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The Effect of Remineralizing Agents With/Without CO_2 Laser Irradiation on Structural and Mechanical Properties of Enamel and its Shear Bond Strength to Orthodontic Brackets

Hannaneh Ghadirian[1], Allahyar Geramy[1], Waleed Shallal[2], Soolmaz Heidari[3], Nooshin Noshiri[4], Mohammad Ali Keshvad[1*]

[1]Department of Orthodontics, School of Dentistry, Tehran University of Medical sciences, Tehran, Iran
[2]Al Karama Teaching Hospital, Kut, Iraq
[3]Department of Operative Dentistry, Dental Caries Prevention Research Center, Qazvin University of Medical Sciences, Qazvin, Iran
[4]Medical Image Processing Laboratory (MIPL), Department of Technology and Engineering, Alzahra University, Tehran, Iran

***Correspondence to**
Mohammad Ali Keshvad, Department of Orthodontics, School of dentistry, North Kargar St, Tehran, Iran.
Tel: +989128026404;
Email: m_ali_keshvad@yahoo.com

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Abstract

Introduction: Remineralizing agents may be used for the treatment of white spot lesions (WSLs) prior to bracket bonding. However, some concerns exist regarding their possible interference with the etching and bonding process, negatively affecting the bond strength. This study aimed to assess the effect of two remineralizing agents with/without CO2 laser irradiation on the mechanical properties and shear bond strength (SBS) of demineralized enamel to the orthodontic bracket.

Methods: This study evaluated 60 premolar teeth in 6 groups (n=10) as follows: (I) sound enamel, (II) demineralized enamel, (III) Nupro remineralizing agent (N), (IV) Nupro and CO2 laser (N/L), (V) Teethmate remineralizing agent (T), and (VI) Teethmate and CO_2 laser (T/L). The remineralizing agents were applied to the enamel surfaces after their immersion in a demineralizing solution for 5 days. In groups IV and VI, the CO_2 laser with a 10.6 μm wavelength, 10 ms pulse duration, a 50 Hz repetition rate, 0.3 mm beam diameter and 0.7 W power was irradiated after applying the remineralizing agents. Brackets were bonded to the enamel surfaces and SBS was measured by a universal testing machine. For the assessment of enamel microhardness, 20 sections of molar teeth were divided into 4 groups (n=5; N, N/L, T, T/L) and their microhardness was measured before demineralization, after demineralization and after remineralization. X-ray diffraction (XRD) analysis, field-emission scanning electron microscopy (FESEM) and energy-dispersive spectrometry (EDS) were carried out to assess the formation of hydroxyapatite. The atomic percentages of the C, O, P, Ca, Na, Si, F and Ca/P ratio were determined by EDS analysis.

Results: The SBS significantly decreased in group II ($P<0.001$). There was no significant difference among the groups I, III, IV, V and VI ($P<0.05$). This finding was similar to the microhardness results, which showed an increase in microhardness after remineralization ($P<0.05$), with no difference among the remineralizing agents. The Ca/P ratio was the highest in the Nupro group and the lowest in the demineralized group.

Conclusion: Remineralizing agents can significantly improve the microhardness and structural properties of demineralized enamel to a level similar to that of sound enamel with no adverse effect on SBS to orthodontic brackets.

Keywords: White spot; CO_2 Laser; Shear strength; Nupro; Teethmate; X-ray diffractions.

Introduction

Orthodontic treatment is performed aiming to improve function, esthetics, and stability. Despite the favorable functional and esthetic results of orthodontic treatment with fixed appliances, this treatment may have unfavorable consequences such as periodontal disease and dental caries, particularly in patients with poor oral hygiene. Furthermore, the presence of archwires, brackets and bands can complicate oral hygiene practice in orthodontic patients.[1] Organic acids caused by acidogenic bacteria can dissolve the enamel minerals and cause porosities in the tooth surface, leading to initial carious lesions known as the white spot lesions (WSLs).[2]

Most studies have reported a prevalence of 23%-73% for WSLs in orthodontic patients, depending on treatment duration.[3-5] Several strategies such as patient education to

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maintain good oral hygiene,[6] the application of adhesives containing remineralizing agents,[7] and the prophylactic use of fluoride varnishes[8] have been suggested to minimize the development of WSLs. In addition to these preventive methods, the use of remineralizing agents is recommended before and during orthodontic treatment, especially for patients who have demineralized enamel prior to the onset of treatment. Some studies have reported a reduction in bond strength when fluoride or casein phosphopeptide amorphous calcium phosphates (CPP-ACP) were applied as a remineralizing agent before acid-etching, while some others did not confirm the adverse effects of remineralizing agents on bond strength of brackets.[9-13]

CPP-ACP can be used intraorally to deliver high concentrations of bioavailable calcium and phosphate ions, prevent demineralization and enhance remineralization as such. The cariostatic activity of CPP-ACP is related to the ability of CPP to localize ACP on the tooth surface and increase the calcium and phosphate levels in dental plaque.[14]

Bioactive glass has been introduced for dental prophylaxis, and many studies have attempted to remineralize the demineralized enamel using bioactive glass.[15-17] Bioactive glass reacts with saliva to release Ca^{2+}, Na^{+}, and PO_4^{3-} ions.[18] It forms a layer highly reach in calcium, phosphate and silica attached to the enamel surface and thus has the potential to remineralize the tooth structure.[19]

Many laser types are also used for preventive purposes in dentistry. The CO_2 laser is suitable for use on enamel surfaces because its wavelength is within the infrared spectrum and is absorbed by phosphate and carbonate bands.[20] The CO_2 laser plays an important role in caries prevention with insignificant adverse effects on tooth structure.[21] Laser irradiation generates heat, which modifies the carbonated hydroxyapatite by the fusion of hydroxyapatite crystals, and the reduction of interprismatic spaces in the enamel structure.[22,23]

Decreased shear bond strength (SBS) can lead to bracket debonding, cause problems for both the clinician and patient, increase the treatment cost, and prolong the course of treatment. Therefore, it is essential to find out whether the remineralizing agents have the ability to treat the demineralized enamel with no adverse effect on their SBS to orthodontic brackets. A safe material should not decrease the bond strength lower than 6 to 8 MPa, which is the minimum bond strength required for orthodontic bracket bonding.[24,25]

This study aimed to evaluate the effect of two remineralizing agents with and without CO_2 laser irradiation on the structural characteristics of demineralized enamel and its SBS to orthodontic brackets.

Materials and Methods

Sixty Extracted human premolars were used for the evaluation of SBS while 10 extracted molars were used for the evaluation of microhardness following demineralization and remineralization processes. The enamel disc-shaped samples were also used to evaluate the structural changes. The exclusion criteria were the presence of caries, cracks, erosion, fluorosis, hypo-calcification and dental restorations. The teeth had normal buccal and lingual surface morphology and were extracted atraumatically. All the teeth were cleaned from debris and soft tissue remnants and they were polished with non-fluoride pumice paste and rubber cups with a low-speed hand-piece. Then they were stored in a 0.5% chloramine-T solution for 7 days for disinfection.[26]

Microhardness Measurement

Sample Preparation

Ten molar teeth were decoronated at the cementoenamel junction, and each crown was longitudinally sectioned in half in the occlusogingival direction using a water-cooled, high-speed saw (Mecatome T201; Presi, France) to obtain buccal and lingual enamel samples with 2-3 mm thickness. Twenty enamel samples obtained as such were mounted in a custom-made wax mold and polished with 800, 1200, 1600 and 2000-grit silicon carbide discs under water coolant to obtain flat enamel surfaces. Two layers of nail varnish were applied to cover the enamel surface, leaving a window of about 4×4 mm for demineralization and remineralization processes.

Demineralization and Remineralization Processes

Twenty enamel samples were divided into 4 groups and underwent demineralization and remineralization processes. The microhardness of the surface was measured in each step using a microhardness tester (Vickers pyramid diamond indenter, MH Tester device, Bareiss, Germany) by applying a 100 g load for 8 seconds. In order to decide upon the proper time for the demineralization process, a pilot study was carried out. According to the obtained results, 5 days of immersion in demineralizing solution was chosen as the proper time to demineralize the enamel surface (a decrease in microhardness value by less than 50%). To evaluate the remineralization ability, the samples were treated with the respective remineralizing agents according to the manufacturers' instructions. The protocols of the application of remineralizing agents were as follows:

- Nupro (N) and Nupro/laser (N/L) groups: Nupro prophy paste was applied to demineralized enamel surfaces using a micro-brush with a rotational movement for 1 minute. The approximate thickness of the paste was nearly 2 mm.
- Teethmate (T) and Teethmate/laser (T/L) groups: The powder and liquid of the Teethmate remineralizing agent were mixed for 15 seconds. The slurry paste was applied to enamel surfaces by a brush in 2 mm thickness for 30 seconds and it was left to dry.

In laser groups, after applying the remineralizing agents, the pulsed CO_2 laser (Smart US-20D, Deka, Italy) was irradiated with a 10.6 μm wavelength, 10 ms pulse duration, a 50 Hz repetition rate, 0.3 mm beam diameter and 0.7 W power in a scanning motion for 10 seconds with a 5 mm distance away from the enamel surface.

Before immersion in remineralizing solution, a thin layer of a 7th generation bonding agent (Clearfil Tri-S Bond, Kuraray, Noritake Dental Inc., Okayama, Japan) was applied passively over the remineralizing agents to protect them from being washed-out.[27,28] The adhesive was applied for 20 seconds and cured using a light-curing unit for 10 seconds. The samples of each group were individually stored in glass containers containing artificial saliva as a remineralizing agent for 7 days.

At the end of the storage period, the adhesive layer that covers the remineralizing agents was gently removed with a tweezer. The samples were then rinsed with deionized water. The surface microhardness of all samples was measured after the remineralization process, similar to the aforementioned protocol. The composition of materials and solutions used for demineralization and remineralization processes is presented in Table 1.

Enamel Characterization

In order to evaluate hydroxyapatite formation, X-ray diffraction analysis (XRD), field-emission scanning electron microscopy (FESEM), and energy-dispersive spectrometry (EDS) were carried out. The enamel samples of molar teeth were prepared according to the method described for the microhardness test.

The XRD (X'Pert PRO-MPD, PANalytical Company, Netherlands) analysis was performed using Cu Kα radiation (k = 1.542 Å) operating at 40 mA and 40 kV in the range of 10-110° 2θ with continuous scanning. FESEM (MIRA3 TESCAN-XMU, Czech Republic) coupled with EDS (SAMX, France) with an accelerated voltage of 15 kV was carried out to assess the morphological changes of the enamel surface and identify elemental changes in the enamel surface of each sample. For FESEM, the enamel samples were cleaned with acetone, dehydrated with ethanol (at a gradient concentration of 70%, 80%, 90%, and 100% for 20 minutes) and left to dry at room temperature for 24 hours. The samples were then sputter-coated with gold.

Shear Bond Strength

Sixty premolars were randomly divided into 6 groups (sound enamel, demineralized enamel, N, N/L, T, and T/L). The remineralizing agents were applied as explained earlier. After the demineralization and remineralization processes, orthodontic brackets were bonded to premolars. Premolar stainless steel brackets (Discovery®, Dentaurum, Ispringen, Germany) were positioned at the center of the buccal surface of the anatomic crown and bonded using Transbond™ XT light-cure adhesive (3M Unitek, Monrovia, CA, USA) according to the manufacturer's instructions. Before photo-polymerization, each bracket

Table 1. Composition of Materials and Solutions Used for Demineralization and Remineralization Processes

Material	Company	Composition	Lot. No.	Method of Application
Demineralization solution	Hand mixing	$CaCl_2$, NaCl, NaH_2PO_4, NaN_3, Acetic acid		Immersion with 5 days
Remineralization solution	Hand mixing	$CaCl_2$, NaCl, NaH_2PO_4, NaN_3		Immersion with 7 days
Nupro prophy paste	Dentsply professional (UAS)	45S5 Bioactive glass (wt%): 45%SiO_2,24.5%Na_2O, 24.5%CaO, 6%P_2O_5	801227	Apply with rotational mixing for 1 min and cover with a bond for 7 days
Teethmate	Kuraray Noritake Dental Inc. (Japan)	1) Powder: Tetra-calcium phosphate, Di-calcium phosphate anhydrous 2) Liquid: Water, Preservative	REF1210-KA	Mix the powder and liquid for 15 s and apply for 30 s and then cover with a bond for 7 days
Clearfil Tri-S Bond	Kuraray Noritake Dental Inc. (Japan)	Bis-GMA, MDP, HEMA, hydrophilic aliphatic dimethacrylate, hydrophobic aliphatic methacrylate, colloidal silica, CQ, ethanol, water	13425	Apply passively over the remineralizing materials for 20 s and then light-cured for 10 s.
Transbond XT Primer	3M Unitek (Germany)	Bis-GMA, TEG-DMA, 4-dimethylaminobenzene, ethanol, CQ, hydroquinone	REF 712-035	Apply a thin layer to enamel
Transbond XT Composite	3M Unitek (Germany)	Bis-GMA, Bis-EMA, Acrylate, Monomers, Filler	REF 712-035	Apply to the bracket base, positioning of the bracket and then light-cured for 40 s.
Unitek Etching Gel	3M Unitek (Germany)	35% Phosphoric acid	REF 704-060	Apply for 15 s on the enamel and wash for 15 s with water-air spray
Nail Varnish	Iran	Acid-resistant paint		Apply over surfaces and leave to dry

Abbreviations: Bis-GMA: bisphenol A diglycidylmethacrylate; MDP: 10-methacryloyloxydecyl dihydrogen phosphate; HEMA: 2-hydroxyethyl methacrylate; NaF: sodium fluoride; CQ: campherquinone; TEG-DMA: triethyleneglycol dimethacrylate; Bis-EMA: ethoxylated bis-phenol-A-glycol dimethacrylate.

was subjected to 300 g compressive force by using a force gauge (Correx Co, Berne, Switzerland), as previously described by Bishara et al.[29] The excess adhesive paste was removed with a small scaler from the margins of the bracket base. All brackets were light-cured for 40 seconds with a dental light-curing unit with 1200 mW/cm^2 intensity. Thermocycling (thermo-cycling device, TC 300, Vafaei Industrial, Iran) was performed for 1500 cycles in deionized water between 5-55°C with 30 seconds of dwell time[30,31] to simulate the oral environment. The roots were embedded in a cubic mold of auto-polymerizing acrylic resin (Acro-pars, Iran) using a mounting jig and a 0.019×0.025 inch straight stainless steel wire to align the buccal surface of each tooth perpendicular to the base of the mold. For SBS testing, the samples were secured in the lower jaw of a universal testing machine (Zwick/Roell Z050, Ulm, Germany) such that the bracket base of the sample paralleled the direction of the shear force. The samples were stressed in an occlusogingival direction with a cross-head speed of 1 mm/min.[13,32] After debonding, the teeth were inspected under a stereomicroscope (SMZ 800, Nikon, Japan) at ×10 magnification to quantify the remaining adhesive, according to the modified adhesive remnant index (ARI).[33] The ARI scores ranged from 1 to 5 as described in Table 2.

Statistics

For surface microhardness evaluation, repeated measures ANOVA followed by a Bonferroni t-test were carried out for pairwise comparisons. A one-way ANOVA and a post-hoc Tukey's test were performed to analyze the SBS data. The differences in ARI scores were analyzed by the Kruskal-Wallis test. $P<0.05$ was considered significant. All tests and statistical analyses were carried out using SPSS version 20.

Results

Surface Microhardness

According to Table 3, the results revealed that the demineralization process caused a significant reduction in the mean microhardness. There was a significant increase in the mean microhardness after the application of remineralizing agents in all groups ($P<0.05$). The ability to improve the condition from demineralization to remineralization was not significant in different groups and the results showed no statistically significant difference among the remineralization groups. However, microhardness values were higher in laser groups (N/L and T/L) compared to no laser groups (N and T).

Enamel Characterization

X-Ray Diffraction

Figure 1 shows the characteristic peaks of the XRD pattern. The peaks (002) at 2θ = 26 and (211) at 2θ = 32.2 are specific peaks for hydroxyapatite in the XRD pattern according to the reference database of the XRD pattern.[34,35] The sound enamel group showed typical characteristics of hydroxyapatite crystals. To compare the spectra of the groups, the ratio of the diffraction peak intensities (002/211) was calculated. This ratio was 2.79 for the sound enamel while it was 3.48 for the demineralized enamel. The obtained ratios for the remineralized enamel in N, N/L, T and T/L groups were 2.78, 2.82, 2.74, and 3.01 respectively.

Table 2. The Ranges of Adhesive Remnant Index (ARI) Scores

Score	Criteria
1	All the adhesive, with tooth impression of the bracket base, remained on the tooth
2	More than 90% of the adhesive remained on the tooth
3	More than 10% but less than 90% of the adhesive remained on the tooth
4	Less than 10% of the adhesive remained on the tooth
5	No adhesive remained on the tooth

Table 3. Means and Standard Deviation of Vicker's Microhardness Number for Different Remineralizing Agents

Groups	N	Stages	Minimum	Maximum	Mean	Standard Deviation
Nupro	5	Sound enamel	315	457	393.00	54.424
	5	Demineralized enamel	64	100	81.00	16.763
	5	Remineralized enamel	134	225	185.00	33.756
Nupro and laser	5	Sound enamel	395	473	441.80	31.060
	5	Demineralized enamel	87	127	107.60	14.792
	5	Remineralized enamel	187	247	219.00	22.327
Teethmate	5	Sound enamel	260	413	362.60	63.752
	5	Demineralized enamel	49	104	82.40	22.534
	5	Remineralized enamel	114	225	166.00	49.209
Teethmate and laser	5	Sound enamel	313	482	402.60	60.682
	5	Demineralized enamel	85	110	101.80	10.803
	5	Remineralized enamel	167	221	199.60	24.956

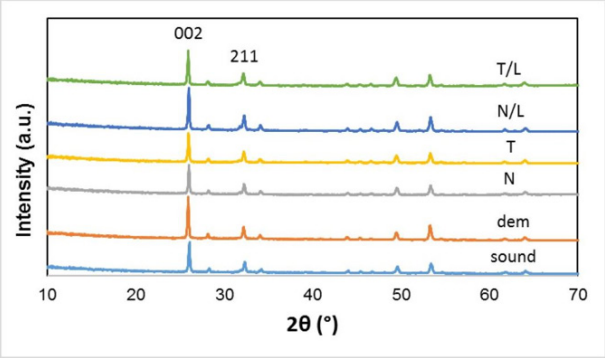

Figure 1. The XRD Pattern of Sound Enamel, Dem (Demineralized), N (Nupro), N/L (Nupro and Laser), T (Teethmate), T/L (Teehtmate and Laser).

The XRD pattern and the peak intensity ratio of the remineralized enamel in all the groups were closer to the sound enamel than the demineralized enamel.

FESEM/EDS

Figure 2 shows the FESEM patterns of the groups. For the sound enamel, a homogeneous smooth appearance with no irregularity was seen. The demineralized enamel surface was rough and eroded. In group N, porosities were covered with a hydroxyapatite deposit. However, the underlying pattern was still visible in some areas. In group N/L, the underlying pattern could not be seen, while a melted and welded appearance caused by laser irradiation was observed. In group T, the enamel surface was entirely covered with granular-shaped hydroxyapatite deposits, while in group T/L, the enamel surface was covered with relatively smooth and more homogeneous crystalline structure compared to group T, due to the typical melting effect of the laser. Figure 2 also shows the chemical composition and atomic percentages of C, O, P, Ca, Na, Si, and F in the groups. The hydroxyapatite deposit was confirmed by EDS analysis in all the groups. The effect of demineralization and remineralization protocols on the enamel surface composition was evaluated by measuring the Ca/P ratio, which was the highest for the Nupro group and the lowest for the demineralized group.

Shear Bond Strength

As indicated in Table 4, the results showed that the SBS was significantly lower in the demineralized group compared to all other groups ($P < 0.001$), while there was no significant difference among other groups ($P > 0.05$). The laser groups showed SBS values lower than the groups without the laser. Table 5 lists the ARI scores for the 6 groups. The demineralized groups showed ARI score 5 in 4 samples out of 10, which was in agreement with the results of the SBS test.

Discussion

Teethmate and Nupro prophy paste were the remineralizing

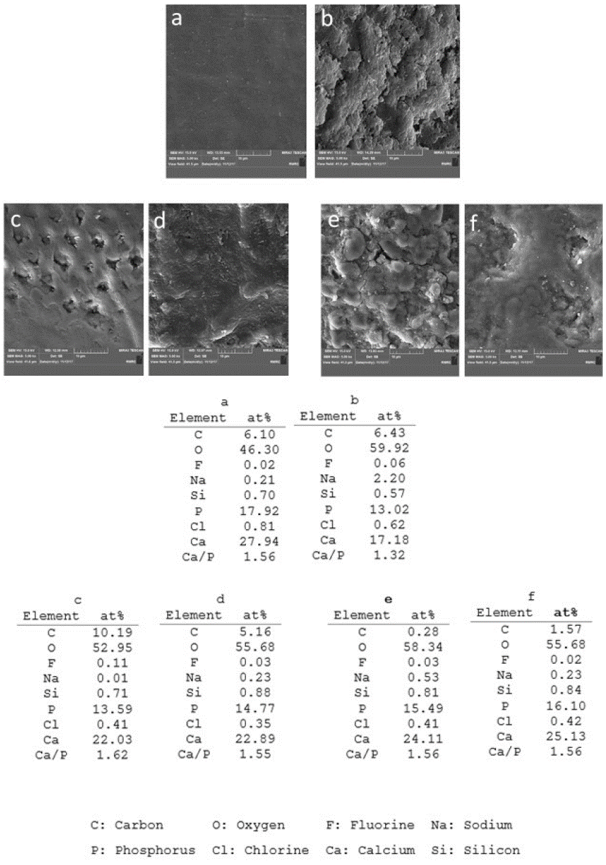

Figure 2. FESEM Images and EDS Analysis of Sound Enamel (a), Demineralized Enamel (b), Nupro (c), Nupro and Laser (d), Teethmate (e), Teethmate and Laser (f).

agents evaluated in this study. Teethmate is composed of two different types of calcium phosphate (tetracalcium phosphate and dicalcium phosphate anhydrous) which react with water to form hydroxyapatite.[36,37] Nupro bioactive glass is mainly composed of SiO_2, Na_2O, CaO, and P_2O_5. According to a study by Gupta et al, sodium release can increase the local pH and facilitate the deposition of additional phosphate and calcium ions.[27,38] Due to the possible role of the CO_2 laser in the remineralization process, the CO_2 laser has also been used to evaluate the probable synergistic effect of the laser on remineralizing agents.[22,39] The safety of controlled irradiation of the CO_2 laser for the pulp and dental structures has previously been confirmed.[40,41]

The chemical analyses of enamel surfaces by EDS revealed that the dominant elements were oxygen (O), carbon (C), calcium (Ca), and phosphorus (P). These elements are the main components of enamel structure and comprise the standard composition of hydroxyapatite. The Ca/P ratio of the sound enamel (1.56 %) decreased to 1.32 % for the demineralized enamel. This obvious difference may refer to the amount of calcium and phosphorus leached out from the enamel during the demineralization

Table 4. Means and Standard Deviation of Shear Bond Strength (MPa) for the Study Groups

Groups	No Samples	Minimum	Maximum	Mean	Standard Deviation
Sound enamel	10	23.56	38.21	28.9820	5.23600
Demineralized enamel	10	9.17	22.35	16.9760	5.07002
Nupro	10	15.17	43.27	30.9750	7.92504
Nupro and laser	10	19.69	29.23	25.0990	3.30448
Teethmate	10	15.41	38.98	29.0990	6.23642
Teethmate and laser	10	19.62	35.85	27.7820	6.14152

Table 5. Adhesive Remnant Index (ARI) Scores of the Study Groups

Groups	Scores				
	1	2	3	4	5
Sound enamel	0	2	3	5	0
Demineralized enamel	0	0	1	5	4
Nupro	0	1	6	3	0
Nupro and laser	0	1	4	5	0
Teethmate	0	0	7	3	0
Teethmate and laser	0	2	4	4	0
Total	0	6	25	25	4

process. This finding was consistent with FESEM images that showed porosities on the enamel surface following the demineralization process. The Ca/P ratios were elevated in all the study groups after the treatment with remineralizing agents and were nearly similar to that of intact enamel with the highest percentage in the N group (1.62%). It means that the nature and pattern of the formed crystals were more similar to those of the intact enamel compared to the demineralized enamel, which was also confirmed by XRD results. This finding was approved by FESEM images, which showed the formation of the HA deposit on the demineralized enamel.

The comparison between the laser-irradiated and non-irradiated groups showed no significant effect of the laser on atomic percentages of Ca and P. The atomic Ca/P ratios for the laser groups showed that laser irradiation had no additional effect on the uptake of calcium and phosphate when combined with the remineralizing agents. On the other hand, El Assal et al indicated that the combination of the CO_2 laser with the hydroxyapatite nano-particles as a remineralizing agent increased the percentage of Ca and P.[42] This synergetic effect is ascribed to the heat produced by the CO_2 laser which can melt the enamel surface and link the remineralizing agent to the enamel surface. The higher power density applied (more than 3 W/cm^2) may be an explanation for this controversy.[43] Clearly visible on FESEM images, the laser facilitated the fusion of the formed hydroxyapatite deposit with the enamel.

According to the findings of FESEM, the sound enamel showed a homogeneous and smooth appearance due to the unexposed enamel rods. In the demineralized enamel, the rough surface was attributed to the exposed hydroxyapatite rods following the removal of peripheral enamel prisms. The more homogeneous enamel surface in the laser groups was related to the coalescence of enamel prisms with each other and with mineral deposits. This finding can be related to the EDS results showing Ca/P ratios similar to those found in the sound enamel. Similar to the study by Ahrari et al, the groups treated with the remineralizing agents alone showed non-homogenous layers with granular or globular precipitations covering the underlying enamel surface.[44]

In XRD analysis, the intensity of diffraction peaks was calculated to compare the hydroxyapatite pattern of the sound enamel, enamel after demineralization, and enamel after remineralization in all groups. The demineralized samples showed an increased intensity of 002 peak, indicative of the improved rearrangement of hydroxyapatite crystals along the c-axis due to the greater removal of hydroxyapatite crystals from the peripheral enamel prisms compared to the core area.[45] The XRD pattern of the treated samples showed a similar pattern to the natural enamel. The comparison of the laser groups and the sound enamel showed that the laser had no significant effect on enamel structure. This finding was in agreement with the results of Nakagaki et al, who showed no change in enamel structure by CO_2 laser irradiation (0.5 W output power for 1 minute) following the use of fluoride. However, laser irradiation with 4 W output power for 1 minute resulted in the formation of an amorphous-like structure.[46] Furthermore, Lin et al showed that the exposure of enamel surface to the CO_2 laser (5 W output power for 5 seconds) did not affect the XRD pattern except for the formation of a small peak of α-TCP.[43] It seems that the phase changes of the enamel surface following exposure to the CO_2 laser highly depended on laser parameters.

The mean surface microhardness values showed that there was a significant decrease in the microhardness of the enamel surface after demineralization. This result was in accordance with the elements leached out from the enamel, determined by EDS. Previous studies have also reported a reduction in the hardness of demineralized enamel.[47-49] The significant increase in microhardness after the remineralization procedure indicates a direct correlation between the Ca/P ratio and the subsequent

surface microhardness of remineralized enamel. According to Amaechi et al, the remineralization of eroded enamel by saliva can take 28 days but as shown by our results, applying remineralizing agents can significantly accelerate this process.[50] It has been demonstrated that the materials with high amounts of calcium and phosphate have high penetration into enamel lesions and as a result, greater remineralization occurs. The ability of bioactive glass to penetrate deeper into the demineralized enamel and improve remineralization in comparison with fluoride was approved by the microhardness test by Bakry et al.[19] Due to the porous and fragile structure of initial carious lesions, only some studies support the use of lasers alone and without the use of remineralizing agents. Furthermore, it was proposed that the heat generated during laser irradiation can effectively fuse the loosely-attached layer formed on the enamel surface.[51] Despite the higher microhardness values obtained in the laser groups, the differences were not significant. As a result, the synergistic effect of the laser with the remineralizing agents was not evident in our findings, which is similar to the findings from the study by Farhadian et al.[52] Melting and recrystallization of the enamel surface may explain higher microhardness values and also the higher intensity of HA peaks for the laser groups. Contrary to our findings, Khamverdi et al and Niyazi et al showed a synergistic effect of the CO_2 laser with CPP-ACP on improving the enamel microhardness, which confirms the facilitating role of lasers in the penetration of remineralizing agents into deeper layers of HA.[39,53]

Calcium-phosphate derivatives transform the hydroxyapatite to stronger crystals by incorporating fluoride into subsurface enamel, which can increase the hardness of demineralized enamel and impede the demineralization process.[54] As a result, both Nupro and Teethmate can increase the resistance of demineralized enamel to further demineralization while the laser can cause welding of enamel prisms; therefore, the application of these materials combined with laser irradiation can interfere with the etching process and decrease the bond strength. The remineralization mechanism may interfere with the acid-etching process, prevent the production of enamel tags, and decrease the bond strength of orthodontic brackets, which means a higher possibility of bracket debonding during the treatment period. Concerning the bond strength, we found that the SBS significantly decreased when the brackets were bonded to the previously demineralized enamel. This result can be explained by the reduction in the mineral content of the demineralized enamel and is supported by the surface microhardness and EDS results. Also, there was a statistically significant improvement in the SBS of the treated groups compared to the demineralized group, similar to the findings from the study by Velİ et al.[55] The higher surface microhardness values in the laser groups can also be related to their lower bond strength, although it was not significant. Different laser settings, the type of remineralizing agent and its frequency and duration of application can explain different results in the literature. The lower bond strength values of the demineralized samples were in agreement with the ARI scores; 40% of the demineralized samples represented score 5, meaning no adhesive remaining on the tooth surface.

Conclusion

According to the results, the application of the remineralizing agents did not interfere with the SBS of orthodontic brackets to enamel and even enhanced the SBS of treated demineralized enamel and improved its structural properties as well. The results of enamel structural analysis, hydroxyapatite formation, and the SBS test were in agreement with each other. The findings indicated that the enamel surface structure has a definite impact on the clinical performance of fixed appliances and the quality of treatment. Future in vivo studies on other commonly used remineralizing agents are required to enhance the existing knowledge about the remineralizing agents and their clinical effects on the performance of orthodontic appliances.

Ethical Considerations

This in vitro study was approved by the ethics committee of Tehran University of Medical Sciences (Ethic code: IR.TUMS.DENTISTRY.REC.1396.2721).

Conflict of Interests

We have no conflict of interest to disclose in the article.

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