The effect of Er:YAG laser and different surface conditioning procedures on microtensile bond strength of the fissure sealant containing amorphous calcium phosphate after artificial aging

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The present study aimed to compare post-aging microtensile values of fissure sealant(FS) containing amorphous calcium phosphate(ACP) with or without bonding after various enamel-etching methods. The buccal enamel surfaces of teeth were prepared and divided into five main groups according to enamel etching (Group 1; acid-etching, Group 2; air-abrasion, Group 3; 2W, Group 4; 3W, and Group 5; 4W of Er:YAG laser). Each main group was subdivided as with(Subgroup B) or without(Subgroup A) a bonding system. We applied FS-containing ACP to etched enamel surfaces. All specimens were aged by Thermocycling and water bath. We then measured microtensile bond strengths and analyzed data with two-way ANOVA and Tukey's tests. We found a statistically significant difference between Group 1 and Group 2, between Subgroup B of Group 5 and Subgroups B of all other groups, and between Subgroups A and Subgroups B of all main groups ($p<0.01$). Er:YAG laser-etching may be an alternative enamel etching method to acid-etching for ACP-containing FS.

Keywords: Fissure sealant, Er:YAG laser, Amorphous calcium phosphate, Aging

INTRODUCTION

In pediatric dentistry, dental caries is disease affecting the oral cavity at the occlusal pits and fissures. Complex morphology of occlusal surfaces that cause bacterial aggregation and proliferation is responsible for 60–85% of carious lesions in these areas¹. Pit and fissure sealants (PFSs) are conservative, painless, and often effective method for preventing occlusal caries^{2,3}. PFSs emerged in Buonocore's first studies of etching enamel in 1955⁴. One develops PFSs by providing marginal integrity and enhancing retention by infiltrating resin-based materials into the enamel, etching in micro-spaces with phosphoric acid¹. In pediatric dental treatments, PFSs are among the most effective materials for oral hygiene training, optimal fluoride intake, healthy diet habits and the prevention of pit and fissure caries¹⁻³.

Newly commercialized Aegis PFSs can release calcium and phosphate due to the presence of amorphous calcium phosphate (ACP). The sealant leaches hydroxyapatite (HAP) from the enamel surface, during a caries formation at or below pH 5.8⁵. At this low pH value, the ACP can break down and saturate the tooth with Ca^{2+} and PO_4 ions⁶. These concentrations help form HAP, which the tooth uses to remineralize its enamel⁷. The ACP not only indicates HAP, but exhibits anti-cariogenic properties and potential remineralization⁸. We preferred Aegis PFS because of inadequate literature about microtensile bond strength on permanent teeth.

The success rate of the PFS depends on its retention on the tooth surface. A number of enamel preparation methods, such as air abrasion, enameloplasty, acid

etching, and laser etching, ensure a more retentive tooth surface. Most widely used is application of 30–40% phosphoric acid. The phosphoric acid forms retentive enamel micro-porosities through decalcification and increases wettability and surface areas⁹. The process of etching forms a porous enamel layer where resin can flow. Porosity not only provides a broad surface area where resin can adhere, but establishes a ground for perfect mechanical retention¹⁰.

Air-abrasion technology (kinetic cavity preparation) is another method suggested for etching the enamel surfaces^{11,12}. Air pressure is used to propel the Al_2O_3 particles and abrade the tooth surface. The advantages of preparing the enamel surface by removing superficial caries and dental plaque from the tooth surface are well known. Al_2O_3 powder could be applied on enamel surfaces with different particle sizes (27, 30, and 50 μm): we used 27 μm .

Laser etching in pediatric dentistry can be an alternative to acid etching. Hard tissue studies began when the FDA approved Er:YAG lasers on enamel and dentin in 1997. The Er:YAG laser, a pulsatile beam of 2.94 μm wavelength, has various advantages on hard tissues. The water in dental hard tissue absorbs its energy¹³. Moreover, the water-cooled system does not harm tooth pulp.

The use of bonding agents prior to PFS application increases bonding to dental tissue, decreases microleakage, obtains the flow of the resin material into pits and fissures and increases clinical success¹⁴⁻¹⁶. This technique minimized salivary contamination of etched surfaces. Both laboratory studies and clinical study demonstrated it increased bond strength on the surface

and effectively decreased microleakage. In the present research, we use an acetone-based and etch-and-rinse bonding agent (Prime & Bond NT)^{17,19}.

The purpose of the present study is to evaluate and compare post-aging microtensile values of fissure sealant (FS) containing ACP, with or without bonding after various enamel-etching methods (phosphoric acid, air abrasion, laser 2W, laser 3W, and laser 4W).

MATERIALS AND METHODS

This study used fifty human permanent mandibular molar teeth, caries-free, non-restored and without other microscopic defects. We polished them with pumice to remove any surface debris or contaminants and kept them in 0.1% thymol solution at room temperature before the experiment. Their roots were cut 2 mm below the cemento-enamel junction. Then, we embedded half the teeth's bucco-lingual distance horizontally into acrylic resins. We polished buccal enamel surface for 60 s under water cooling with 600-grit silicon carbide (SiC) paper to obtain a smooth, homogenous, and standard enamel surface.

The study tested one commercial acid etching (phosphoric acid), one air-abrasion system [aluminium oxide (Al_2O_3)], and three different Er:YAG laser (Smart 2940D Plus; DEKA Laser, Firenze, Italy) irradiation power energies.

We sorted specimens randomly into five groups ($n=10$ teeth) according to enamel-etching methods:

Phosphoric acid group (Group 1): We used 35% phosphoric acid gel (Bosworth Acid Etch, Bosworth Co, Illusiana, USA) to etch the teeth's buccal enamel surfaces for 30 s, rinsed them thoroughly for 20 s and dried them using a mild, oil-free air spray for 10 s to get a uniform chalky-white appearance.

Air abrasion group (Group 2): The buccal enamel surfaces of the teeth were etched with air abrasion (Rondoflex-plus 360 Kavo®, Germany) at 80 psi with 27 micrometer aluminium oxide (Al_2O_3) particles and in 2 mm distance from the specimen's surface for 30 s. Thirty seconds of thorough water spray rinse removed residual aluminium particles.

Laser 2W (Group 3): An Er:YAG laser pulsed infrared radiation at a wavelength of 2,940 nm through an articulated arm. At the end of the articulated arm on the Smart2940 D system is a handpiece used in the non-contact (window) tip. Additionally, with the laser beam in focus at 1 mm from the window of the handpiece, presents a much smaller spot size than 1mm, due to its proximity. The laser was used with a handpiece head positioned 1 mm above and perpendicular to the enamel surface. 2W power energy of Er:YAG laser was applied on buccal enamel surfaces for enamel-etching. A pulse energy of 100 mJ, a repetition rate of 20 Hz, a pulse length of 230 μs and sweeping fashion with a water flow of 5 mL/min were the parameters of the laser for 30 s.

Laser 3W (Group 4): 3W power energy of Er:YAG laser was applied on buccal enamel surfaces for enamel-

etching. A pulse energy of 150 mJ, a repetition rate of 20 Hz, a pulse length 230 μs and sweeping fashion with a water flow of 5 mL/min were the parameters of the laser for 30 s.

Laser 4W (Group 5): 4W power energy of Er:YAG laser was applied on buccal enamel surfaces for enamel-etching. A pulse energy of 200 mJ, a repetition rate of 20 Hz, a pulse length 230 μs and sweeping fashion with a water flow of 5 mL/min were the parameters of the laser for 30 s.

After these procedures, we placed cylindrical transparent gelatins (diameter 4 mm, height 8 mm) on etched enamel surfaces. Then we divided each main group into two subgroups: A, to which we only applied fissure sealant, and B, to which we also applied a bonding agent, Prime & Bond® NT (Dentsply Detrey, Konstanz, Germany).

Subgroup A: We applied Aegis fissure sealant (each 2-mm layer polymerized with Bluephase, with light intensity 1,200 mV/cm², for 20 s) on these teeth, in accordance with the instructions of the producing company (Table 1).

Subgroup B: We applied Prime & Bond, again in accordance with the instructions of the producing company, and then Aegis fissure sealant (Table 1).

Then we built up approximately 4-mm universal hybrid composite resins (Filtek™ Z250, colour A2, 3M ESPE) on the fissure sealant to obtain an adhesion surface for microtensile tests.

Thermocycling and water-aging procedures

Just after curing, we kept teeth in distilled water for 24 h and thermocycled them 10,000 times at 5–55°C in water baths for one min each. Following the thermocycling, the teeth were kept in the water bath at 37°C (Nüve BM 402, Ankara, Turkey) for 12 months.

After aging procedures, we kept samples in 37°C distilled water for 24 h, and divided into rectangular prism-shaped sections measuring 1 mm×1 mm, using a water-cooling cutting device (Isomet Buhler Ltd, Lake Bluff, IL, USA). A microtensile bond strength (μTBS) test was applied on 200 chosen sticks, including 20 from each group ($n=20$).

We then fixed the sticks to the universal testing machine with cyanoacrylate and an accelerator (Zapit, Dental Ventures of America, Corona, CA, USA). In a universal test device (Lloyd LF Plus; Amatek Inc, Lloyd Instruments, Leicester, UK) μTBS applied 1 mm of power per min until failure occurred. We recorded the loading amount in the failure moment as megapascal (MPa) and assessed results statistically.

A stereomicroscope at X40 (SZ-PT Olympus, Japan) helped identify slabs' fracture mode as either cohesive (within enamel or sealant), adhesive (between sealant and the enamel), or mixed (cohesive and adhesive fracture). A single observer (MÜ) blinded to the groups performed all analyses.

Scanning electron microscope analysis

One stick obtained from each group (adhesive, cohesive,

Table 1 Materials used in the study

Materials	Composition	Application procedures	LotNumber
AEGiS Bosworth®Company (USA)	UDMA, mono-and di methacrylates resins TLV-TWA: 15 mg/m ³ TWA for ACP N/A for resin	1. After etching of enamel 2. Apply sealant 3. Wait for approximately 15 s 4. Light-cure (Bluephase, Ivoclar Vivadent, Liechtenstein) for 20 s	0608-398
Prime& Bond NT (Dentsply DeTrey, Germany)	PENTA, UDMA, Resin R5-62-1, T-resin, D-resin, nanofiller, cetylaminehydrofluoride and acetone.	1. Apply adhesive under pressure for 20 s 2. Dry with a gentle air stream for 10 s 3. Light-cure (Bluephase, Ivoclar Vivadent, Liechtenstein) for 10 s	0703000381

Bluephase, Ivoclar Vivadent, Liechtenstein; light intensity: 1,200 mW/cm².

Abbreviations: Bis-GMA=bisphenol-A-glycidyl methacrylate; N/A=Not available; TEGDMA=triethyleneglycol dimethacrylate; TLV-TWA=Threshold Limit Value-Time-Weighted Average; UDMA=urethane dimethacrylate.

Fig. 1 SEM images of fracture [1a: Group 1(Subgroup A), 1b: Group 1 (Subgroup B), 1c: Group 2 (Subgroup A), 1d: Group 2 (Subgroup B), 1e: Group 5 (Subgroup A), 1f: Group 1 (Subgroup B)]

and mixed) was separated for analysis after exposure to the microtensile process. A detailed imaging process in these samples probed the superficial changes on the joining area of enamel and fissure sealant. A sputter coater (SCD 005; Bal-Tec, Balzers, Liechtenstein), coated the specimens with a thin layer of gold, and the scanning electron microscope (SEM) (LEO 1430 VP, Carl Zeiss, Cambridge, England) evaluated and photographed the results to determine morphological differences between enamel and fissure sealant surface. The samples were examined at 1,000× magnification (Fig. 1). In the present study, Fig. 1 shows cohesive failure in Groups 1, 2, and 5 (Subgroups A and B).

Statistical analysis

μTBS values were calculated (MPa) and analyzed using two-way ANOVA. Multiple comparisons were performed using Tukey's test, with a significance level of 0.05. SPSS for Windows, Version 14.0 (SPSS Inc, Chicago, IL, USA) helped conduct statistical analysis.

RESULTS

Table 2 illustrates the statistical assessment of data.

In Subgroup A; there were statistical significant differences between Group 1 and Group 2 ($p<0.01$). Furthermore, the statistical significant differences were

found between Group 5 and Group 2, and 3 ($p<0.01$).

In Subgroup B; there was the statistical significant difference between Group 1 and Group 2 ($p<0.01$). Moreover, the statistical significant differences were found between Group 5 and the all other groups ($p<0.01$).

There were statistically significant differences between Subgroups A and B in all main groups ($p<0.01$).

Table 3 illustrates types of fracture. Recorded fractures were mainly cohesive (55–95%) in all experimental groups.

Stereomicroscopic images obtained from sticks from Groups 1, 2, and 5 by universal test device are shown in Fig. 1. We indicated cohesive failure, the most common failure type.

The SEM image of Group 1 (Fig. 1a) without bonding agent showed a honeycomb-like appearance on the surface following organic debris cleaning. The SEM image of Group 2 (Fig. 1c) without bonding agent exhibited unevenness and irregularity of the enamel surface. The SEM image of Group 5 (Fig. 1e) without bonding agent showed the surface of the enamel almost entirely covered with fissure sealant.

The SEM image of Group 1 (Fig. 1b) with the bonding agent showed a honeycomb-like appearance on the surface, but intervals caused by bonding at the

Table 2 Mean and standard deviations of the microtensile bond strength for each group

	Surface Pretreatment Procedure				
	Group 1 (Acid etching)	Group 2 (Air Abrasion)	Group 3 (Laser 2W)	Group 4 (Laser 3W)	Group 5 (Laser 4W)
Aegis (SA)	17.91 (3.08) ^{A,a}	13.87 (3.55) ^{B,a,b}	15.64 (3.20) ^{C,c}	17.06 (3.39) ^D	20.29 (2.83) ^{E,b,c}
Bond+Aegis(SB)	23.55 (2.51) ^{A,d,e}	19.14 (2.85) ^{B,d,f}	19.73 (2.77) ^{C,g}	22.40 (3.06) ^{D,h}	32.12 (2.05) ^{E,e,f,g,h}

$n=20$ specimens per experimental condition.

Standard deviations are shown in parentheses.

By the two-way ANOVA: $F=246.412$ $P=0.000$, $p<0.01$ Tukey's test indicates statistical difference ($p<0.01$) for means followed by the same letters; capital letters in the column are for the comparison of subgroup A and subgroup B with the same surface pretreatment; small letters in the rows are for the comparison of different surface pretreatment with the same subgroup.

Table 3 Fracture types of each group

	Surface Pretreatment Procedure									
	Group 1 Acid etching		Group 2 Air Abrasion		Group 3 Laser 2W		Group 4 Laser 3W		Group 5 Laser 4W	
	SA	SB	SA	SB	SA	SB	SA	SB	SA	SB
Adhesive	3	0	8	7	1	1	2	0	1	0
Cohesive	16	17	10	12	19	17	14	19	18	20
Mix	1	3	2	1	0	2	4	1	1	0

$n=20$ specimens per experimental condition.

(SA: Subgroup A, SB: Subgroup B)

enamel-sealant junction. The SEM image of Group 2 (Fig. 1d) with the bonding agent showed chalky surfaces and irregular porosity. The SEM image of Group 5 (Fig. 1f) with the bonding agent showed complete penetration of the enamel surface fissure sealant.

DISCUSSION

Conventional tensile and shear tests have evaluated the bond strength of fissure sealants to the enamel. Many current researchers prefer the microtensile technique, accepted as a more accurate method for evaluating bond strength^{20,21}.

During clinical trials, *in-vivo* assessment of materials presented challenges. To imitate the natural aging process, *in-vitro* assessment was more favorable to simulations of oral cavity conditions (thermocycling, water aging, and chewing forces) in the laboratory environment. In microtensile tests conducted with fissure sealants, aging was ignored and omitted in almost all of the studies. Yet we know resin-containing materials might be affected by thermal changes in the oral environment, affecting test results obtained. This study differs from other microtensile bond strength studies in that all specimens were aged using thermocycling (10,000 times) and water bath (at 37°C for 12 months).

The preventive success of fissure sealants depends on such factors as enamel surface properties, duration of acid-etching, type of acid and surface pre-treatment techniques. But the more important factor is fissure sealant retention, which depends on micro-mechanical and physico-chemical interactions between the resin and the etched enamel. Conventional acid etching has some disadvantages such as technical sensitivity, isolation problems and excessive time consumption. Researchers have thus sought alternative etching methods.

One such method is air abrasion. This non-traumatic method prevents heat and vibration that may otherwise cause microfractures. Knobloch *et al.*²² investigated the effect of different surface preparation techniques (air abrasion, etching with 37% phosphoric acid, and a combination of both) on the bond strength of the resin-based fissure sealant. They found combined air-abrasion and acid-etching improved the bonding strength of the resin-based fissure sealant.

Moslemi *et al.*²³ reported that pre-treatment of the enamel surface with air-abrasion increased the bond strength of the fissure sealant; however, pre-treatment with the Er,Cr:YSGG laser did not increase the effectiveness of conventional acid-etching of enamel. In the present study, the microtensile values of air abrasion groups varied significantly from phosphoric acid groups ($p < 0.01$). Air abrasion is shown the lowest microtensile values, which the SEM images support (Fig. 1).

The potential drawbacks of phosphoric acid etching has made laser etching more popular. The Er:YAG laser roughs the surface of enamel effectively, including hydroxyapatite and water. Hard tissue evaporates

rapidly during irradiation. The Er:YAG laser also involves water cooling, ablating surrounding tissue with minimal thermal side effects¹⁵.

Chimello-Sousa *et al.*²⁴ compared the combined Er:YAG laser system and conventional phosphoric acid with phosphoric acid alone on permanent tooth enamel and the tensile bond strength of fissure sealants after etching. They confirmed that the conventional method led to higher tensile bond strength.

Shahabi *et al.*²⁵ investigated tensile bond strength of the fissure sealants on permanent molar teeth enamel surfaces after three different etching methods: 37% phosphoric acid, Er:YAG laser (1W) and both combined. The significant differences were found between the laser-only group and the other groups. In the present study, Group 5 yielded the highest microtensile values: its SEM images showed the deepest craters, cracks, and grooves on enamel surfaces.

Moisture or saliva contamination hinders the bonding of hydrophobic resin sealants to etched enamel². This problem is the most common reason for unsuccessful sealing²⁶. Other researchers have increased bond strength by adding a bonding agent layer between the etched enamel and fissure sealant^{2,17-19}. Feigal *et al.*¹⁹, also reported a bonding agent is useful in increasing fissure sealant retention on teeth contaminated with saliva. Feigal and Donly¹⁴ showed that applying an intermediate bonding layer reduces microleakage on saliva-contaminated enamel.

Perdigao *et al.*²⁰ compared the μ TBS values of fissure sealants after application of phosphoric acid or self-etching adhesives on permanent molar teeth. They found both groups similar.

Venker *et al.*²⁷ retrospectively investigated a self-etching primer (Prompt L-Pop) and phosphoric acid etching in a school-based fissure sealant program for a 12-month period. They demonstrated that fissure sealant retention with the use of the phosphoric acid etch was six times as likely to have retention of all the sealed tooth surfaces as those sealed with a self-etching primer.

In a randomized, controlled and split-mouth trial, Burbridge *et al.*²⁸ compared the clinical success of fissure sealants by applying a self-etching priming agent (Xeno III Dentsply, Germany) and phosphoric acid etching together with total-etching adhesive (Prime Bond NT, Dentsply, Germany) on permanent molar teeth. They found the second group more successful, concluding that self-etching adhesives could not be recommended for enamel preparation prior to fissure sealant placement.

Tunç *et al.*²¹ investigated the efficiency of phosphoric acid, self-etch adhesive, and etch-and-rinse adhesive systems on μ TBS values of Aegis fissure sealant on bovine teeth, and found the differences between the three methods insignificant.

The bonding agent with primer and adhesive components together may enhance the initial bond to enamel and also improve adhesion between the

adhesive and fissure sealant²⁹). Water-based primers and adhesives could be less effective in bonding to etched and dry enamel surfaces than acetone-based adhesives³⁰. Acetone, a volatile solvent in the adhesive system (Prime & Bond NT), can remove all residual moisture from the etched enamel, carrying resin monomers into close adaptation with the surface. In the present study, Subgroup B of Group 5 showed the highest μ TBS mean value among all groups. In our opinion, this cleansing effect of acetone solvent may have caused the higher microtensile bond strength in the present study. These results could make great contributions for further studies. Since microtensile bond strength test is only one of numerous factors affecting dental sealants' clinical behavior, other *in vitro* studies should comprehensively analyze the enamel surface of lased permanent teeth. New comprehensive clinical studies are also needed to review caries prevention after different enamel preparations.

CONCLUSIONS

The following conclusions could be obtained based on results of this study:

- The Er:YAG laser may be a useful alternative to acid etching for application of fissure sealant in pediatric dentistry.
- Etch-and-rinse adhesive systems may be used with Er:YAG laser on permanent teeth.
- Amorphous calcium phosphate (ACP) containing fissure sealant, showed high microtensile bond strength values when used with 4 W Er:YAG laser etching.

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