Effect of Laser and Air Abrasion Pretreatment on the Microleakage of a Fissure Sealant Applied with Conventional and Self Etch Adhesives

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Aim: *The purpose of this study was to investigate the effects of different pretreatment protocols along with different bonding agents on the microleakage of a fissure sealant material.* ***Method:*** *A total of 144 freshly extracted noncarious human third molars were used. The teeth were randomly assigned into three groups with respect to the pretreatment protocol employed: A. Air Abrasion B. Er,Cr:YSGG laser C. No pretreatment (Control). In each group specimens were further subjected to one of the following procedures before application of the sealant: 1. %36 Phosphoric acid-etch (AE) (DeTrey Conditioner 36/ Denstply, UK) 2.AE+Prime&Bond NT (Dentsply,UK) 3.Clearfil S^3 Bond (Kuraray, Japan) 4.Clearfil SE Bond (Kuraray, Japan). All teeth were sealed with the same fissure sealant material (Conseal F/ SDI, Australia). Sealed teeth were further subjected to thermocycling, dye penetration test, sectioning and quantitative image analysis. Statistical evaluation of the microleakage data was performed with two way independent ANOVA and multiple comparisons test at p=0.05. For qualitative evaluation 2 samples from each group were examined under Scanning Electron Microscopy.* ***Results:*** *Microleakage was affected by both the type of pretreatment and the subsequent bonding protocols employed (p<0.05). Overall, the highest (Mean=0.36mm) and lowest (Mean=0.06 mm) microleakage values were observed in samples with unpretreated enamel sealed by S^3+Conseal F and samples with laser pretreated enamel sealed by Acid Etch+Prime&-Bond+Conseal F protocols, respectively (p<0.05). In the acid-etch group samples pretreated with laser yielded in slightly lower microleakage scores when compared with unpretreated samples and samples pretreated with air abrasion but the statistical significance was not important (p=0,179). Similarly, when bonding agent is applied following acid-etching procedure, microleakage scores were not affected from pretreatment protocol (p=0,615) (intact enamel/laser or air-abrasion). For both all-in one and two step self etch adhesive systems, unpretreated samples demonstrated the highest microleakage scores.* ***Conclusions:*** *For the groups in which bonding agent was utilized, pretreatments did not effected microleakage. Both the tested pretreatment protocols and adhesive procedures had different effects on the sealing properties of Conseal F in permanent tooth enamel.*

Keywords: *teeth, pit and fissures, pretreatment, air abrasion, microleakage Er,Cr:YSGG laser*

INTRODUCTION

The pits and fissures are enamel faults, which are the major contributing reason why occlusal caries constitutes an increasing proportion of children's caries occurrence. Regarding prevention of fissure caries, evidence based data reports that pit and fissure sealants the best preventive measure being more effective than fluoride application.[1]

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The preventive benefit of fissure sealant depends on the ability of the material to promote an appropriate obstruction of all pits, fissures or eventual enamel defects, and remain completely intact and bonded to enamel surface.[2] Lack of proper sealing results in marginal leakage which in turn, can prompt caries lesion progression underneath the sealant material.[3] Conventionally, resin sealants are placed after cleansing and etching of enamel with various concentration of phosphoric acid. Overall, the acid etch procedure enhances bonding to enamel by, *i*. removal of surface debris, *ii*. raising the free surface energy of the enamel to exceed the surface tension of the bonding material, *iii*. producing spaces into which the resin penetrates and interlocks mechanically when set; and *iv*. increasing the surface area of enamel available to the bonding material.[4] On the other hand, the technique is technically sensitive and the clinician may face problems such as isolation and inadequate removal of debris and pellicle by conventional prophylaxis and the etching process.[5] In order to overcome this clinical problem, the use of hydrophilic adhesive resins as an intermediate layer under sealant materials has been suggested.[6] The technique is so-called "bonded sealant" concept and has been reported successful in both laboratory and clinical research.[6,7] The recent introduction of self-etching adhesives may further improve the bonded sealant technique, as the clinical procedure is simplified.[8]

Table 1. Composition and application procedures of the materials.

PRODUCT	COMPOSITION		APPLICATION
DeTrey Conditioner	35% phosphoric acid gel		Apply and leave for 30 s, rinse 15 s, air dry for 10 s.
Prime&Bond NT	Di- and trimethacrylate resins, PENTA, nanofillers, amorphous silicone dioxide, photoinitiators, stabilizers, cetylamine hydrofluoride, acetone		Apply and leave for 20 s, gently air dry, light cure for 20 s.
Clearfil Tri-S Bond	10-Methacryloyloxydecyl dihydrogen phosphate, Bis-phenol Adiglycidylmethacrylate, 2-Hydroxyethyl methacrylate, Hydrophobic dimethacrylate, dl-Camphorquinone, Ethyl alcohol, Water, Silanated colloidal silica		Apply and wait 20 s, Dry with high-pressure air for 5 seconds, light-cure for 10 s.
Clearfil SE Bond	*Primer* Primer A: water, acetone photoinitiator Primer B: 4-AET, HEMA, 4-AETA, İnitiator	*Adhesive* 4-AET, HEMA, UDMA, TEG-DMA, SiO2 microfillers	Mix primers A and B 50:50 ratio, apply, leave 10 s and air dry. Apply adhesive, light cure for 10 s.
Conseal F	Ester methacrylate (93 wt%); inorganic fillers, sodium fluoride (7 wt%)		Apply from the margin into the fissure, remove air bubbles, let penetrate for 15 to 20 s, light cure for 20 s.

Abrevations: HEMA: 2-hydroxyethyl methacrylate; bis-GMA: bisphenol-A-dimethacrylate; UDMA: urethane dimethacrylate; 4-AET: 4-acryloyloxyethyl trimellitate; 4-AETA: 4-acryloxyethyl trimellitate anhydride; MDP: 10-methacryloyloxydecyl dihydrogen phosphate; PENTA: dipentaerythritol penta acrylate monophosphate.

Other than bonded sealants, alternative techniques applied in order to increase sealant effectiveness include surface preparation with burs, air abrasion or laser pretreatment and utilizing four handed dentistry.[5,9,10]

The conditioning effects of the Er,Cr:YSGG laser on dental hard tissues have been investigated in detail by means of microleakage evaluation and mechanical testing of composite restorations in primary and permanent tooth cavities.[11,12] However, few data is present concerning the laser pretreatment prior to the application of fissure sealants. A single research compared the effectiveness of Er,Cr:YSGG pretreatment with bur and air abrasion pretreatments in permanent teeth.[10] Er,Cr:YSGG pretreatment was tested along with self-ecth adhesives in primary teeth previously in two *in vitro* manuscripts.[9,13] Conversely, no data is present regarding the effect of air abrasion or Er,Cr:YSGG pretreatment on the microleakage of fissure sealants placed with self-etch adhesives on permanent teeth fissures.

Air abrasion is a method for tooth cleaning with a stream of aluminum oxide particles directed through a handpiece and powered by compressed air or nitrogen gas. There is limited evidence that cleaning teeth with air abrasion prior to acid etching improves sealant retention.[5]

The aim of this study was to evaluate the effects of laser and air abrasion pretreatments on the microleakage of a fissure sealant applied with conventional and self-etch adhesives.

MATERIALS AND METHOD

A total of 144 freshly extracted non carious human third molars were used. The teeth were randomly assigned into three groups with respect to the pretreatment protocol employed:

A. Air Abrasion (CoJet Prep, 3M ESPE, Seefeld, Germany) procedure, Cojet prep devices abraded specimens for 20 seconds (s) with a Al_2O_3 particle size of 30 µm.

B. Er,Cr:YSGG laser occlusal fissures were irradiated with an Erbium, Chromium: Yttrium Scandium Gallium Garnet (Er,Cr:YSGG) hydrokinetic laser system (Millenium System, Biolase Technology Inc., San Clemente, CA, USA). Before specimen preparation, the power output was set at 3,5 W (Output parameters: Wavelength=2.78 µm, Pulsed with duration from 140 to 200 µs and a repetition rate of 20 Hz). Air and water was sprayed through the handpiece at a level of 85% water and 90% air to prevent enamel surfaces from overheating. The laser beam was delivered on non-contact mode, with the handpiece positioned perpendicular to the fissures. The duration of exposure depended on the time needed to evenly guide the laser beam across the pits and fissures to be irradiated. Fissures were, then, rinsed and air-dried.

C. No pretreatment (Control).

In each main group, samples were randomly assigned into 4 subgroups (n=12 each). The materials and procedures applied in each subgroup before sealant (Conseal F, SDI,Australia) application, are as follows;

1. Phosphoric acid-etch (AE), (DeTrey Conditioner 36, Dentsply UK)
2. AE+Prime&Bond NT (Dentsply,UK)
3. Clearfil S^3 (Tri-S) Bond (Kuraray,Japan)
4. Clearfil SE Bond (CLSE) (Kuraray,Japan).

All materials were applied according to manufacturer's directions (Table 1). Following pretreatment methods, all teeth were sealed with a resin-based fissure sealant (Conseal F, SDI,Australia) for all groups and was light-cured for 20s with a quartz-tungsten-halogen curing unit (Hilux,Benlioglu Dental,Turkey). After completion of curing procedures, all specimens were subjected to thermocycling (1000X, in 5 ± 2°C to 55 ± 2°C with a dwell time of 15 s and a transfer time of 10 s).

Table 2. The pairwise comparisons of mean values for all tested groups.

Material	Pretreatment (I)	Pretreatment (II)	Mean Difference (I-II)	Std. Error	Siga	%95 Confidence Interval For Difference	
						Lower Bound	Upper Bound
AE	Control	Laser	,042*	,011	,001	,014	,069
		Air Abrasion	,007	,011	1,000	-,021	,035
	Laser	Control	-,042*	,011	,001	-,069	-,014
		Air Abrasion	-,035*	,011	,008	-,062	-,007
	Air Abrasion	Control	-,007	,011	1,000	-,035	,021
		Laser	,035*	,011	,008	,007	,062
AE+Bond	Control	Laser	,021	,011	,215	-,007	,048
		Air Abrasion	,001	,011	1,000	-,027	,028
	Laser	Control	-,021	,011	,215	-,048	,007
		Air Abrasion	-,020	,011	,238	-,048	,007
	Air Abrasion	Control	-,001	,011	1,000	-,028	,027
		Laser	,020	,011	,238	-,007	,048
Tris	Control	Laser	,167*	,011	,000	,140	,195
		Air Abrasion	,051*	,011	,000	,023	,078
	Laser	Control	-,167*	,011	,000	-,195	-,140
		Air Abrasion	-,117*	,011	,000	-,144	-,089
	Air Abrasion	Control	-,051*	,011	,000	-,078	-,023
		Laser	,117*	,011	,000	,089	,144
CLSE	Control	Laser	,113*	,011	,000	,085	,141
		Air Abrasion	,021	,011	,189	-,006	,049
	Laser	Control	-,113*	,011	,000	-,141	-,085
		Air Abrasion	-,092*	,011	,000	-,119	-,064
	Air Abrasion	Control	-,021	,011	,189	-,049	,006
		Laser	,092*	,011	,000	,064	,119

*The mean difference is significant at the ,50 level. a Adjustment for multiple comparisons: Bonferroni.

Microleakage was evaluated with conventional dye-penetration method. For this purpose, the apices of teeth were sealed with sticky wax two consecutive layers of nail varnish was applied up to 1mm from the sealant margins. Then, samples were immersed in 0.5% aqueous basic fuchsin solution (Wako Pure Chemical Industry, Osaka, Japan) for 24 hours. After thoroughly rinsing with distilled water, the samples were air-dried and embedded in epoxy resin (Struers, Copenhagen, Denmark) for sectioning. Then, the specimens were sectioned buccolingually (3 section/tooth) under water cooling and photographed digitally at 20X magnification (1280x1024 resolution) under a stereo-microscope (Olympus, Tokyo, Japan). Photographs were saved as TIFF images and processed with a MacBook device. The extent of dye penetration on the tooth-sealant interface was measured (in mm) using an open source software (ImageJ, V.1.42, National Institutes of Health, Bethesda, MD). The microleakage value for each section was calculated by dividing the sum of buccal and lingual dye penetration values by the sum of the lengths of buccal and lingual enamel-sealant interfaces (9). The measurements were made by single operator, blinded to treatment groups. Microleakage value for each specimen, and thereafter, for each tooth and subgroup was calculated as means±SD. Statistical evaluation of the data was performed by two way independent ANOVA and multiple comparisons test at p=0.05.

Following microleakage evaluation, samples were randomly selected from each group and processed for evaluation under a scanning electron microscope (SEM)((JSM-6400 V, JEOL, Tokyo, Japan) to demostrate the sealant-enamel interface. Evaluation was performed after sputter-coating with gold and under accelerating voltage of 20 kV.

RESULTS

Results of Dye penetration test

None of the tested materials or pretreatment protocols could prevent microleakage totally (Table 2). The extent of dye penetration under sealants bonded with the tested self-etching adhesives was significantly higher than those achieved with the etch-and-rinse adhesive systems irrespective to the pretreatment performed.

Microleakage was affected by both the type of pretreatment and the subsequent bonding protocols employed ($p<0.05$). Overall, the highest (Mean=0.36mm) and lowest (Mean=0.06 mm) microleakage values were observed in samples with unpretreated enamel sealed by TriS+Conseal F and samples with laser pretreated enamel sealed by Acid Etch+Prime&Bond+Conseal F protocols, respectively ($p<0.05$). In the acid etch group samples pretreated with laser yielded in slightly lower microleakage scores when compared with

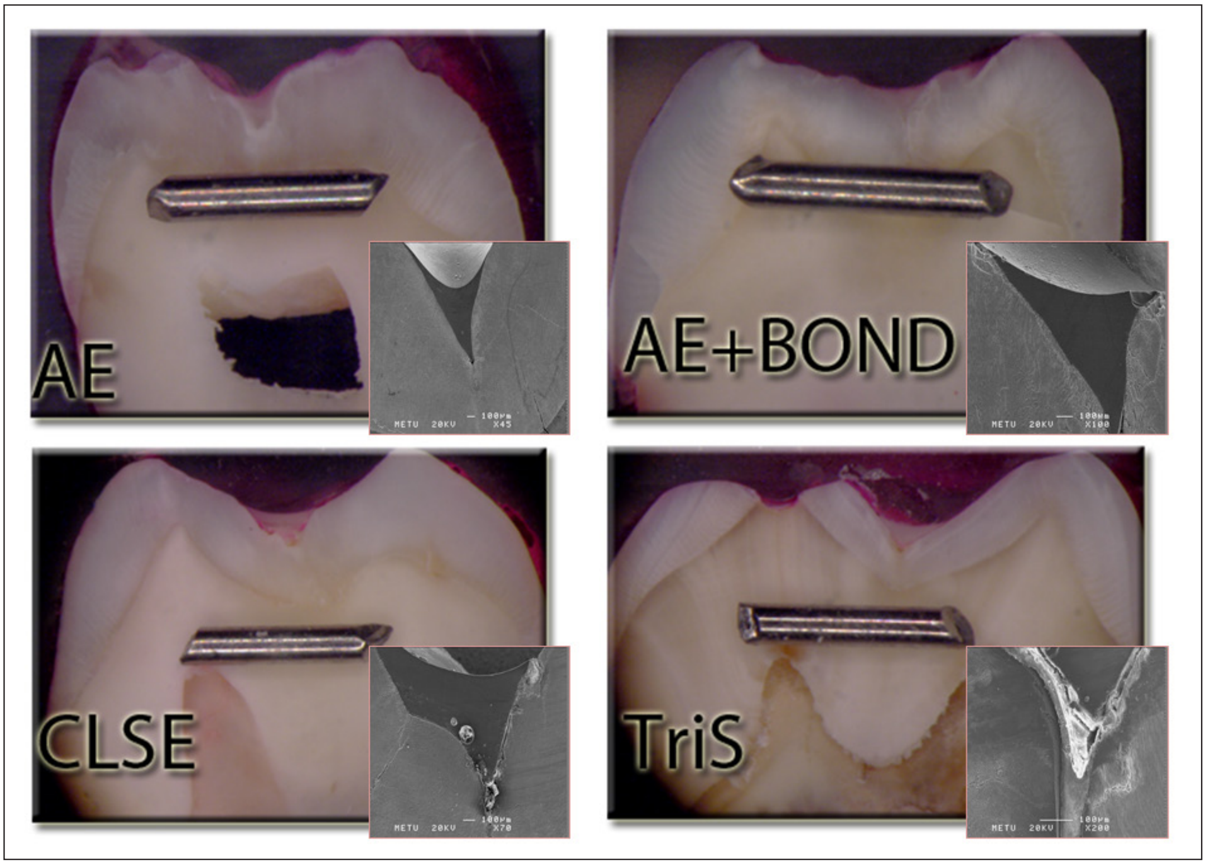

Figure 1. Photographs and SEM micrographs of Control (intact enamel) group.

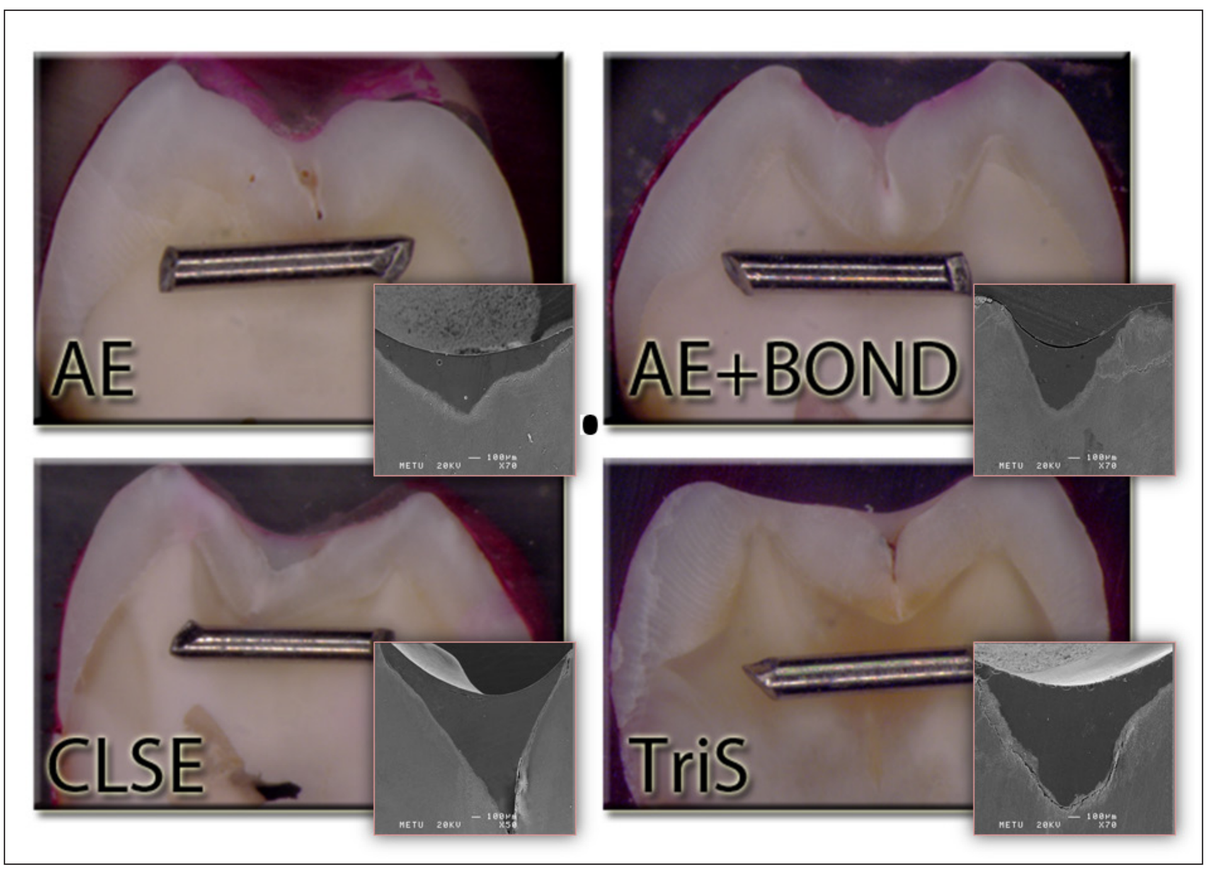

Figure 2. Photographs and SEM micrographs of Er,Cr:YSGG laser pretreatment group.

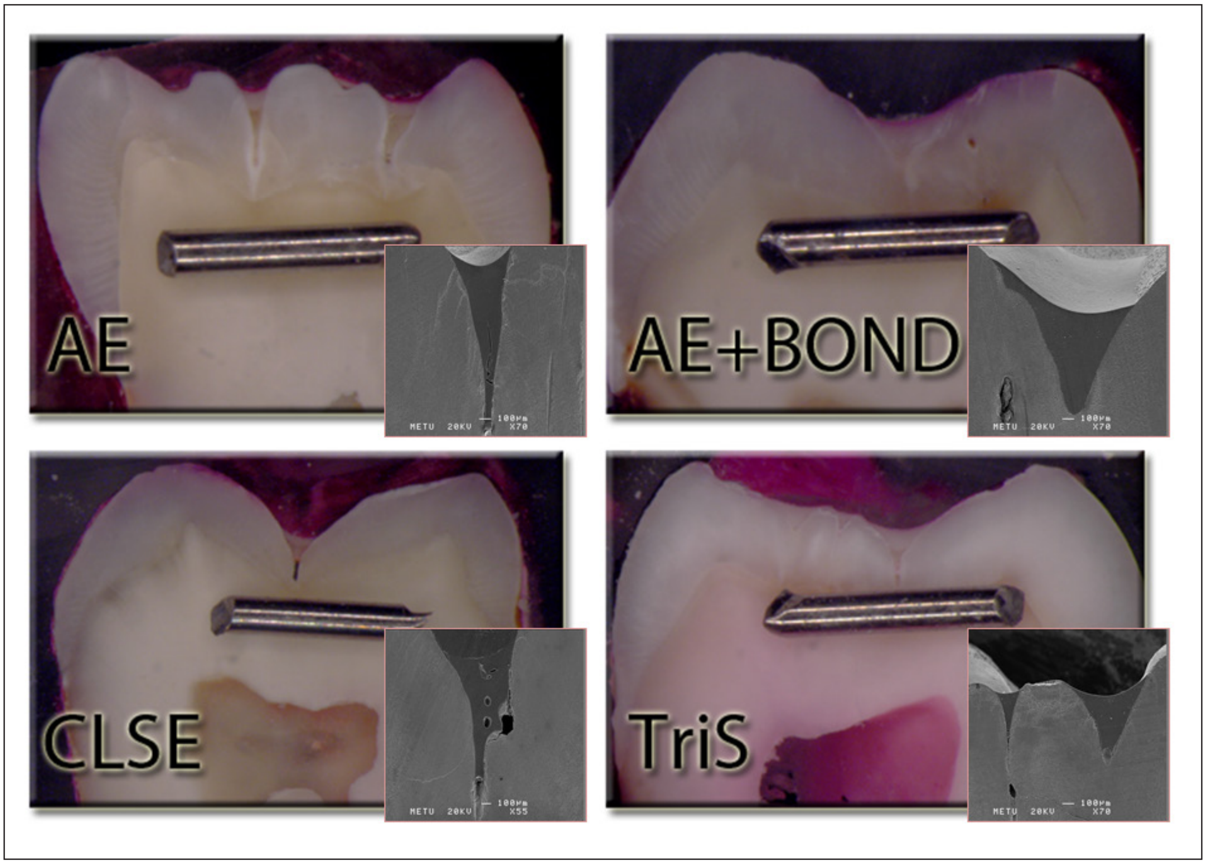

Figure 3. Photographs and SEM images of Air Abrasion pretreatment group.

unpretreated samples and samples pretreated with air abrasion but the statistical significance was not important (p=0,179). Similarly, when bonding agent is applied following acid-etching procedure, microleakage scores were not affected from pretreatment protocol (p=0,615)(intact enamel/laser or air-abrasion). For both all-in one and two step self-etch adhesive systems, unpretreated samples demonstrated the highest microleakage scores. For the fissure sealants bonded with all in one adhesive (TriS) both laser and air abrasion pretreatment helped in reducing the microleakage ($p<0.001$). On the other hand, for the fissure sealants bonded with a two step self-etch adhesive system (Clearfil SE), pretreatment with air abrasion failed to reduce the microleakage scores, whereas pretreatment with laser improved the results of the material.

Results of SEM evaluation

SEM results resemble the results of quantitative evaluation. The representative SEM images of each group were shown in Figures 1-3. For the acid etched specimens, the enamel-bonding agent or enamel sealant interface did not showed any gap formation (Figure 4). Qualitative evaluation with SEM demonstrated that no gap was present between the enamel and bonding system in the specimens pretreated with laser and bonded with self-etch adhesives (Figures 5-6). When the specimens pretreated with air abrasion are evaluated, a gap between enamel and all-in one self etch adhesive system was observed (Figure 7) whereas the two-step self etch adhesive did not yielded in gap formation (Figure 8). On the other hand, on unpretreated specimens a gap was observed within the enamel and bonding system in both self-etch adhesives (Figures 9-10).

DISCUSION

The major finding of the present study is that, irrespective from the pretreatment protocol, the lowest microleakage scores were obtained when orthophosphoric acid etching is applied. None of the tested self-etch adhesive systems or surface pretreatments which are used without phosphoric acid etching step demonstrated comparable scores to that of acid etched specimens. Thus, when microleakage is of concern, the need for acid etching prior to fissure sealant application cannot be eliminated or replaced with a self-etching adhesive protocol. On the other hand, when self-etching adhesive would be used in “bonded fissure sealant” concept, surface pretreatment with Er,Cr:YSGG laser or air abrasion enhances the material’s durability to microleakage depending on the self-etch adhesive system. The two-step self etch adhesive system yielded in better results when compared to the all-in one adhesive tested herein. On the other hand, in order to confirm the results of the present research further in vivo and in vitro studies are recommended. The “aging” concept is an important factor for all restorative materials. In a previous *in vitro* research, Cehreli *et al* [14] reported that four-year water storage significantly increased the extent of microleakage under fissure sealants applied with or without a bonding agent. On the other hand, the same authors showed that after 4-year of aging in water, all tested self-etching adhesives showed better sealing performance compared to phosphoric acid etching only. Thus, the marginal integrity of the fissure sealants placed with self-etch adhesives on intact, laser pretreated or air abrasion pretreated enamel should further be tested following aging procedures or in situ conditions.

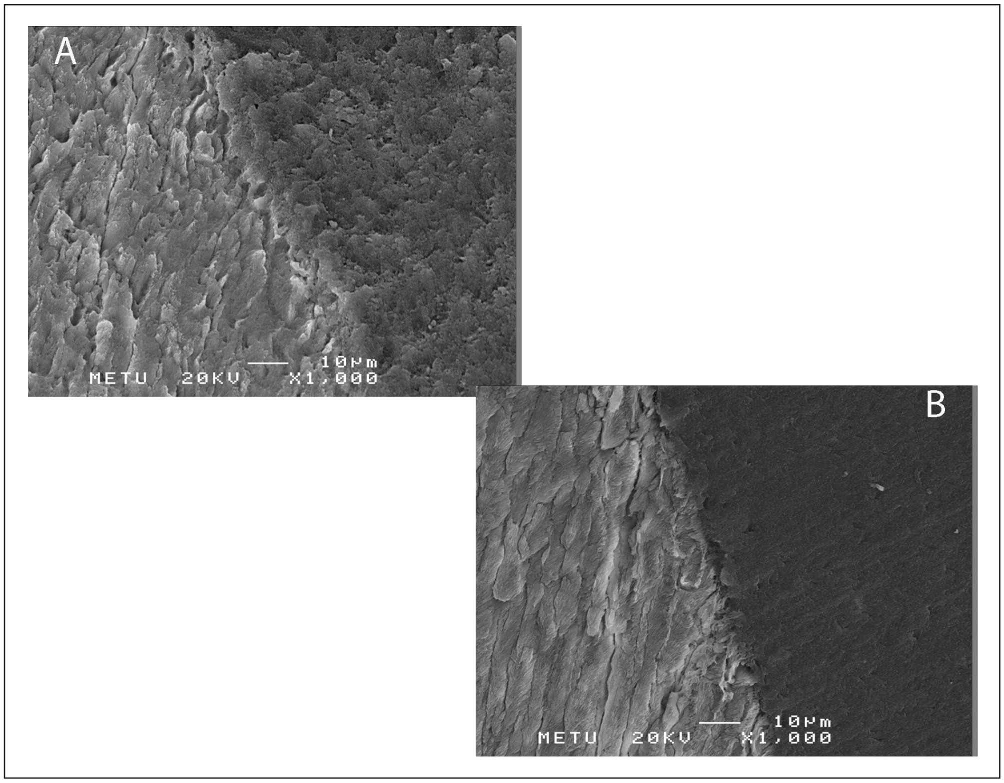

Figure 4. a.SEM image of sealed enamel with acid etch and bond. **b**. SEM micrograph of enamel-resin interface in acid-etch+fissure sealant group.

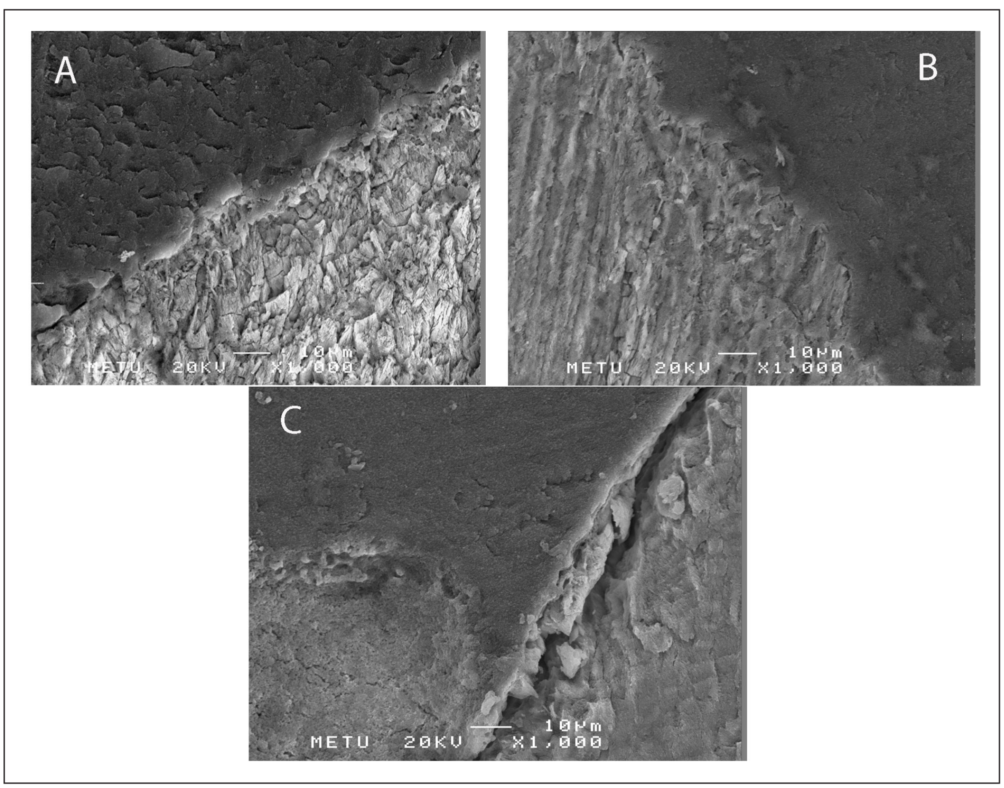

Figure 4. a. SEM micrograph of sealed enamel pretreated with laser and bonded with self-etch adhesives. **b.** SEM micrograph of sealed enamel pretreated with laser and bonded with self-etch adhesive (CLSE). **c.** SEM micrograph of sealed enamel pretreated with air abrasion and bonded with all-in one self-etch adhesive (TriS).

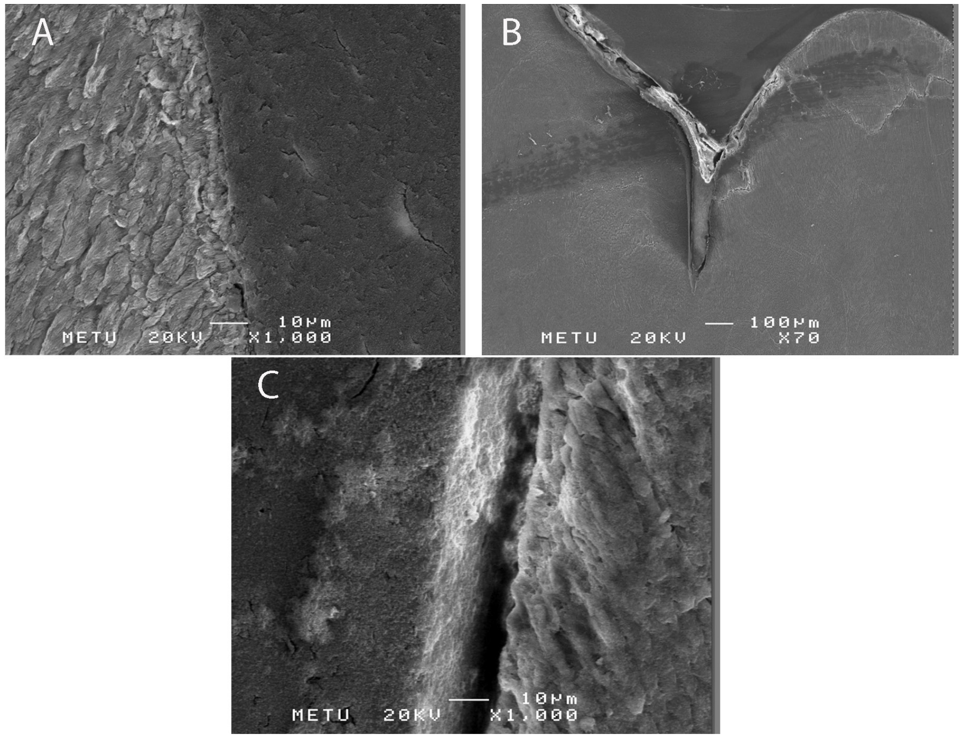

Figure 6: a. SEM micrograph of sealed enamel pretreated with air abrasion and bonded with two-step self-etch adhesive (CLSE). **b.** SEM micrograph resin-enamel intreface in unpretreated enamel bonded with self-etch adhesive (CLSE). **c.** SEM image of sealed unpretreated enamel and bonded with self-etch adhesive (TriS).

The second factor that could influence the results obtained herein is the presence of saliva contamination. The present study showed that when ortophoshoric acid is used, the use of an intermediate hydrophilic bonding agent or the use of laser pretreatment would not affect the extent of microleakage significantly. The previous research states that this would not be the case in a clinical scenario, when the fissures are contaminated with saliva proteins.[15] To the authors' knowledge, there is no data in dental literature regarding the effectiveness of laser or air abrasion pretreatment on sealants applied to saliva contaminated enamel. The effect of self-etch adhesives on fissure sealants applied to saliva contaminated enamel was not evaluated either. Thus, instruments materials tested herein should further be evaluated on the saliva contaminated enamel.

Regarding enamel pretreatment with air abrasion or Er,Cr:YSGG laser, the results of the present study is in compliance with some previous research. Higher microleakage scores were seen following the use of laser or air abrasion alone compared to that following either acid etching alone or tooth preparation using a bur preparation along with acid etching.[15-19] Hatibovic- Kofman *et al* [20] indicated that microleakage may be prevented most effectively with a combination of mechanical air abrasion and chemical acid etching. This can be attributed to the possibility that aluminum oxide particles might be retained in the fissures and prevent penetration of the sealant material.

The Er,Cr:YSGG laser (emitting at a wavelength of 2.79 µm) and the Er:YAG laser (emitting at a wavelength of 2.94 µm) are effective tools for the removal and modification of dental hard tissues.[9,10,13]

Previous articles have mainly dealt with the Er:YAG laser,[21,22] and to date, there have been few investigations regarding the effect Er,Cr:YSGG laser on enamel and dentin.[23] In a recent study, although a acid-etched–like chalky appearance was observed on enamel, etching with phosphoric acid was recommended for higher bond strengths in both dentin and enamel if an Er,Cr:YSGG laser is used for tooth preparation or surface treatment.

The sixth and seventh generation bonding systems, which are two-step and all-in-one self etch adhesive systems respectively, are claimed to have less operative steps and a shorter chair times particularly when treating pediatric patients.[8,11] On the other hand, both have weaker bond strengths especially on uncut enamel.[8] However, an *in vivo* study reported favorable results with Clearfil S3 bond applied prior to fissure sealant. Clearfil S3-bond used in the present study is considered as a weak self-etching primer (pH 2.5) and its hydrophilic acid functional monomer (10-MDP) reported to have an intense chemical interaction with the hydroxyapatite. The authors reported clinically acceptable retention rates which may be the result of a two-fold mechanism: increased micromechanical retention in addition to the chemical interaction.[24]

Clinical evidence tends to support the use of conventional acid etching prior the sealant application. In a 24 month clinical evaluation conducted with a nanofilled fissure sealant material, it was reported that sealants placed with etch-and-rinse adhesive showed better retention rates than those placed with self-etch adhesive.[25] On the other hand, air abrasion pretreatment along with conventional acid etching increased the efficacy of dental sealant *in vivo*. A split-mouth randomized trial involving 16 participants aged 16–17 found that 91% of teeth cleaned with air abrasion had completely retained sealant after two years compared to 76.5% of teeth cleaned without air abrasion.[5] However, in the present *in vitro* study, air abrasion failed to decrease microleakage scores particularly with all-in-one adhesive system. This could be attributed to chemical interactions between the formulation of the bonding agent and the aluminum oxide particles.

Both air abrasion and laser pretreatments require a special device, and probably additional time and additional precautions. Although the routine use of air abrasion or ErCrYSGG laser prior to etching on the sound enamel is not supported by the present study, both techniques improved the microleakage resistance of the fissure sealants placed with self-etch adhesives. On the other hand, as stated by previous researchers[9,26] acquired caries resistance of lased fissures is another issue that needs to be confirmed.

If this concept is true, Er,Cr:YSGG pretreatment would significantly aid in the prevention of inevitable microleakage-oriented secondary caries under sealants particularly applied with self-etch adhesives. Besides, clinician should keep in mind that this effect might be material dependent.

CONCLUSION

The use of Er,Cr:YSGG pretreatment might be utilized when applying bonded fissure sealants with particular self-etch adhesive systems.

Acid-etch protocol is still the golden standard in application of fissure sealants.

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