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Effect of Sodium Hypochlorite in Ground Fluorotic Enamel: Shear Bond Strength and Surface Analysis

Efecto del hipoclorito de sodio en esmalte fluorótico tallado: resistencia adhesiva al cizallamiento y análisis de la superficie

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ABSTRACT: Evaluate the effect on the shear bond strength (SBS) of 5% NaOCl applied after acid etching on ground anterior teeth with fluorosis and analyze the surface with scanning electron microscopy (SEM), also compare fluorotic and healthy ground enamel by atomic force microscopy (AFM) and Vickers microhardness (VM). For the SBS test 30 anterior teeth with moderate and severe fluorosis according Dean index were selected by an examiner previously calibrated with an expert in fluorosis by using the intraclass correlation coefficient (ICC). With the help of a calibrated high speed diamond bur for veneer preparation and a parallel chamfer high speed diamond bur the vestibular face was prepared with a uniform reduction of .3 mm under water cooling. In one half of the vestibular face of the teeth the conventional adhesive procedure was carried out while in the other half was added an additional step with 5% NaOCl applied for 1 minute and washing after acid etchant. A resin post was adhered in each half and load was applied until fracture. The failure mode was evaluated and a SEM analysis was made. Posteriorly 10 samples of fluorotic and healthy ground enamel were used to measure the nanostructural characteristics by AFM (roughness average and absolute depth profile) and the VM in three operative steps (after ground, after etchant and

after deproteinization). The Shapiro-Wilks and Brown-Forsythe methods were used to test the distribution of variables. The Paired Student's t-test was used to compare the differences between mean bond strength (MPa) in the two groups for SBS test. Chi-square analyzes were performed to compare the failure modes between groups. One-way ANOVA analysis and Tukey-Kramer post hoc test were used to compare groups for roughness average, absolute depth profile and Vickers microhardness. A greater SBS (32.17±4.20 MPa) and a surface more homogeneous and less contaminated were observed in the deproteinization subgruoup compared to the conventional subgroup (27.74±4.88 MPa). AFM parameters were greater in fluorosis subgroup than in healthy enamel subgroup. VM was lower in the fluorotic enamel in each operative step in comparation with healthy enamel. The use of 5% NaOCl as a deproteinizing agent after acid etchant in ground fluorotic enamel results in better adhesion, which may imply greater success in adhesive treatments. The ground surface of fluorotic enamel shows higher values of roughness and depth and lower values of VM which proves that there is a more irregular and less hard surface.

KEYWORDS: Dental fluorosis; Dental enamel; Dental bonding.

RESUMEN: Evaluar el efecto en la resistencia adhesiva al cizallamiento (SBS) de la aplicación de 5% de NAOCl después del grabado ácido en dientes anteriores fluoróticos tallados y analizar la superficie con microscopía electrónica de barrido (SEM), también comparar esmalte tallado fluorótico y sano por microscopía de fuerza atómica (AFM) y microdureza Vickers (VM). Para la prueba SBS 30 dientes anteriores con fluorosis moderada y severa de acuerdo al índice de Dean fueron seleccionados por un examinador previamente calibrado por un experto en fluorosis usando el coeficiente de correlación intraclase (ICC). La cara vestibular se preparó .3mm con la ayuda de una fresa calibrada para la preparación de carillas y una paralela con punta en chamfer de diamante a alta velocidad e irrigación. En una mitad de la superficie vestibular de los dientes se llevó a cabo el procedimiento adhesivo tradicional, mientras que en la otra mitad se incluyó un paso adicional con la aplicación de NaOCl al 5% por un minute y lavado posterior al grabado ácido. Un poste de resina fué adherido en cada mitad y se aplicó carga hasta la fractura. Se evaluó el modo de fallo y se realizó un análisis con SEM. Posteriormente se utilizaron 10 muestras de esmalte tallado fluorótico y sano para medir las características nanoestructurales por medio de AFM (rugosisdad promedio y perfil de profundidad absoluta) y la VM en tres diferentes pasos operativos (posterior al tallado, posterior al grabado ácido y posterior a la desproteinización). Se utilizaron los métodos de Shapiro Wilks y Brown-Forsythe para conocer la distribución de las variables. Una T de student pareada se utilizó para comparar la diferencia entre los promedios de la fuerza de unión (MPa) entre grupos para la prueba SBS. Se llevaron a cabo análisis de chi cuadrada para comparer los modos de fallo entre los grupos. Análisis de ANOVA de una vía y Tukey-Kramer post hoc fueron utilizados para comparar los grupos para las variables rugosidad promedio, perfil de profundidad absoluta y microdureza Vickers. Se observó una mayor SBS (32,17±4,20 MPa) y una

superficie más homogénea y menos contaminada en el subgrupo con desproteinización en comparación con el subgrupo convencional (27,74±4,88 MPa). Los parámetros de AFM fueron mayores en el subgrupo de fluorosis que en el subgrupo de esmalte sano. La VM fue más baja en el esmalte fluorótico en cada paso operativo en comparación con el esmalte sano. El uso de NaOCl al 5% como agente desproteinizante después del grabado ácido en el esmalte fluorótico tallado da como resultado una mejor adhesión, lo que puede implicar un mayor éxito en los tratamientos adhesivos. La superficie tallada del esmalte fluorótico muestra valores más altos de rugosidad y profundidad y valores más bajos de VM lo que prueba que existe una superficie más irregular y menos dura.

PALABRAS CLAVE: Fluorosis dental; Esmalte dental; Adhesión dental.

INTRODUCTION

Dental fluorosis is a disturbance in the development of dental enamel that leads to a malformation of the same, caused by successive exposure to high concentrations of fluoride during dental development (1), Kuhns (2), was the first to describe a condition that seemed fluorosis in endemic areas of Mexico in 1888, but it was not until 1931 that the correlation between fluoride in drinking water and dental fluorosis was discovered (3). The prevalence of dental fluorosis has increased in many parts of the world, even in non-endemic areas (4). The severity of the condition depends on when and for how long overexposure to fluoride occurs, the individual response, weight, degree of physical activity, nutritional factors and bone growth among other factors (5). Fluorotic enamel is characterized by a hypermineralized outer layer more resistant to acid and retention of areas of more porous enamel in the hypomineralized subsurface. The pores are occupied by water and enamel secreting proteins that were retained (6). In its milder form the enamel is characterized clinically by white lines due to the accentuated periquimaties, while in the most severe cases, the enamel contains larger areas that may be discrete or confluent brown. Because of the post-eruption trauma, the surface layer of the enamel can be detached resulting in areas of irregular enamel (7). For its treatment, therapeutic measures are carried out, ranging from a dental bleaching in its milder forms, through microabrasion, placement of vestibular porcelain or resin veneers and even crowns in their most severe presentation (2). Thanks to advances in adhesive dentistry and aesthetic requirements, conservative restorative procedures are increasingly accepted, restorations such as buccal veneers are treatment options that clinicians frequently use as they represent an effective and reliable procedure in the long term (8,9) being the adhesion a very important point for its success. Fluorotic enamel due to its altered composition represents an adhesive challenge, it has been observed that the preparation of the external surface of .3mm, the same reduction used for veneers of minimum preparation, presents good adhesive results (10,11) since it eliminates the 50 µm of the hypermineralized outer layer (12) which exposes the hypomineralized porous subsurface and with an organic network that occurs in more advanced cases of fluorosis and that is formed by proteases and proteins of the enamel matrix, mainly amelogenins (13). One of the proposed options for the removal of this organic network is the use of an organic solvent (4). The union between abnormal enamel and the restoration depends on the alterations of the enamel, the removal of the excess of proteins can give an advantage and achieve a better adhesion with the restoration, sodium hypochlorite is known to be an excellent protein denaturant (14). Deproteinization of the

enamel with 5% NaOCl after the acid etchant has worked in other conditions where higher amounts of enamel proteins are found as in imperfect hypocalcified amelogenesis (15-17), as well as in primary and immature teeth (18). The aim of this *in vitro* study was to compare the adhesive performance of deproteinization with sodium hypochlorite applied after acid etching during the adhesive protocol in moderate and severe fluorotic enamel with reduction of .3mm and analyze the surface characteristics of the ground fluorotic enamel (roughness, depth and microhardness) comparing it with ground healthy enamel.

MATERIALS AND METHODS.

SUBJECTS AND SAMPLE PREPARATION

Patients undergoing dental extraction of anterior teeth due to periodontal disease or orthodontic reasons at hospital and private clinics of Villa de Reyes (San Luis Potosí, Mexico) where water fluoride level between 0.7 and 2ppm, were asked to donate their teeth. Autonomous University of San Luis Potosi Ethics Committee approved the research Project grant CEIFE-034-017. The teeth were stored for a period of no more than two months in thymol solution at .2%. All samples were subsequently cleaned in an ultrasonic bath (Biosonic UC300-115B, Coltene/Whaladent, Cuyahoga, Falls, Ohio, USA), then with an ultrasound (NSK, Multi-Task Ultrasonic System, Various 350) to eliminate the remnants of calculus, soft and periodontal tissue, then washed in tap water and stored in distilled water (Milli-Q, Millipore Co., Billerica, MA, USA) at 4°C until the beginning of the experimental procedures. The collected teeth were analyzed by an expert according to the severity of fluorosis using the Dean index (19). Examiners were calibrated with an expert in fluorosis by using the intraclass correlation coefficient (ICC). 30 fluorotic anterior teeth with moderate and severe fluorosis were used in the SBS test and 2 of them were observed in SEM. For AFM measurements 10 healthy and 10 fluorotic teeth were selected and other 10 healthy and 10 fluorotic teeth were included in this study for Vickers microhardness test.

ADHESIVE PROCEDURE

30 fluorotic anterior teeth (incisives and canines) were mounted in fast-curing acrylic with the help of a plastic mold 1mm below the enamel cement junction. They were placed with its vestibular face perpendicular to the floor for the shear bond strength test.

With the help of a calibrated high speed bur for veneer preparation (#834, 016 medium size grain; Komet USA LLC, Rock Hill, South Carolina, USA) and a parallel chamfer high speed bur (#882, 012 medium size grain; Komet USA LLC, Rock Hill, South Carolina, USA) the middle third of the vestibular face was prepared with a uniform reduction in the of .3mm under water cooling trying to achieve a flat surface perpendicular to the floor as shown in Figure 1. To avoid individual teeth differences and spilling to the other half in the 5% NaOCl application, a deeper groove in the middle part of the teeth reaching to the dentin was made to divide the vestibular face into two parts. At random in one of the halves the conventional adhesive procedure was carried out according to the instructions of the manufacturer: syringe application of 35% phosphoric acid for 30 seconds (Etchant Gel S, Coltene/Whaledent, Altstätten, Switzerland), washing for 5 seconds and drying for 2 seconds with air. Posteriorly, application of a layer of adhesive (One Coat Bond SL, Coltene/Whaledent Altstätten, Switzerland) for 20 seconds by rubbing with the help of a microbrush, air for 2 seconds and light curing for 1 minute with a power of 1200 mW/cm² (Iled, Woodpecker, Guilin, China). In the other half the step of deproteinization (5% Sodium Hypochlorite applied with a microbrush for 1 minute, washing for 5 seconds and dry with air for 2 seconds) was added after the acid etching and before the adhesive application, careful application,

the deep groove and the use of a paper tip like a barrier served to prevent the passage of NaOCl to the other half of the tooth.

A plastic mold with a circular hole 3mm in diameter and 4mm deep was positioned on each of the treated sections of the teeth. Resin (Brilliant NG, Coltene/Whaledent, Altstätten, Switzerland) was placed in 2mm increases and light-cured with a power of 1200 mW/cm² (Iled, Woodpecker, Guilin, China) in the mold forming cylindrical post perpendicular to the enamel surface. Each post was light curing for 40 seconds for increment, for 2 increments. They were stored in distilled water at 37°C for 2 weeks. After that period, 1000 thermal cycles were performed in baths between 5°C and 55°C with a dwell time of 30 seconds and a transfer time of 10 seconds.

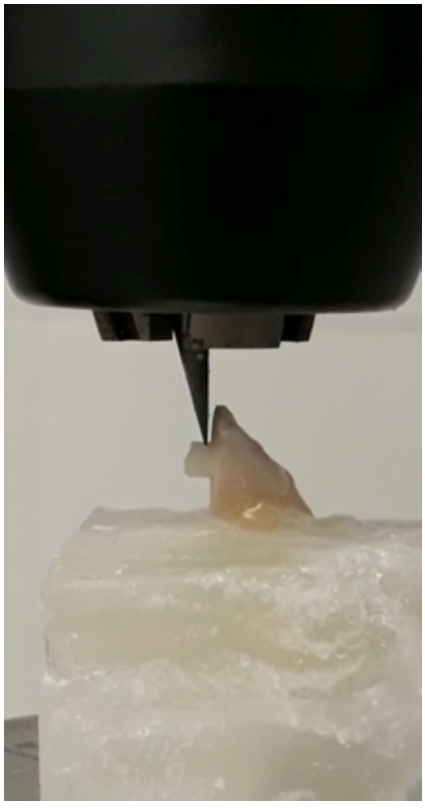

Figure 1. Mounting of samples for the SBS test.

SHEAR BOND STRENGTH TEST

The test was carried out for the 2 subgroups of study: fluorotic teeth with normal procedure of adhesive protocol and with deproteinization with the help of a universal test machine (Instron 3365, Norwood, Massachusetts, USA) and the analysis of the data with the help of Bluehill software (Instron 3365, Norwood, Massachusetts, USA). A knife-edge that was aligned to .2mm of the adhesive interface was used as a loading device (Figure 1). The distance of the loading device to the enamel surface was maintained using the space of two celluloid bands. The load was applied in a controlled displacement mode at a speed of .5mm/min until the failure occurred. The adhesive force of shear was calculated in MPa.

FAILURE MODE EFFECT ANALYSIS

The analysis of the surfaces of the enamel after the fracture of all the samples was carried out by an examiner previously calibrated on the total area using a stereomicroscope (SZ-PT Olympus, Tokyo, Japan) at magnifications of 10X and 40X at an angle of 90 degrees. The classification of the faults was the following:

- Adhesive between tooth and adhesive
- Cohesive in composite
- Cohesive in adhesive
- Cohesive enamel
- Mixed

SCANNING ELECTRON MICROSCOPE (SEM) EVALUATION

In order to observe the surface characteristics of the enamel treated with the different techniques under the SEM, 2 enamel samples 3mm in width were obtained and mounted in acrylic blocks,

were grounded .3mm with the help of a calibrated high speed bur for veneer preparation (#834, 016 medium size grain; Komet USA LLC, Rock Hill, South Carolina, USA) and a parallel chamfer high speed bur (#882, 012 medium size grain; Komet USA LLC, Rock Hill, South Carolina, USA) and etched for 30 seconds (application of 35% phosphoric acid, Etchant Gel S, Coltene/Whaledent, Altstätten, Switzerland, washing for 5 seconds and drying for 2 seconds with air) and one of the samples was then deproteinized (5% Sodium Hypochlorite applied with a microbrush for 1 minute, washing for 5 seconds and dry with air for 2 seconds) Then, each specimen was immersed in ascending concentrations of ethanol (25%, 50%, 75%, 80%, 90% and 95%) for 20 min each and 100% for 1 hr. The samples were fixed with double-faced stickers on the specimen holder and gold sputtered (S150A sputter coater, Edwards; London, UK) to render the samples electrically conducting, using a very thin layer of gold. The samples were examined using SEM (JEOL JSM-6510 Tokyo, JAP), operated at 10kv. The surface was examined at x250 and x1000.

ATOMIC FORCE MICROSCOPY (AFM)

10 samples (3mm in width) of healthy enamel and 10 of fluorotic enamel were obtained; the enamel was grounded .3mm as described previously using a calibrated high speed bur for veneer preparation (#834, 016 medium size grain; Komet USA LLC, Rock Hill, South Carolina, USA) and a parallel chamfer high speed bur (#882, 012 medium size grain; Komet USA LLC, Rock Hill, South Carolina, USA). All samples were measured after each of the 3 operative times to which they were subjected: after grounded .3mm, after the application of 35% phosphoric acid (Etchant Gel S, Coltene/Whaledent, Altstätten, Switzerland, washing for 5 seconds and drying for 2 seconds with air), and after the application or 5% sodium hypochlorite (applied with a microbrush for 1 minute, washing for 5 seconds and dry with air for 2 seconds) at the same scan size (50×49.5mm²) by triplicate in different areas, all of them selected at random, and a mean value was obtained for each sample. The subgroups of study for AFM and VM are shown in Table 1. The enamel surface roughness and absolute depth profile of enamel were carried out using AFM (Nanosurf Easy Scan 2, SPM Electronics, Liestal, Switzerland) in contact mode with a silicon nitride (SiN) scanning rate of 49.5µm/s. The values used for the short cantilever were as follows: spring constant 0.1 N/m; resonant frequency 28 kHz; length 225µm; mean width 28µm; thickness 1µm; tip height 14µm and radius <10nm. A calibration grid of silicon oxide on a silicon substrate (Nanosurf AG, CH-4410, SPM Electronics, Liestal, Switzerland) with *XY* periodicity of 10µm and a *Z* height of 119 nm was used to calibrate the instrument before the evaluation. The Nanosurf Easy Scan 2 software (version 1.6) was used to measure the AFM parameters.

The enamel surface roughness was quantified using Sa and Sy parameters. Sa (roughness average) represents the arithmetical mean of the absolute values of the scanned Surface profile and Sy (peak-valley height) represents the average value of the absolute values of the heights of the five highest profile peaks and the depths of five deepest profile valleys within the sampling length on the scanned surface. Commonly, Sy is known as the peak-to-valley height. In this article we call it the absolute depth profile. The AFM was used in the contact mode because it is a suitable method for surface roughness measurements from hard tissues.

VICKERS MICROHARDNESS ANALYSIS

The Vickers Microhardeness tester (HVS-1000Z, Sinowon, DongGuan, CHN) was used to determine the Vickers microhardness. 10 samples (3mm in width) of healthy enamel and 10 of fluorotic enamel were used for this analysis. Three indentations were performed with a load of 50Kgf for 30 seconds and a separation of approximately

100µm in each sample to obtain a mean value. Each sample was measured after each of the 3 operative times to which they were subjected: after grounded .3mm using a calibrated high speed bur for veneer preparation (#834, 016 medium size grain; Komet USA LLC, Rock Hill, South Carolina, USA) and a parallel chamfer high speed bur (#882, 012 medium size grain; Komet USA LLC, Rock Hill, South Carolina, USA), after the application of 35% phosphoric acid (Etchant Gel S, Coltene/Whaledent, Altstätten, Switzerland, washing for 5 seconds and drying for 2 seconds with air), and after the application or 5% sodium hypochlorite (applied with a microbrush for 1 minute, washing for 5 seconds and dry with air for 2 seconds). The subgroups of study for AFM and VM are shown in Table 1.

Table 1. Surface preparation for each study subgroup for AFM and Vickers Microhardness.

Study subgroup	Preparation
Healthy enamel after ground	Ground .3mm, washing and drying
Healthy enamel after etchant	Ground .3mm, acid etchant 30 seconds, washing and drying
Healthy enamel after deproteinization	Ground .3mm, acid etchant 30 seconds, washing and drying, sodium hypochlorite 5% 1 minute, washing and drying
Fluorotic enamel after ground	Ground .3mm, washing and drying
Fluorotic enamel after etchant	Ground .3mm, acid etchant 30 seconds, washing and drying
Fluorotic enamel after deproteinization	Ground .3mm, acid etchant 30 seconds, washing and drying, sodium hypochlorite 5% 1 minute, washing and drying

STATISTIC ANALYSIS

All data is expressed by mean and standard deviation. The Shapiro-Wilks and Brown-Forsythe methods were used to test the distribution of variables. The Paired Student's t-test was used to compare the differences between mean bond strength (MPa) in the two groups. Chi-square analyzes were performed to compare the failure modes between groups. One-way ANOVA analysis and Tukey-Kramer post hoc test were used to compare groups for roughness average, absolute depth profile and Vickers microhardness. The JMP program, version 10.0 (SAS Institute, Cary, NC, USA) and Stata version 11.0 (StataCorp LP, College Station, TX, USA) were used for statistical analysis with statistical significance set at $\alpha=0.05$.

RESULTS

The interobserver reproducibility analysis fluorosis achieved by the examiner and the expert revealed an ICC of 0.99.

SHEAR BOND STRENGTH

The shear adhesion resistances in MPa (mean and standard deviation) for the two adhesive protocols used on each side of the fluorotic enamel for the 30 samples are shown in Table 2.

Table 2. Mean shear bond strength of study subgroups.

Subgroups	Shear bond strength
Conventional procedure	27.74 ± 4.88
Deproteinization procedure	32.17 ± 4.20
P	<0.05

Results show means ± SD expresed in Megapascals. Statical test Paired Student's t.

When the results of both groups were compared, the mean values of the shear adhesion resistance showed statistically significant differences between the two ($P<0.05$) showing greater resistance to shear in the group where deproteinization was included in the adhesive protocol (32.17±4.20 MPa) than in the group with the conventional adhesive protocol (27.74±4.88 MPa).

FAILURE MODE EFFECT ANALYSIS (FMEA)

The FMEA found after the shear adhesion resistance test is summarized in Table 3.

Table 3. Failure mode of specimens of the study subgroups after shear bond strength test.

Subgroups	Adhesive (tooth and adhesive)	Cohesive (resin)	Cohesive (adhesive)	Cohesive (enamel)	Mixed
Conventional procedure	2	5	5	3	15
Deproteinization procedure	1	6	5	6	12

Chi-square analyses ($p < 0.05$).

Among the failures found, the most prevalent failure in both groups was the mixed failure, however the analysis of Chi Square did not reveal significant differences in the failure mode between both groups.

SCANNING ELECTRON MICROSCOPY (SEM)

Figure 2 (A and B) show the morphology observed in the representative samples at 250 and 1000x of the ground and etchant fluorotic enamel. The ground and etchant fluorotic enamel with application of 5% NaOCl is shown in Figure 3 (A and B). A predominantly type II acid etching pattern is observed in all images, characterized by a variable central protrusion and loss of peripheral margins of the enamel prisms, existing diverse irregular areas that can be mainly explained by the presence of fluorosis and lines of variable depth caused by the milling of the surface. A closer view shows that etching surface seems to be a little more homogeneous and with less presence of impurities in deproteinized group Figure 3 (A and B) and a greater contamination is observed in the images of the group without deproteinization after acid etching Figure 2 (A and B).

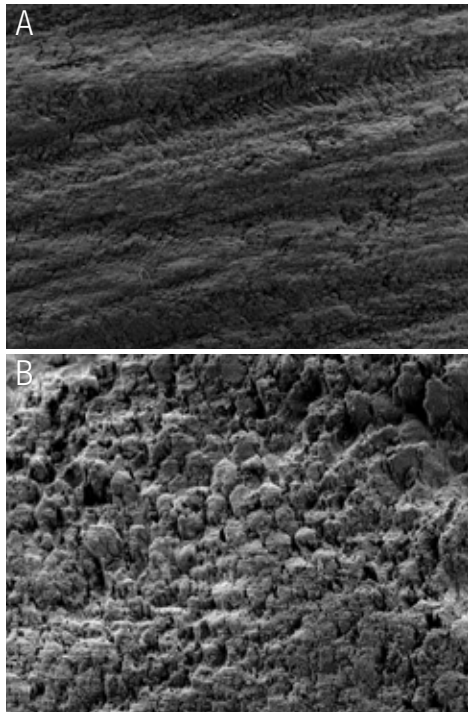

Figure 2. (A) Ground and etched enamel at 250x. (B) Ground and etched enamel at 1000x.

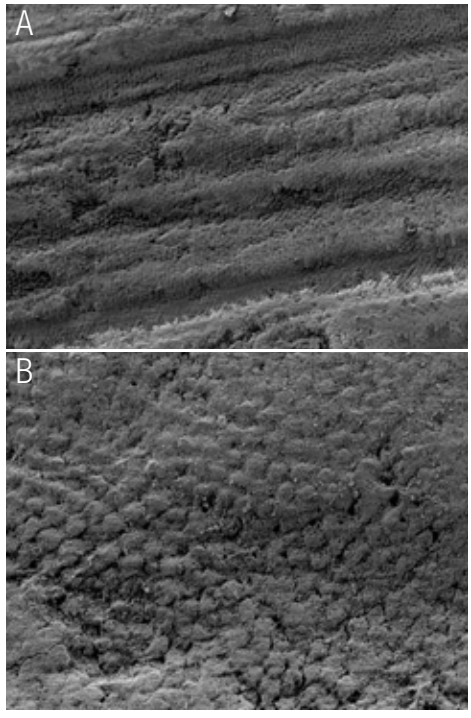

Figure 3. (A) Ground, etched and deproteinized enamel at 250x. (B) Ground, etched and deproteinized enamel at 1000x.

ATOMIC FORCE MICROSCOPY (AFM)

Table 4 shows the roughness average of study subgroups in each operative time. Healthy and fluorotic enamel showed decreased roughness after acid etching and a slight non-significant increase after deproteinization. The fluorotic enamel after ground subgroup showed a statistically significant highest roughness value than the rest of the subgroups, which decreased significantly after acid etching, approaching the value of the healthy enamel subgroup after acid etching.

The absolute depth profile results in each operative time are shown in Table 4. Healthy and fluorotic enamel subgroups behave similarly in the three operative times, decreasing their value after acid etching and again after deproteinization without being significant. The fluorotic enamel subgroup after ground showed a higher value compared to the rest of the subgroups, which decreased significantly after the etching acid, falling to values close to the healthy enamel subgroup after acid etching.

VICKERS MICROHARDNESS ANALYSIS

The microhardness mean values and standard deviations of the total number of indentations are shown in Table 4. In Table 5 the Tukey-Kramer post hoc test showed that the healthy enamel subgroup after ground had the highest value with significant differences with all the other groups. The fluorotic enamel after ground showed a statistically significant difference with the value obtained after acid etching, but there was no significant difference with the fluorotic enamel after deproteinization subgroup. Between the different enamel subgroups there was a significant difference between the groups after acid etching as well as in the groups after deproteinization.

Table 4. Descriptive statistics (mean and standard deviation) of roughness, absolute depth profile and vickers microhardness of study subgroups in each operative time.

ROUGHNESS			
Subgroups	After ground	After etchant	After deproteinization
Healthy enamel	474.14 ± 71.2 (b)	440.12 ± 62.1 (b)	460.29 ± 74.4 (b)
Fluorotic enamel	667.77 ± 179.3 (a)	469.85 ± 92.1 (b)	528.48 ± 96.2 (b)
ABSOLUTE DEPTH PROFILE			
Subgroups	After ground	After etchant	After deproteinization
Healthy enamel	4.44 ± .38 (b)	4.31 ± .79 (b)	4.08 ± .74 (b)
Fluorotic enamel	6.25 ± 1.86 (a)	4.76 ± .76 (b)	4.47 ± .81 (b)
VICKERS MICROHARDNESS			
Subgroups	After ground	After etchant	After deproteinization
Healthy enamel	387.95 ± 19.7	285 ± 32.1	313.65 ± 53.5
Fluorotic enamel	272.86 ± 32.8	223.62 ± 36.3	253.09 ± 25.5

Results for roughness expressed in nanometers; for absoluteh depth profile in micrometersandfor vickers microhardness in HVN. Different letters indicates a statiscally significant difference ($p<0.05$) between subgroups by using Tukey-Kramer post hoc test.

Table 5. Surface microhardness *p* values comparisons between subgroups.

Subgroups[a]	*P*
HEG versus FEE	<.0001*
HEG versus FED	<.0001*
HEG versus FEG	<.0001*
HEG versus HEE	<.0001*
HED versus FEE	<.0001*
HEG versus HED	0.0002*
HEE versus FEE	0.0032*
HED versus FED	0.0038*
FEG versus FEE	0.0305*
HED versus FEG	0.1129
HEE versus FED	0.3340
FED versus FEE	0.4233
HED versus HEE	0.4548
FEG versus FED	0.8029
HEE versus FEG	0.9704

[a]Healthy enamel after ground (HEG), healthy enamel after etchant (HEE), healthy enamel after deproteinization (HED), fluorotic enamel after ground (FEG), fluorotic enamel after etchant (FEE), fluorotic enamel after deproteinization (FED). Asterisc (*) indicates a statiscally significant difference ($p< 0.05$) between subgroups by using Tukey-Kramer post hoc test.

DISCUSSION

To minimize the effect of different types of teeth in this study only canine teeth and human incisors were used, since they are the teeth where the placement of buccal veneers with minimal preparation is usually carried out. Teeth with moderate and severe fluorosis according to the Dean index (19) were selected to be teeth with a degree of fluorosis where a treatment with minimal preparation veneers (.3mm) can be carried out (2). Teeth with moderate fluorosis present all the affected surface and brown spots may appear, while in teeth with severe fluorosis discrete or confluent holes may occur (20), in both cases less invasive treatments such as whitening or micro abrasion may not completely satisfy the aesthetic demands of patients. Since there is a lack of knowledge about some specialized procedure to improve the adhesion in the hypomineralized subsurface of the enamel, in this study the removal of .3mm of enamel was decided, as it is done in some minimally invasive preparations for vestibular veneers, and, in this way, the elimination of the outermost hypermineralized layer that occurs in fluorosis, since it has been observed that the hypermineralized external layer is about 50µm of the affected enamel (12). In another study where the same preparation of .3mm was performed, no statistically significant differences were found between the total etch and self-etch adhesives used (11). In another recent study, better adhesive results were observed when performing .3mm reduction in fluorotic enamel (10) but no specific treatment protocol was proposed to improve adhesion in this altered substrate. In this study, it was decided to use a One Coat Bond SL (Coltene/Whaledent, Altstätten, Switzerland), a total etch adhesive since it is the type of system that is used with the best adhesive results in the veneering of veneers. Two of the most commonly used artificial aging techniques were carried out. All samples were stored in distilled water for a period of 2 weeks immediately after resin placement. After this period all samples were thermocycled. Some studies have reported significant decreases in adhesion forces, even after relatively short periods due to the increase in water and the consequent hydrolysis that occurs in the adhesive interface and that leads to the decrease of adhesion forces, simulating the oral environment (21,22). The use of an agent for the removal of the organic network that can be found by eliminating the hypermineralized outer layer in some cases of fluorosis had already been proposed previously (4). In this study, statistically significant differences were found in achieving an adhesive improvement in the group where the deproteinization was incorporated with 5% sodium hypochlorite after acid etching (32.17±4.20MPa) compared to the group where the conventional technique was performed (27.74± 4.88 MPa); this coincides with

the good results found in studies in other conditions such as hypocalcified imperfect amelogenesis (15-17) as well as in primary and immature teeth (18) when using deproteinization in the adhesive protocol. The explanation may be that the removal of the hypermineralized outer layer of fluorosis leaves us a hypomineralized subsurface with a greater amount of proteins that could resemble enamel in hypocalcified imperfect amelogenesis and in immature teeth, and where the use of deproteinization when eliminating this organic network shows adhesive advantages. Regarding the failure mode, no significant differences were found between the groups, with the mixed failure being the most predominant, coinciding with another article where the same classification was used (10). The higher prevalence of mixed failure can be explained because the totality of the evaluated surface is observed, and therefore with small non-homogeneous portions on the surface, a mixed fault is considered. As observed in the images obtained by scanning electron microscopy, a less contaminated and more uniform surface is observed after the use of the deproteinizing agent compared with the surface without this treatment. These observations agree with the results obtained in the shear bond strength test in this study, since better adhesive results were obtained on the most uniform surface and with less contamination, but it differs from the results obtained in another study where there were no statistical differences in the shear bond strength test were found (14), however in the before mentioned study healthy enamel was used, which explains these differences since in our study using fluorotic enamel can be find an abnormal porous enamel with more protein and water, where the effect of sodium hypochlorite would be greater. In this study most of the ground enamel treated with fosforic acid at 35% for 30 seconds shows a well defined type II pattern (23) which coincides with previous articles where well-defined etching patterns on both ground and unground enamel surfaces were found (24).

The results obtained by AFM (roughness average and absolute depth profile) in fluorotic enamel after ground are higher than the values found in healthy enamel, agree with what was previously reported6 mentioning that fluorotic enamel is characterized by a hypermineralized outer layer more resistant to acid and retention of areas of more porous enamel on the hypomineralized subsurface. A study was found where they observed the roughness in healthy teeth with interproximal reduction, with similar results to our group of healthy enamel after ground (25). Regarding teeth with fluorosis, two articles were found where roughness was evaluated by AFM, with results lower than ours in both groups (healthy and fluorotic enamel) even after acid etching, which can be explained since they measured enamel without carving while in our study we use ground enamel (26,27).

In relation to Vickers microhardness, we found a lower microhardness in ground fluorotic enamel compared to healthy enamel, our results agree with that described in the literature that says that when eliminating the external 50 microns (12) a hypomineralized zone is found and with an organic network greater than normal enamel (13). A study that analyzed the microhardness of the surface of healthy and fluorotic enamel found results comparable to ours but slightly inferiors, unlike in our study they observed intact enamel and posterior teeth (28).

CONCLUSION

Within the limitations of this in vitro study, it is concluded that: the use of sodium hypochlorite as a deproteinizer after acid etching in teeth with moderate and severe fluorosis and grounded for veneers (.3mm), improved the adhesive resistance to shear bond strength. Regarding the failure mode, no differences were found between the uses or not of the deproteinization after acid etching in teeth

with moderate and severe fluorosis. The roughness in ground fluorotic enamel is greater compared to healthy enamel and Vickers microhardness is less in ground fluorotic enamel than in healthy enamel.

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