and resulted in SEM findings nearly similar to normal enamel surfaces.
- There was no statistically significant difference between Group M and Group B and they were more efficient than other groups providing clinically acceptable SBS values for the bonding of orthodontic brackets to previously treated demineralized enamel surfaces.

Conflict of interest

The authors have no conflict of interest to declare.

Financial Disclosure

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