Resin Replica in Enamel Deproteinization and its Effect on Acid Etching

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Purpose: *The goal of this in vitro study was to identify the topographical features of deproteinized (NaOCl) and etched with phosphoric acid (H_3PO_4) enamel surface, compared to phosphoric acid surface alone with a Resin Replica model.* ***Materials:*** *Ten extracted lower first and second permanent molars were polished with pumice and water, and then divided into 3 equal buccal sections having similar physical and chemical properties. The enamel surfaces of each group were subjected to the following treatments: Group A: Acid Etching with H_3PO_4 37% for 15 seconds. Group B: Sodium Hypochlorite (NaOCl) 5.25% for 60 seconds followed by Acid Etching with H_3PO_4 37% for 15 seconds. Group C; No treatment (control). All the samples were treated as follow: Adhesive and resin were applied to all groups after A, B and C treatment were performed; Then enamel/dentin decalcification and deproteinization and topographic SEM Resin Replica assessment were used to identify resin tags enamel surface quality penetration.* ***Results*** *showed that group B reached an area of 7.52mm^2 of the total surface, with a 5.68 mm^2 (73%)resin tag penetration equivalent type I and II etching pattern, 1.71 mm2 (26%) equivalent to type III etching pattern and 0.07 mm^2 (1%) unaffected surface. Followed by group A with 7.48 mm^2 of the total surface, with a 3.47 mm^2 (46 %)resin tag penetration equivalent to type I and II etching pattern, 3.30 mm^2 (45 %)equivalent to type III etching pattern and 0.71 mm^2, and (9 %) unaffected surface. Group C did not show any resin tag penetration. A significant statistical difference (P <0,001) existed between groups A and B in resin quality penetration, leading to the* ***conclusion*** *that when the enamel is deproteinizated with 5.25% NaOCl for 1 minute prior H_3PO_4, the surface and topographical features of the replica resin penetration surface increases significantly with type I-II etching pattern.*

Keywords: *Enamel, deproteinization, sodium hypochlorite, phosphoric acid, etching, resin replica, permanent teeth.*

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INTRODUCTION

Since Buonocore in 1955,[1] enamel etching and later adhesion system in dentistry has been deep-seated.[2,3] The different etching patterns first seen and reported by Gwinnett (1971) [3-4] and Silverstone (1975) [5] showed the morphological changes produced in the enamel surface using a sweep electron microscope (SEM) identifying 3 different patterns. The type I and II offered retentive areas of greater size and depth, while type III etching pattern, did not alter the deeper strata where the enamel prisms are located, lacking major micromechanical retention. These 3 etching patterns appear randomly at any point on the enamel[6] and can be found closely bound in the same enamel zone.[6] Clinically, however one can only see an opaque surface, exhibiting the quantity but not quality of the etched surfaces.[4,5]

Today we know that etching quality depends on the etching agent, acid concentration, etching time, and composition of the enamel surface.[7-13]

It has been firmly established that the essence of adhesion lies in achieving the best acid etching, with a generalized retentive morphological condition over the enamel surface.[12,14-15]

However, recent studies have shown that the topographic quality of enamel etching with phosphoric acid (*H3PO4*) is not achieved over the entire adhesive surface; more than 69% of this surface was no etched, while 7% presented tenuous etching and only 2% was ideally etched.[16, 17]

To counteract these limitations some authors have suggested abrading the enamel in order to increase retention. This invasive technique offered apparently an increased

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surface retention and removed part of the organic material present.[24]

Espinosa *et al* [20] showed that removing the organic content from the enamel surface with 5.2% sodium hypochlorite (NaOCl) as a deproteinizing agent prior to phosphoric acid etching, doubles significantly enamel's retentive surface to 94.47% and increased the type I and II etched enamel. This technique could optimize significantly adhesion removing organic elements of both the enamel structure and acquired pellicle.

Thus, this *in vitro* study was undertaken to support the results of our preceding study[20] and evaluate the qualitative and quantitative resin tag penetration with a resin replica after NaOCl enamel deproteinization prior to 37% phosphoric acid (H_3PO_4).

MATERIALS AND METHODS

Ten human mandibular first and second permanent molars extracted for periodontal reasons were chosen, with the following exclusions: Teeth with enamel cracks or fractures along their buccal aspect, dental pathology, malformations, carious lesions, restorations or erosions. This study was conducted in accordance with the guidelines established by the Mexican Ministry of Health's Code of Bioethics for Dentists, in the Official Mexican Standard, and in the bioethics regulations enforced by the University of Guadalajara. Patients who agreed to participate in the study gave their written authorization.

After extraction, all samples were stored in saline solution at 37ºC. Each tooth was polished with pumice and rinsed with distilled water for 10 seconds. Roots were amputated with a low-speed double sided diamond disk (Shofu #S23-1164 Japan), under continuous water spray irrigation.

To obtain enamel samples comparable among themselves and with uniform physical and chemical characteristics, each crown was sectioned horizontally from mesial to distal along the mid coronal buccal aspect of the molar using the same disk. This section was then divided vertically into 3 comparable enamel blocks. Each of the 30 fragments was encoded for identification purposes and prepared to receive one of the following 3 treatments: ***Group A (Acid)***: The enamel surface was etched with 37% H3PO4 gel (3M ESPE Scotchbond etching gel, St Paul, MN) applied with a microbrush for 15 seconds, washed with sterile water and air spray for 20 seconds, then dried with oil free compressed air. Two separate adhesive layers of Single Bond 2 (3M ESPE, St Paul,MN) were applied and photo polymerized separately for 10 seconds.

A one millimeter layer of composite Filtek™ Z350 (3M ESPE, St Paul,MN) was applied over the polymerized adhesive agent and photopolymerized for 20 seconds. This was done to create a body and tag over the adhesive. ***Group B*** (***Sodium Hypochlorite + Acid):*** The enamel surface was treated with 5.25% NaOCl applied with sterile cotton pellet for 60 seconds, washed, then dried with sterile water for 10 seconds, and etched as for *Group **A***. Two separate adhesive layers of Single Bond 2 (3M ESPE, St Paul,MN) were applied and photo polymerized for 10 seconds. A one millimeter layer of composite Filtek™ Z350 (3M ESPE, St Paul,MN) was applied over the polymerized adhesive agent and photopolymerized for 20 seconds. ***Group C*** No treatment was done, but the adhesive and composite were applied over the untreated enamel following the same model used for *Groups **A** and **B***.

To obtain resin replicas, all samples were decalcified and deproteinized for a 48 hours period, submerging the samples in eight cycles with 10% chlorhydric acid, during 5 hours each and one hour with 5.25% Sodium Hypochlorite, until the enamel was completely dissolved.[25-32]

All samples were coated with gold electrodepositing, using a Sputtering Effacoater (Ernest Fullam 18930 N.Y.USA) and prepared for surface SEM analysis (JEOL JSM 5400LV, Japan). The observation zone for all samples was standardized at the middle upper section (2mm) of the tooth, between the apex and equator of the clinical crown. 20 microphotographs at 500x magnification were obtained from each resin replica specimen covering the entire treated sample surface. A total of 60 microphotographs for each molar were obtained in a consecutive order, generating a total of 600 images or 200 images *per* group for its analysis.

To maintain a standard between the samples (keeping in mind that each tooth was divided into 3 sections, which formed the 3 groups), each tooth was subjected to the two different treatments ensuring that this handling was applied to teeth with the same enamel quality. The images were subjected to a double-blind evaluation by 2 investigators, with a ($r = 0.78$ correlation). To obtain quantitative results, the samples were evaluated using Auto-CAD 2005 Software (Microsoft Corporation, Macrovision Corp.) to grade each of the images.

RESULTS

The total replica surface area of each image (μm^2) was determined, characterized and analyzed qualitatively and quantitatively.

Resin tag enamel penetration equivalent to type I-II etch patterns can be seen in tables 1-3 and Graph 1, 4. The area with resin tag equivalent to type III etched pattern and control samples were determined separately (Tables 1-5 and Graph 2-4).

Data shows that groups ***A*** and ***B*** presented the greater total resin tag surface equivalent to type I-II pattern. However, the furthermost pattern was found in *Group **B***. From a total surface of 7.52mm^2, 5.68 mm^2 (73%) produced a resin tag equivalent type I-II etched pattern, followed by *Group **A***, 3.47 mm^2 (46%), out of a total surface of 7.48mm^2. (Tables 1-5 and Graph 1-4)

Tables 1, 2, 4 and Graphs 2 and 4 show the data for resin tag equivalent type III pattern of total etched surface. From a total surface area o 7.52mm^2 ***Group A,*** displayed 3.3 mm^2 (45%). On the other hand, the same resin tag equivalent type III etching pattern was found in ***Group B,*** with 1.76mm^2 surface (26%) out of a total of 7.52 mm^2 (Tables 1, 2, 4 and Graphs 2, 4).

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Table 1. Descriptive statistics for Group A (H3PO4) according total area in microns and resin tag equivalent to type I and II, type III and control (no etched pattern).

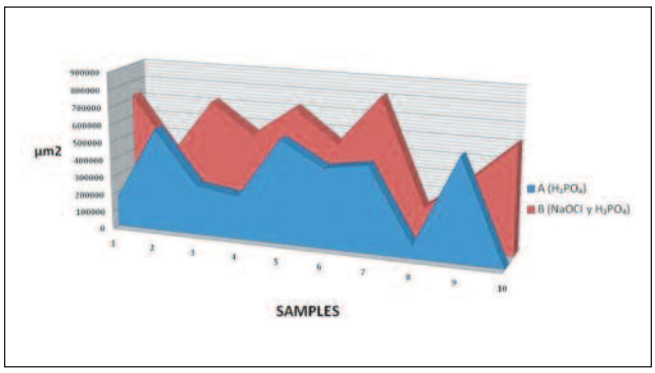
Resin tag equivalent	N	Min	Max	Average	Std Dev
Total area	10	652,320	994,280	747,950	90,470
type I & II Etch	10	0	588,736	347,304	2.224 30E5
type III Etch	10	155,266	579,108	330,026	1.472 68E5
None	10	0	389,562	71,595	1.294 54E5

Table 2. Descriptive statistics for Group B (NaOCl and (H_3PO_4) according total area in microns and resin tag equivalent to type I and II, type III and control (no etched pattern).

Resin tag equivalent	N	Min	Max	Average	Std Dev
Total area	10	645,220	987,560	752,506	89310
type I & II Etch	10	222,622	813,584	568,677	1.844 03E5
type III Etch	10	21,629	422,598	176,209	1.340 14E5
None	10	0	69,084	7,621	21647

Table 3. Descriptive statistics for ten samples according resin tag equivalent for type I and II Total etched surface patterns in microns for Group A (H_3PO_4) and Group B (NaOCl and H_3PO_4)

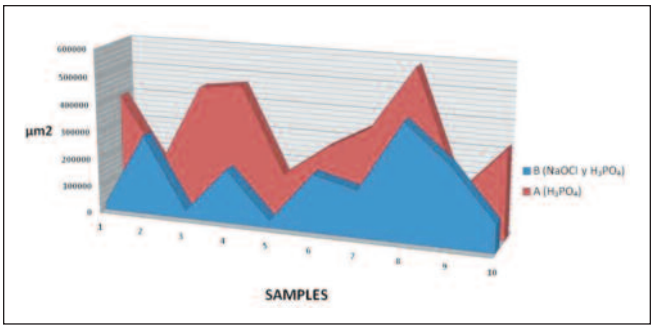
	Group-A	Group-B
1	165,968	718,351
2	584,994	400,138
3	284,201	714,039
4	247,973	547,674
5	588,736	716,852
6	453,143	535,215
7	489,824	813,584
8	73,212	222,622
9	584,994	400,138
10	0	618,153
	3,473,045	5,686,766



Graphic 1. Total surface resin tag equivalent type I and II etched patterns distribution in square microns for Group A (H_3PO_4) and Group B (NaOCl and H_3PO_4)

Table 4. Descriptive statistics for ten samples according resin tag equivalent for type III Total etched surface patterns in square microns for Group A (H_3PO_4) and Group B (NaOCl and H_3PO_4)

	Group-A	Group-B
1	165,968	718,351
2	584,994	400,138
3	284,201	714,039
4	247,973	547,674
5	588,736	716,852
6	453,143	535,215
7	489,824	813,584
8	73,212	222,622
9	584,994	400,138
10	0	618,153
	3,473,045	5,686,766



Graphic 2. Total surface resin tag equivalent type III etched patterns distribution in square microns for Group A (H_3PO_4) and Group B (NaOCl and H_3PO_4)

*Group **A**,* showed no etching in 9% (0.71mm²)of its surface while group B only showed a 1% (0.07 mm²) non etched surfaces (Table 5 and Graphic 3).

The results displayed significant differences for the *Groups **A*** and ***B*** in resin tag equivalent type etched area distribution Pearson's correlation test showing totally different tendencies. (Table 5)

Table 5. Pearson Correlation test.

Resin Replica tag Equivalent Groups	Group A I and II Etch	Group A III Etch	Group A No Etch
Group B I and II Etch	.083	-.089	.384
Group B III Etch	.051	.119	-.358
Group B No Etch	.367	-.418	-.144

No relationship between any of the groups

Table 6. Descriptive statistics for ten samples for none resin tag surface pattern in square microns for Group A (H_3PO_4) and Group B (NaOCl and H_3PO_4)

	Group-A	Group-B
1	165,364	0
2	0	69,084
3	9,748	0
4	0	0
5	0	0
6	0	0
7	151,273	3,968
8	0	0
9	0	0
10	389,562	3,156
	715,947	76,208

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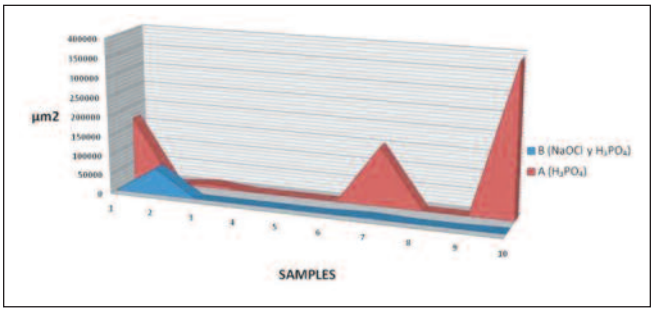
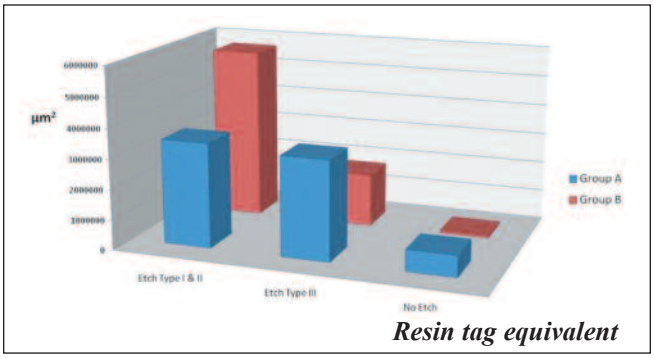


Graphic 3. Total surface for no resin tag patterns distribution in square microns for Group A (H_3PO_4) and Group B (NaOCl and H_3PO_4)



Graphic 4. Resin replica tag type equivalent patterns differences in square microns for Group A (H_3PO_4) and Group B (NaOCl and H_3PO_4)

Taking into account the 3 resin replica tags, equivalent types of etching patterns, one can notice a greater response from a type I and II etching patterns. However, this replica topographical analysis is found in *group B* with the greatest total surface (73%). (Graph 4)

DISCUSSION

It has been shown that proper enamel etching depends on the type and acid concentration, etching time, composition of the enamel surface and organic removal. Unfortunately after all these years we still face adhesive failures and do repetitive dentistry.[18,19, 21-22] We sometimes need to struggle with insurance companies, who pose a restriction of re-application prior 5 years of restoration´s initial placement.[30]

Two key factors encountered for adhesive failure reside in the quantity of the etched surface as well as in the quality of the etched pattern. Adhesion to enamel depends on achieving the maximum retentive capacity of the surface from the effect of acid etching. It is important to realize that the action of H3PO4 over the enamel surface occurs mostly on mineralized tissues (inorganic matter) and does not eliminate the organic matter.[11] The morphological changes generated vary from tooth to tooth with a prevalence of a type III etching pattern, which decreases significantly the ability of materials to bond effectively to enamel.[4-7]

Retentive morphology should be homogeneous over the entire treated surface.[12,18,19] Notwithstanding, the topographic quality of enamel etching with H3PO4 is not achieved over the entire adhesion surface.[4,5,7] Our previous study (Espinosa R *et al.)*[20] showed more than 50% of the treated surface was not etched; however, enamel deproteinization with 5.25% NaOCl for 60 seconds prior enamel etching with H3PO4 exhibited the best results, obtaining I and II etch patterns up to 94.47% of its surface compared to 49.3% of type III pattern produced by acid action alone. The etched surface areas from our previous study (94.4%) compared to 73% in this study could be from small differences in the enamel composition, laboratory procedures in the resin replica technique or even the SEM AutoCAD figure evaluation.

This resin replica technique supported our earlier study showing qualitative and quantitative assessment on the resin tags penetration. Enamel surfaces etched with phosphoric acid showed an irregular poor resin tag formation (Fig. 1). However, deproteinization significantly increased resin tags penetration in type I and II surfaces (Fig. 2). No tags were seen from controls (Fig.3). Deproteinization with 5.25% NaOCl for 60 seconds prior enamel etching increases significantly the quality and depth of the resin replica which could increase significantly the retention of all adhesives restorations. A recent study confirmed clinically our results, indicating that the deproteinization with 5.25% NaOCl for 60 seconds prior enamel etching increased significantly the retention of all adhesives restorations.[33] Hence, a new frontier opens in front of us and is ready to be tested.

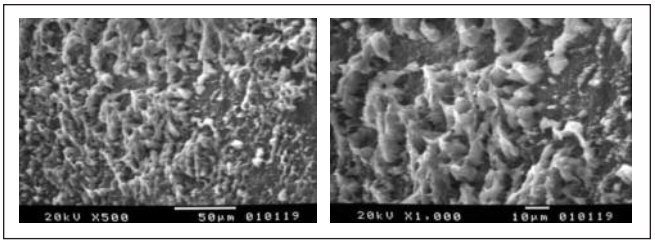


Figure 1. Group A: A(X500) B (X1000). Resin replica tags from the enamel surface etched with phosphoric acid for 15 seconds, showing poor resin tag penetration .

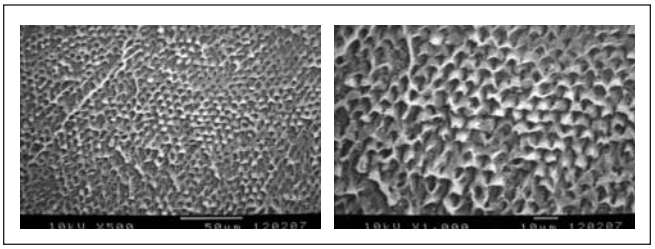


Figure 2. Group B: B(X500) B (X1000). Resin replica tags from the enamel surface deproteinized with 5.25% NaOCl for 30 seconds and etched with 37% H3PO4 for 15 seconds, showing good, organized resin tag penetration.

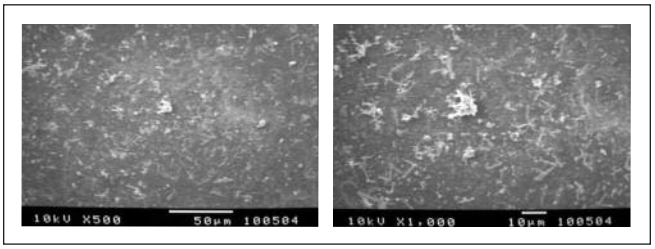


Figure 3. Control Group: A(X500) B (X1000). Resin replica from no etched enamel surface, showing no tag penetration over the entire surface v

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CONCLUSIONS

- Conventional H3PO4 enamel etching has significant limitations, etching less than 46% of the total enamel's surface.
- Enamel deproteinization prior to phosphoric acid etching almost doubles enamel's retentive surface to 73%.
- The topographical features of the replica resin penetration surface increases significantly with type I-II etching pattern, when deproteinization is done with 5.25% NaOCl for 1 minute prior phosphoric acid etching.

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