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ORIGINAL ARTICLE / ARAŞTIRMA MAKALESİ

The Effects of Different Surface Preparation Techniques on Enamel Surface Properties and Bond Strength

Farklı Yüzey Hazırlama Tekniklerinin Mine Yüzey Özelliklerine ve Bağlanma Dayanımına Etkisi

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ABSTRACT

Objectives: The purpose of this study was to investigate the morphological and chemical changes and bond strength on enamel surfaces subjected to different surface preparation.

Material and Methods: The samples were evaluated in *in vitro* tests with 7 different groups(acid etching, Er:YAG laser QSP mode, and Er:YAG laser MSP mode, Air-Flow, and combinations with acid). Surface profilometer and AFM were used to evaluate the surface roughness. After the surface treatment, the chemical changes were analyzed using SEM-EDS. The effects of preparation technique on the bond strength to the enamel surface were evaluated by using micro tensile and shear bond strength tests.

Results: The values obtained the surface roughness evaluation of surface profilometer and AFM were compatible with each other. The highest roughness value was found in QSP when all groups were compared. Ca/P ratios of the surfaces were evaluated as a result of SEM-EDS analysis and it was observed that Ca/P ratio increased in all groups except Acid. It was seen that the results obtained for all groups of micro tensile and shear bond strength tests were consistent with each other.

Conclusion: In conclusion, it is considered the use of Er:YAG laser QSP settings with acid can effect the long-term success of restorations clinically.

Keywords: AFM; Enamel; SEM-EDS; Surface Preparation.

ÖZ

Amaç: Bu çalışmanın amacı, farklı yüzey hazırlığına tabi tutulan mine yüzeylerdeki morfolojik ve kimyasal değişimler ile bağlanma mukavemetinin araştırılmasıdır.

Gereç ve Yöntemler: Örnekler in vitro testlerde kullanılmak üzere 7 farklı gruba (asit dağlama, Er:YAG lazer QSP modu ve Er:YAG lazer MSP modu, Air-Flow ve asitle kombinasyonlar) ayrıldı. Yüzey pürüzlülüğünü değerlendirmek için yüzey profilometresi ve AFM kullanıldı. Yüzey işleminden sonra kimyasal değişiklikler SEM-EDS kullanılarak analiz edildi. Preparasyon tekniğinin mine yüzeyine bağlanma dayanımı üzerindeki etkileri mikro gerilim ve makaslama bağlanma dayanımı testleri kullanılarak değerlendirildi.

Bulgular: Yüzey profilometresi ve AFM'nin yüzey pürüzlülüğü değerlendirmesinden elde edilen değerler birbiriyle uyumluydu. Tüm gruplar karşılaştırıldığında en yüksek pürüzlülük değeri QSP'de bulundu. SEM-EDS analizi sonucunda yüzeylerin Ca/P oranları değerlendirilmiş ve Asit dışındaki tüm gruplarda Ca/P oranının arttığı görülmüştür. Mikro gerilim ve makaslama bağlanma dayanımı testlerinde tüm gruplar için elde edilen sonuçların birbiriyle uyumlu olduğu görüldü.

Sonuç: Sonuç olarak, Er:YAG lazer QSP ayarlarının asitle birlikte kullanılmasının klinik olarak restorasyonların uzun vadeli başarısını etkileyebileceği düşünülmektedir.

Anahtar kelimeler: AFM; Mine; SEM-EDS; Yüzey Hazırlığı

INTRODUCTION

The eruption of the permanent dentition occurs at an early age (~6 years), when the first permanent molar erupts, and is almost complete by ~12 years, with the eruption of the second molars. The teeth are often at highest risk to caries during this process, as the enamel is not fully matured, parents are often unaware of the newly erupting teeth, and cleaning difficulties can arise, due to tender gums. It may also be a challenge for children to brush all surfaces of the new erupting teeth, effectively, as molars exhibit a complex morphology, with pits and fissures covering 12% of the tooth surface (Subramaniam, Konde & Mandanna, 2008).

Occlusal pits and fissures vary morphologically, therefore, they are often irregular and narrow allowing the accumulation of bacteria and debris. This may cause caries due to demineralization of the occlusal surfaces. Currently, minimal restorative intervention aimed at the protection

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of healthy tooth structure as much as possible is used for the treatment of occlusal caries. The progress in adhesive dentistry and understanding of the caries process allow the successful restoration (Tyas & Burrow, 2004).

Various restorative materials have been used to date. Today, composite resins are the most preferred clinically among minimally invasive approaches. The most important factor affecting the clinical success of composite resin restorations is the quality of the adhesion between the dental hard tissues and the restorative materials, and herewith long-term retention. The quality of retention and adhesion is directly related to the etching technique (Matei, Popescu & Suciu, 2014).

Phosphoric acid is utilized conventionally for conditioning of enamel surface. However, this method is time-consuming and has an unacceptable taste for children, requires technical precision and hereby caused evolving new alternative methods which increase the surface intensity of enamel. One of these alternative methods, the air-polishing system carries out a mechanical abrasive effect removing the organic debris when applied to the enamel surface. It has been reported that it can be used to etch the enamel surface prior to the restoration (Burnett, Shinkai & Eduardo C de, 2004).

The laser has a widespread clinical use in paediatric dentistry as well as contemporary dentistry. Laser treatment that is increasingly used in dentistry is considered as an enamel surface conditioning technique in recent years (Sagır, Usumez, Ademci & Usumez, 2013; Karandish, 2014).

The properties of sound enamel tissue must first be known for understanding caries, one of the common health problems nowadays. The inorganic part of enamel consists of hydroxyapatite crystals, which are a special form of calcium and phosphate substantially. The inorganic components of enamel also include calcium, phosphate, sodium and magnesium. Other inorganic components exist on lower proportions are; iron, zinc, strontium, fluorine, rubidium, bromide, vanadium, copper, manganese, gold, silver, chromium, cobalt (Weatherell, Robinson & Hallsworth, 1974).

The filler particle size in composite resin materials affects the character of the material. The flowable composite resins are used successfully in paediatric dentistry because of low viscosity, easy adaptation of the cavity shape, and easy application (Jackson & Morgan, 2000).

It is frequently preferred for restoration of pit and fissure caries due to the fact that the shape of the cavity is not ideal and, therefore, penetration into the cavity and prevents the polymerization shrinkage. The efficient bonding depends on the wettability of the tooth tissues on which the adhesive is applied. Accordingly, the wettability of enamel, which is composed of hydroxyapatite crystals with high surface energy, is high and the adhesion to enamel is more successful. The earliest accepted method is the total-etch approach, which is based on the principle of completely removing the smear layer. Firstly, the etching of the dental hard tissue is performed. Three-step ethanol-water based total-etch adhesives are considered the gold standard in terms of bond strength (De Munck et al., 2005).

The bond strength of composite resin materials to enamel tissue can be influenced by various factors. The enamel surface must be prepared before treatment to ensure that the restorative material is adhered to the enamel surface. Thus, the surface energy of enamel is improved. Depending on the different preparation techniques and the adhesive system used, the surface properties of the dental tissue can affect the bond strenght (Sattabanasuk, Vachiramon, Qian & Armstrong, 2007; Firat, Gurgan & Gutknecht, 2012).

In vitro tests are frequently used for the investigation of newly developed materials and methods (Van Meerbeek et al., 2003). Various methods are used to evaluate the morphological and chemical effects of different dental treatments. The use of bond strength analysis as well as surface roughness and chemical composition analyzes allows for leading of the clinical success of the materials and techniques.

Nowadays, different methods are used to improve the adhesion between the dental hard tissues and the composite resins frequently used clinically. In this respect, researchers have been interested in the use of laser, which have been increasingly used in dentistry recently, as a method of the enamel etching.

Based on this information, it was aimed to investigate the morphological and chemical changes of the enamel surfaces on which prepared with different etching techniques and the bond strength with a flowable composite resin *in vitro*. The purpose of this study was to compare the conventional (acid) and novel (air-polishing and laser) enamel etching techniques. The initial hypothesis was admitted that the use of QSP setting of Er: YAG laser for the enamel etching was statistically significant in terms of surface roughness and bond strength in the study.

MATERIALS AND METHODS

Ethical committee approval for the study was obtained from the Faculty of Dentistry Ethics Committee (2016/011).

This study was conducted in two processes *in vitro*. Various tests were applied to evaluate the effects of different surface preparation techniques on the enamel composition and bond strength.

Firstly, the evaluation of the surface roughness of enamel before and after etching was done using Atomic Force Microscopy and Surface Profilometer. The structural changes of enamel samples after etching were examined by Scanning Electron Microscopy. Subsequently, the changes in the mineral content of the enamel that was prepared by different etching techniques were evaluated by Energy Diffraction X-Ray Spectroscopy.

Secondly, the effects of preparation technique on the bond strength between the flowable composite resin with total-etch adhesive and the enamel surface were evaluated by using micro tensile and shear bond strength tests.

Preparation of teeth to be used for in vitro study

A total of 322 impacted human third molars were collected from the patients referred to Department of Oral and Maxillofacial Surgery with the verbal and written consent for this *in vitro* study. The teeth were cleaned of organic debris and stored in distilled water at room temperature.

Preparation of Teeth for the Investigation of Surface Properties

The root portions of the teeth were separated from the crown portions by the precision sectioning device under water cooling (Isomet 1000, Buehler Lake Bluff, IL, USA). Then, the buccal surfaces of the crown parts of the teeth were formed into smooth surfaces under water cooling with silicone carbide waterproof sandpaper (600, 1000, 1200 grit). The samples were checked with a x40 magnification under the stereomicroscope in terms of defect presence and the appearance of dentin tissue. Subsequently, the samples with the buccal surfaces exposed were placed on a plastic cylinder mold with an internal diameter of 10 mm and a height of 2 mm and over a red wax, and the plastic molds were filled with the self-curing acrylic resin (Panacryl, Arma, Istanbul, Turkey). The samples removed from the molds were checked for surface parallax. The surfaces of the specimens were marked as 2 equal parts, and one side was subjected to etching while the other side was not processed. ‘T’ indicating ‘tested’ was written on the etching side and ‘C’ indicating ‘controlled’ was written on the non-processed side.

Preparation of Teeth for the Investigation of Bond Strength

The sample preparation was performed same as the investigation of surface properties. The only difference was that the plastic molds used had an inside diameter of 10 mm and a height of 20 mm.

A total of 154 specimens were prepared for surface profilometer, AFM and SEM-EDS studies. A total of 168 specimens were prepared for micro tensile and shear bond strength tests. The samples were kept in glass bottles with distilled water at room temperature until the testing time. The samples were randomly divided into 7 groups according to enamel surface preparation techniques.

Surface preparation procedures

Acid etching

The samples were first washed and dried. The surface was then prepared with 37% phosphoric acid (N-etch, Kerr Italia, S.R.L., Scafati, Salerno, Italy) in accordance with the manufacturer’s instructions for 20 seconds. The surface was washed with water for 20 seconds to remove the acid and air-dried for 10 seconds.

Laser etching

The samples were prepared with the cylindrical sapphire tip attached to H14C for 15 seconds with horizontal movements at a distance of 1 mm from the surface and perpendicular to the surface using two different settings (QSP mode and MSP mode at 10 Hz, 1.2W, 120 mJ) of Er:YAG laser (LightWalker AT, Fotona, Slovenia).

Air-polishing etching

The samples were prepared with Air-Flow Master Piezon (EMS, Nyon, Swiss) device and Air-flow Classic Powder for 30 seconds with horizontal movements at a distance of 2 mm from the surface with pressure air and water (33.2 ± 1.7 mL/min and 10.8 L/min).

The Implementation of Restorative Material to The Samples

A conventional adhesive system and a nanohybrid composite were applied to the surface of etched enamel specimens which was prepared for use in micro tensile and shear bond strength tests.

The samples were dried slightly for 3 seconds, Prime in OptiBond FL (Kerr Italia, S.r.l., Scafati, Salerno, Italy) kit was applied to the surface with a small brush for 15 seconds. Afterwards, low-pressure air was applied for 5 seconds. Then, Adhesive was applied with a small brush for 15 seconds and thinned for 3 seconds by low pressure air to spread to the enamel surface. Subsequently, it was polymerized for 20 seconds.

Standard teflon molds with 3 mm diameter and 4 mm height were placed on the enamel surfaces where the adhesive system was applied. Tetric N-Flow (Ivoclar-Vivadent, Schaan, Liechtenstein, a nanohybrid flowable composite, was applied to these cavities in 2 mm layers and polymerized for 20 seconds. The mold was carefully removed and polymerized for 20 seconds.

Surface Roughness Analysis

Twenty samples from each group were used for the surface roughness analysis. The evaluations were made on two different surfaces, one of which was the controlled and tested portions of each sample. Surftest SJ-210 (Mitutoyo Corporation, Tokyo, Japan) was used to measure the surface roughness of the samples. The device calibration before each measurement was made in accordance with the manufacturer's recommendations. The surface roughness was measured from randomly selected three different points and each measurement was repeated 3 times (0.5 mm/sec). Then, the obtained values are recorded in micrometers. The evaluations were made by taking the arithmetic mean of these 3 values (Ra = Roughness average).

AFM Analysis

One sample from each group was used for the AFM analysis. The evaluations were made on two different surfaces, one of which was the controlled and tested portions of each sample. The surface topography of the samples was studied with AFM XE-100 (PSIA Corp, Sang – Daewon-dong, Korea) in non-contact mode. For each sample, the sample surface was scanned at two randomly chosen 10 μm × 10 μm areas (0.5 Hz). Three-dimensional images and numerical surface roughness (Ra) of the scanned areas were obtained.

SEM-EDS Analysis

One sample from each group was used for the SEM-EDS analysis. The evaluations were made on two different surfaces, one of which was the controlled and tested portions of each sample. SEM-EDS examinations of samples were performed with SEM SU-1510 (Hitachi High Technologies Corporation, Tokyo, Japan). The samples were fixed on discs with double sided carbon adhesive. For SEM evaluations, samples were first coated with gold-palladium on a Denton Vacuum Desk V Cold Sputter / Etch Unit (Desk V Cold Sputter/Etch Unit, Denton Vacuum LLC, NJ, USA) with a thickness of 50 A° for 60 seconds (30 mA, 0,05 torr). Digital images were obtained at different magnification ratios (x2000 and x5000). EDS analysis of the same samples were carried out in order to determine mineral content and weight as %. Ca% P% Na% and O% were calculated by surface element analysis on both sides of the enamel surface by evaluating randomly selected 6 different points of each surface. All the obtained values were recorded in written form.

Shear Bond Strength Test

Twenty samples from each group were analyzed by universal test device (Shimadzu AGSX, Shimadzu Corporation, Tokyo, Japan) for shear bond strength. The samples were fixed on the auxiliary metal pieces. The rounded separator tip, 0.5 mm thickness, was placed perpendicular to the connection surface between enamel and composite resin. The separator tip was applied at a crossing speed of 0.5 mm / min until the bonding failure occured (Jafari, Shahabi, Chiniforush & Shariat, 2013). The obtained values were recorded with the computer connected to the device as Newton and later converted to Megapascal (mPa). Afterwards, the specimens were examined with a stereomicroscope at x40 magnification in order to determine the type of failures (adhesive, cohesive, mixed).

Micro Tensile Bond Strength Test

First, 1 x 1 x 4 mm sticks were prepared under water cooling with the precision sectioning device. Microtensile Test Device MTD-500 (SD Mechatronic MTD 500, Germany) was used for the test. The sticks were fixed on the metal holding part of the device with a cyanoacrylate based adhesive. The tensile strenght was applied to the specimens at a speed of 1 mm / min until the bonding failure occured (Phrukkannon, Burrow & Tyas, 1998). The results were recorded in Newtons, then converted to mPa. The specimens were examined by stereomicroscope (Leica Stereo Explorer, Leica Microsystems Ltd, Heerbrugg, Swiss) to determine the failure types.

Statistical Evaluation

Statistical evaluations of all obtained data were performed with the SPSS 21.0 program (SPSS, Chicago, USA). One-way ANOVA was used to determine whether there was a difference between the groups if each variable had a normal distribution, and the Bonferroni test was used for the binary comparison of the groups. Kruskal-Wallis and Mann-Whitney U tests were used for non-normal distribution variables. The descriptive statistics; arithmetic mean, standard deviation, median, minimum and maximum values were shown. Statistical significance was set at $p<0.05$.

RESULTS

The initial hypothesis was accepted based on the results of the study. The results were presented in the following headings.

Results of Surface Roughness Analysis

The descriptive statistics values of all groups are shown in Table 1. The highest Ra values were obtained in QSP, while the lowest Ra values were observed in the control groups.

The roughness values of the 'control' subgroups of all groups were found statistically significantly lower than the 'test' subgroups. Moreover, the effect of surface preparation on the roughness values was statistically considerable. The increase in surface roughness values between 'control' and 'test' subgroups of Acid, Air-Flow and Air-Flow + Acid were not statistically significant while the values between 'control' and 'test' subgroups of MSP, QSP, MSP+Asit ve QSP+Asit were significant.

Table 1. Surface roughness analysis for all groups.

Groups		N	R_{max}	R_{min}	R_{medium}	$R_a \pm SD$
Acid	**Control**	20	0,319	0,293	0,302	0,304±0,01
	Test		0,582	0,384	0,408	0,436±0,12
MSP	**Control**	20	0,365	0,276	0,299	0,316±0,04
	Test		8,773	5,881	8,198	7,438±0,40
QSP	**Control**	20	0,699	0,376	0,459	0,496±0,16
	Test		9,610	8,702	8,992	9,045±0,43
Air-Flow	**Control**	20	0,643	0,341	0,438	0,471±0,14
	Test		2,153	0,780	1,028	1,283±0,79
MSP+Acid	**Control**	20	0,296	0,203	0,243	0,253±0,03
	Test		8,058	6,817	7,097	7,257±0,67
QSP+Acid	**Control**	20	0,880	0,585	0,766	0,748±0,08
	Test		8,091	6,706	7,336	7,346±0,53
Air-Flow+Acid	**Control**	20	0,300	0,196	0,271	0,259±0,02
	Test		0,892	0,684	0,814	0,798±0,05

**Bonferroni test*
MSP: Medium-Short Pulse
QSP: Quantum-Square Pulse

Results of AFM Analysis

The dark areas denoted pits and the light areas signified hills on the images. The highest surface roughness values were observed in QSP while the lowest values were monitored in the control groups similarly to the results of the surface roughness analysis (Table 2). There was also created three-dimensional image of the surface enamel. The effect of surface preparation on the increase of surface roughness values in all groups was found statistically significant.

Table 2. AFM analysis for all groups.

Groups		Average	
		R_a	R_q
Acid	**Control**	16,552	20,472
	Test	28,013	60,601
MSP	**Control**	13,311	16,748
	Test	311,210	344,045
QSP	**Control**	37,260	48,577
	Test	332,012	398,321
Air-Flow	**Control**	23,873	30,390
	Test	36,565	46,377
MSP+Acid	**Control**	19,845	26,894
	Test	123,373	139,845
QSP+Acid	**Control**	19,598	24,266
	Test	195,074	243,901
Air-Flow+Acid	**Control**	121,925	145,438
	Test	196,733	218,983

**Bonferroni test*
MSP: Medium-Short Pulse
QSP: Quantum-Square Pulse

The investigation of the samples in 'control' groups with AFM revealed that the enamel crystals were arranged on the surface in a shallow and regular manner. The enamel crystals were arranged irregularly and the regional cavities were monitored due to the dissolution of the crystals on the 'test' samples of Acid group (Fig.1a).

It was observed the irregular slight depths on the images of the 'test' samples of MSP and QSP groups. In addition, the micro-cracks were found on the samples etched with only laser. The 'test' samples of Air-Flow group showed an irregular and non-uniform structure (Fig. 1b, 1c, 1d).

The 'test' samples of MSP + Acid and QSP + Acid groups showed more regular texture than Acid group. It was observed less irregularity on the 'test' samples of Air-Flow + Acid groups than laser groups but more amorphous than Air-Flow group (Fig.1e, 1f, 1g).

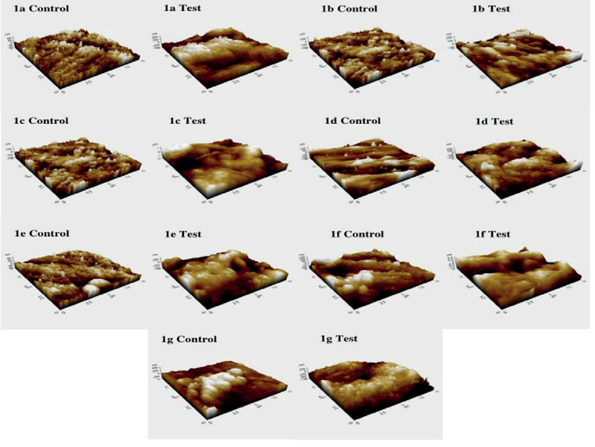

Figure 1: 1a control: AFM images of 'Control' enamel sample of Acid group.1a test : AFM images of 'Test' enamel sample of Acid group. 1b control: AFM images of 'Control' enamel sample of MSP group. 1b test: AFM images of 'Test' enamel sample of MSP group. 1c control: AFM images of 'Control' enamel sample of QSP group. 1c test: AFM images of 'Test' enamel sample of QSP group. 1d control: AFM images of 'Control' enamel sample of Air-flow group. 1d test: AFM images of 'Test' enamel sample of Air-flow group. 1e control: AFM images of 'Control' enamel sample of MSP+Acid group. 1e test: AFM images of 'Test' enamel sample of MSP+Acid group. 1f control: AFM images of 'Control' enamel sample of QSP+Acid group. 1f test: AFM images of 'Test' enamel sample of QSP+Acid group. 1g control: AFM images of 'Control' enamel sample of Air-flow+Acid group. 1g test: AFM images of 'Test' enamel sample of Air-flow+Acid group

Results of SEM Analysis

The mean values of EDS analysis applied to examine the amount and distribution of the minerals on the surface of the specimens evaluated by SEM were shown in Table 3. Different morphological changes were observed on the enamel surfaces etched with different procedures on the SEM images. The honeycomb appearance was clearly visible on the surface of the 'test' samples in Acid group. It was present that the dissolution of the crystals and the gaps between enamel crystals (Fig. 2a).

The SEM images of the 'test' specimens in MSP and QSP groups showed that the obstruction of the gaps and the formation of irregular craters (Fig. 2b, 2c).

The smear layer was observed on the SEM images of the 'test' samples in Air-Flow group due to the ingredient of Air-flow Classic Powder (Fig. 2d).

The SEM images of the 'test' specimens in MSP+Acid and QSP+Acid groups showed that the obstruction of the gaps and the formation of irregular craters with the honeycomb appearance (Fig. 2e,2f).

The smear layer with the irregular honeycomb appearance was observed on the SEM images of the 'test' samples in Air-Flow group due to the ingredient of Air-flow Classic Powder (Fig. 2g).

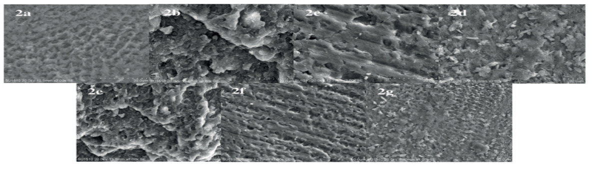

Figure 2: 2a. SEM images of 'Test' enamel sample of Acid group. 2b: SEM images of 'Test' enamel samples MSP group. 2c: SEM images of 'Test' enamel samples QSP group. 2d: SEM images of 'Test' enamel samples Air-flow group. 2e: SEM images of 'Test' enamel samples MSP+Acid group. 2f: SEM images of 'Test' enamel samples QSP+Acid group. 2g: SEM images of 'Test' enamel samples Air-flow+Acid group

Wilcoxon test, a nonparametric test, was performed on the data that had no normal distribution. The weight and atomic percentages of the elements (Ca, O, P, Na) and Ca / P ratios in all groups were compared with the 'control' and 'test' groups and the difference between them was evaluated (Table 3).

There was no statistical difference between the 'control' and 'test' samples in Acid, Air-Flow, MSP+Acid, QSP+Acid,

Table 3. Ca/P ratio and weight percentages of mineral distributions of enamel samples.

		Weight %				
Groups	**Subgroups**	**Calcium**	**Phosphate**	**Oxigen**	**Sodium**	**Ca/P**
Acid	**Control**	21,82±2,30	11,58±0,97	62,79±3,06	1,14±0,15	1,88±0,09
	Test	23,55±2,19	13,02±0,50	65,45±3,86	0,63±0,15	1,80±0,12
MSP	**Control**	26,34±1,10	14,77±1,03	58,14±1,47	0,69±0,14	1,78±0,05
	Test	23,93±0,19	12,36±1,55	62,28±0,68	0,44±0,05	1,93±0,07
QSP	**Control**	22,94±0,78	12,01±1,33	64,38±0,89	0,70±0,13	1,89±0,14
	Test	20,80±0,81	9,44±0,65	66,45±1,13	0,59±0,08	2,43±0,29
Air-Flow	**Control**	23,38±1,23	12,51±1,09	63,18±0,96	1,08±0,33	1,84±0,03
	Test	21,47±1,04	11,62±1,05	65,66±0,46	0,93±0,19	1,86±0,04
MSP+Acid	**Control**	21,56±1,02	12,25±0,63	65,43±0,49	0,70±0,11	1,73±0,02
	Test	21,00±1,15	12,12±0,60	66,17±0,19	0,75±0,09	1,76±0,03
QSP+Acid	**Control**	21,92±0,77	12,15±0,68	67,30±0,22	0,77±0,02	1,80±0,07
	Test	20,75±0,28	11,19±0,49	65,15±0,18	0,76±0,02	1,85±0,09
Air-Flow+Acid	**Control**	21,79±0,58	11,99±0,26	65,37±0,65	0,83±0,09	1,81±0,01
	Test	21,17±0,21	11,15±0,70	65,53±0,67	1,22±0,24	1,89±0,03

**Wilcoxon test*
MSP: Medium-Short Pulse
QSP: Quantum-Square Pulse

Air-Flow+Acid groups in terms of EDS analysis (Chart). The increase in Ca / P ratio was found to be statistically higher than the other groups comparing the 'control' and 'test' samples in MSP and QSP groups.

Results of Microtensile and Shear Bond Strength Analysis

The 'test' samples of QSP+Acid group yielded the highest shear bond strength while the 'test' samples of Air-Flow exhibited the lowest values. The descriptive statistics of shear bond strength values of all groups are given in the Table 4.

Table 4. Descriptive statistics of shear bond strenght values.

Groups	N	Min (mPa)	Max (mPa)	Mean(mPa) ± Standard Deviation
Acid	20	4,25	8,11	5,86 ± 2,00
MSP	20	4,76	6,66	5,45 ± 1,05
QSP	20	4,99	7,17	6,19 ± 1,10
Air-Flow	20	3,37	3,98	3,72 ± 0,31
MSP+Acid	20	4,52	7,47	6,25 ± 1,54
QSP+Acid	20	7,06	8,99	8,07 ± 0,96
Air-Flow+Acid	20	4,26	6,13	5,1 ± 0,94

**Shapiro-Wilk analysis, one-way ANOVA, Bonferroni test*
MSP: Medium-Short Pulse
QSP: Quantum-Square Pulse

Shapiro-Wilk analysis resulted in the assumption of normality ($p > 0.05$). The difference between Air-Flow and QSP + Acid group ($p < 0,05$) was significant based on one-way ANOVA, but no significant difference was observed between the other groups. It was seen that the results of the microtensile bond strengths were in compatible with the shear bond strength. The descriptive statistics of microtensile bond strength values are given in the Table 5.

Shapiro-Wilk analysis resulted that the data had normal distribution ($p > 0.05$). There was a significant difference between all groups according to the one-way ANOVA and Bonferroni's multiple comparison analysis.

Table 5. Descriptive statistics of microtensile bond strenght values.

Groups	N	Min (mPa)	Max (mPa)	Mean(mPa) ± Standard Deviation
Acid	10	5,63	10,36	7,61 ± 0,01
MSP	10	5,84	10,71	6,92 ± 0,02
QSP	10	5,74	10,93	7,73 ± 0,23
Air-Flow	10	4,25	7,95	5,45 ± 0,14
MSP+Acid	10	6,42	12,31	10,11 ± 0,06
QSP+Acid	10	6,10	12,64	11,82 ± 0,45
Air-Flow+Acid	10	4,36	8,85	6,84 ± 0,09

**Shapiro-Wilk analysis, one-way ANOVA, Bonferroni test*
MSP: Medium-Short Pulse
QSP: Quantum-Square Pulse

DISCUSSION

This study was planned in order to investigate the surface properties, composition and bond strength of enamel etched with several procedures and thus, to increase the success of restorations.

The pits and fissures are the most sensitive surface to caries (Cehreli, Gungor & Karabulut, 2006). The most important issue in clinical evaluation is to decide whether the restoration is necessary for suspected fissure. Various restoration materials have been used in paediatric dentistry. The widespread use of composite resins and the adoption of minimally invasive approaches have resulted in more satisfactory results both in esthetics and function. The role of adhesive systems in bonding of composite resins is precious. Adhesive systems are classified as total-etch, self-etch and glass ionomer adhesive systems according to the effects on the smear layer. These systems form the basis of adhesives (Unlu, Ermis, Sener, Kucukyilmaz & Cetin, 2010). The adhesive system used in current study was a three-step total-etch adhesive that requires the separate etching and washing steps. This process was followed by primer and adhesive resin application.

Several studies exist in the literature reporting that the traditional three-step total-etch adhesive systems exhibit more successful results than the newly developed, simplified adhesive systems in laboratory tests (De Munck et al., 2003; Shirai e al., 2005; Imai et al., 2017). The results of these studies confirm that the three-step total-etch adhesive systems can be used as a gold standard in assessing the clinical performance of newly developed adhesives (De Munck et al., 2003). A number of laboratory studies has been reported that providing stronger bond to enamel with total-etch adhesive systems (Pashley & Tay, 2001; Perdigao & Swift, 2006; Kalra, Suprabha, Rao, Shenoy & Lewis, 2015; Schwendicke, Doméjean, Ricketts & Peters, 2015).

Kalra et al. (2015) applied three different surface treatments (total-etch and self-etch adhesive systems, only acid etching) to the enamel surfaces with initial caries lesions, before performing the resin-based fissure sealant. It was noticed that the highest bond strength values were obtained in the total-etch adhesive system group.

Schwendicke et al. (2015) implemented composite resin restorations with three different self-etch and total-etch adhesive to the extracted human teeth. In the study, it was assessed the integrity of restorations, microleakage and resistance to fracture and attained statistically higher values in total-etch groups. In our study, Optibond FL, a total-etch adhesive system, was used based on the literature.

The improvements in modern dentistry facilitate the essential function and aesthetic with minimal loss of dental tissue. The use of flowable composites is one of the major advances for dental material technology (Bonilla, Yashar & Caputo, 2003).

The use of flowable composites in paediatric dentistry is encouraged due to the low viscosity, consistency, easy adaptation and application (Jackson & Morgan, 2000). It was investigated the radiopacity of six different flowable composite resins. The supreme values were indicated in Tetric N Flow and Clearfil Majesty Flow groups. It has been suggested that the radiopacity of the composite resins is considerable for detecting new caries formation (Ergücü, Türkün, Onem & Güneri, 2010). Therefore, Tetric N-Flow, a nanohybrid composite, was preferred for our study (Jackson & Morgan, 2000; Baroudi & Rodrigues, 2015).

The intent of the enamel surface conditioning is to create a proper tooth surface for chemical and micro-mechanical bonding of the adhesive systems (Espinosa et al., 2010). In our study, the acid etching, a standard and recognized method, was used based on the literature. However, it has led to search for alternative methods that would further increase the enamel surface energy because of the unpleasant taste and technical sensitivity. For this purpose, it was considered that different methods such as laser and air polishing systems could be used besides acid (Boyde, 1984; Usumez & Aykent, 2003).

Agrawal and Shigli (2012) prepared the occlusal surfaces of the teeth with only brush, brush with prophylactic paste, burs, air-polishing, air-abrasion and acid etching with time extension methods before applying resin-based fissure sealant. In this study in which microleakage was evaluated, the lowest values were observed in the groups that burs, air-abrasion and air-polishing systems were used respectively while there was no statistically difference between the groups.

Brocklehurst, Joshi, and Northeast (1992) evaluated the effectiveness of different cleaning methods such as prophylactic paste, only water, air-polishing systems prior to fissure sealant on the success of restoration. It has been reported that the use of air-polishing system increases the depth of penetration of the resin to the enamel surface. In our study, it was intended to compare with the studies in the literature using the only air-polishing and combination with acid.

Nowadays, erbium lasers are preferred for the enamel etching while Nd:YAG and CO_2 lasers have been used in the past. Er:YAG laser can be absorbed at high rate by the water and hydroxyapatite of dental tissue (Karandish, 2014).

Keller and Hibst (1993) prepared the bovine enamel prepared with acid and Er:YAG laser at different energy levels for SEM observations and bond strenght of a composite resin. The acid and laser etched surfaces demonstrated similar morphological changes on SEM images. There was no statistically significant difference between acid and laser groups in terms of bond strength of the composite resin.

Sagır et al. (2013) investigated the bond strength of the orthodontic brackets to the enamel etched with acid and Er:YAG laser at MSP and QSP settings. Significant differences were not observed between the laser MSP and QSP settings while higher bond strength values were obtained in both laser groups than the acid group.

In another study, it was investigated the bond strenght of the orthodontic brackets to the enamel etched with different methods (acid, Er:YAG laser MSP and QSP settings). The use of QSP etching with self-etch adhesive system has been reported to exhibit the highest statistical values (Akin, Veli, Erdur, Aksakalli & Uysal, 2016).

The outcomes of studies in which used Er:YAG laser etching may differ slightly (Gurgan et al., 2008). The conflict on this issue is considered depend on the variable parameters of laser used in the studies (Firat et al., 2012). In current study, it was evaluated the effect of two different settings of Er:YAG laser and conventional acid etching to the success of composite resins. In literature review, it has not been observed any study investigating the conventional and novel ettching techniques together.

Different surface conditioning methods can induce changes in content and structure of the enamel. The solubility and permeability of the enamel may also vary if the ratios between the organic and inorganic components of the enamel change. Hence, the alternative etching methods should have minimal effect on the structure and surface properties of the enamel (Rohanizadeh, LeGeros, Fan, Jean & Daculsi, 1999).

Clinical trials are the most effective method to demonstrate the long-term success of adhesive systems and composite resin restorations. Nevertheless, the fact that clinical trials are difficult and time-consuming, and the results are not determinable when considering ethical reasons. However, *in vitro* tests give more accurate results with shorter time and lower cost (Van Meerbeek et al., 2003). Our study was conducted as *in vitro* because of controlling variables and resulting in a short time.

The teeth used in the current study were collected among the impacted third molars intended to imitate the immature permanent teeth. The plane of teeth used in the laboratory studies may affect the results. The buccal surfaces of teeth are commonly used because the thickness of occlusal enamel differs. Based on this information, the impacted third molar teeth were used for the current study and stored in distilled water until the test time.

The surface roughness is a substantial parameters used to assess the success of composite resins *in vitro* (Mikulewicz, Szymkowski & Matthews-Brzozowska, 2007). Contact and non-contact profilometers are used for the evaluation of surface rougness (Wan Bakar & McIntyre 2008; Barkmeier, Erickson, Kimmes, Latta & Wilwerding, 2009; Ganss, Lussi, Sommer, Klimek & Schlueter, 2010). In the present study, we used the contact profilometer performing precise measurements and “Ra” values was used. In this respect, there is an oppurtunity to compare with other studies (Silva & Zuanon, 2006). Kim et al. (2007) examined the enamel surfaces of premolar teeth with a surface profilometer and obtained Ra values of 0,45 ± 0,52 µm. It is also compatible with the results of our study that the individual differences in Ra values exist.

Barkmeier et al. (2009) investigated the surface roughness of the enamel (prepared with total-etch and self-etch adhesives) with profilometer, and the bond strength of a composite resin. The total-etch adhesive group exhibited the highest values in all respect.

AFM enables to investigate the surface roughness in micro and nano levels. AFM was preferred for our study due to the reliability on the monitoring of surface changes (White et al., 2010). AFM ensures a three-dimensional image of dental surfaces. The most commonly used parameter for the evaluation of the data obtained with AFM is Rq values (Raposo, Ferreira & Ribeiro, 2007).

The morphological and structural changes on the enamel surfaces etched with Er: YAG laser by AFM and SEM-EDS were evaluated in a study. It was observed the triangular recesses on the laser etched surfaces (Rodriguez-Vilchis, Contreras-Bulnes, Olea-Mejìa, Sánchez-Flores

& Centeno-Pedraza, 2011). In our study, AFM images consistent with the literature were obtained.

In dentistry, SEM is often used as a supporting analysis in studies. EDS serves to analyze the chemical composition of a structure. In our study, it was observed regular and small pores on the enamel surfaces of Acid group while irregular and various sized pores on the enamel surfaces of laser groups. The results of AFM and SEM analysis were compatible with each other. EDS analysis was performed to standardize the mineral content of the specimens prior to the surface preparation and there was no statistically significant difference between the groups (de-Melo et al., 2011). It has been reported that various etching methods may change the mineral ratios of enamel surface (Rodriguez-Vilchis et al., 2011; Keinan, Mass & Zilberman, 2010).

Alcantara-Galeana et al. (2017) examined the structural and morphological changes on the deciduous teeth etched with acid, Er: YAG laser and self-etch adhesive system with SEM-EDS. It has been reported that Er:YAG laser etching increased the mineral ratios.

This study aims at the integrated evaluation of the different surface conditioning techniques all in one which is not covered in the previously reviewed literature. The SEM-EDS results of this study are consistent with the research performed by Rodriguez-Vilchis et al. (2011) and Kwon, Kwon, Kim, and Kim (2003) The distribution of minerals in the enamel are influenced by the different surface conditioning techniques. It was observed that Ca / P ratio increased in all groups except the Acid group. The increase in MSP and QSP groups was significant while the increase in the other groups was not significant.

The most essential consideration in the success of composite resins is the adhesion between the dental hard tissue and material. The bond strength tests are often preferred used to evaluate the success of resin-based dental materials (Heintze & Zimmerli, 2011). In our study, micro tensile and shear bond strengths were performed.

Sasaki et al. (2008) investigated the micro tensile bond strength of the composite resin material to the enamel surface on which etched with acid, Er:YAG laser and combined. It has been reported that the highest bond strength values were obtained with the combined use of acid and Er:YAG laser.

Firat et al. (2012) evaluated the micro tensile bond strength of the composite resin and a total-etch adhesive system to the enamel and dentin on which etched with acid and Er:YAG laser at the different pulse duration and energy level. It was stated the bond strength values were decreased as the pulse duration increased in the laser etched groups. However, it has indicated that the use of laser with acid etching increased the bond strength.

In our study, it was observed that the use of Er:YAG laser QSP setting with acid significantly increased the micro tensile and shear bond strenght in concordance with the literature (Akin et al., 2016; Altunsoy, Botsali, Korkut, Kucukyilmaz & Sener, 2014; Buyukhatipoglu, Ozsevik, Secilmis & Usumez, 2016).

CONCLUSION

It was predicted that besides the conventional acid etching, the use of laser, air-polishing systems, and the combination with acid may be alternative promising methods in terms of the increase for the bond strenght. The use of Er:YAG laser QSP setting was prompted to be a method for improving the success of the restorations as it has increased the Ca / P ratio and bond strength. The fact remains that these *in vitro* studies need to be supported by *in vivo* studies for the effectiveness.

Conflict of Interest

The authors declare that they have no conflict of interest related to this

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Authors' contributions

BA performed the literature review and collected the data. YS created the study design. YS consulted on idea and hypothesis. BA carried out the laboratory study and wrote the manuscript. YS critically revised the manuscript. All authors have made substantive contribution to this study and manuscript, and all have reviewed the final paper prior to its submission.

Disclosure statement

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