Enamel Deproteinization and Its Effect on Acid Etching: An in vitro Study

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Purpose: The goal of this in vitro study was to identify the topographical features of the enamel surface deproteinized and etched with phosphoric acid (H₃PO₄) compared to phosphoric acid alone. Materials and method: Ten extracted lower first and second permanent molars were polished with pumice and water, and then divided into 4 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 H₃PO₄ 37% for 15 seconds. Group AH1 : Sodium Hypochlorite (NaOCl) 5.25% for 30 seconds followed by Acid Etching with H₃PO₄ 37% for 15 seconds. Group AH2 : Sodium Hypochlorite (NaOCl) 5.25% for 60 seconds followed by Acid Etching with H₃PO₄ 37% for 15 seconds. Results showed that group AH2 etching technique reached an area of 76.6 mm² of the total surface, with a 71.8 mm² (94.47%), type 1 and 2 etching pattern, followed by group AH1 with 55.9 mm² out of 75.12 mm² (74.1%), and finally group A with only 36.8 mm² (48.83%) out of an area of 72.7 mm². A significant statistical difference (P <0.05) existed between all groups, leading to the conclusion that enamel deproteinization with 5.25% NaOCl for 1 minute before H₃PO₄, etching increases the enamel conditioning surface as well as the quality of the etching pattern.

Keywords: Enamel, deproteinization, sodium hypochlorite, phosphoric acid, etching, permanent teeth


INTRODUCTION

The cutting edge in dentistry at the end of the XX century came with the advent of esthetic, adhesive dental materials. This effect was discovered in 1955 by Buonocore, who demonstrated an increased adhesion of acrylic resins on enamel treated with 85% phosphoric acid (H₃PO₄). Further research was fundamental to the understanding and acceptance of enamel etching and the adhesion system in dentistry. The morphological changes produced in the enamel surface using a sweep electron microscope (SEM) was first reported by Gwinnett (1971) and Silverstone (1975), who identified the enamel micromorphology and classified enamel etching into 3 patterns. In the type 1 etching pattern, H₃PO₄ dissolves the head of the prism, with the peripheral material or interprismatic substance remaining intact. In type 2, the acid dilutes the peripheral zone of the prisms, leaving the prism head relatively intact. In type 3, the surface change has no specific features but displays generally some superficial dissolution that does not alter the deeper strata where the enamel prisms are located. These 3 etching patterns appear randomly at any point on the enamel and can be found together in the same enamel zone. Clinically, however one can only see a white, opaque surface, exhibiting the quantity but not quality of the affected surfaces. Silverstone, later showed that the most retentive etching patterns were types 1 and 2, because the porous surface offered retentive areas of greater size and depth. The type 3 etching pattern, which did not present a defined and deep morphology and lacked the micromechanical retention, offered by the previous two.

Etching quality depends on the etching agent, acid concentration, etching time, and composition of the enamel surface. Mechanical elements, such as air abrasion and laser have also been analyzed with no good results.

The common aim of all these investigations has been to improve the retentive properties of the enamel for the best possible adhesion.

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.\textsuperscript{12,18-19} However, recent studies have shown that the topographic quality of enamel etching with H\textsubscript{3}PO\textsubscript{4} is not achieved over the entire adhesion surface, that more than 69% of the treated surface had no etching whatsoever, 7% presented tenuous etching, and only 2% was ideally etched.\textsuperscript{20,21} These results are generally seen in the clinical environment where sealants, adhesive restorations as well as orthodontic brackets are failing.\textsuperscript{22-27}

To counteract these limitations some authors have suggested grinding or abrading the enamel in order to increase retention. This invasive technique offered apparently an increased surface retention and removed part of the organic material present.\textsuperscript{28}

On the other hand, a non invasive technique successfully employed in endodontics, utilizes sodium hypochlorite (NaOCl) as an irrigating solution to disinfect, remove debris, as well as organic materials from the canals.\textsuperscript{29,30}

Sodium hypochlorite (NaOCl), has an antibacterial effect and does not damage healthy tissue or tooth structure. Its mechanism of action has been shown by Solera and Silva-Herzog, 2006\textsuperscript{31} (Diagram 1).

- pH similar to calcium Hydroxide (CaOH\textsubscript{2}).
- NaOCl + HO\rightarrow Na OH (Sodium Hydroxide) + HClO (Hypochlorous acid). Na OH acts on fatty acids forming soap (saponification) which reduces surface tension. The Hipochlorous acid (HClO) etches and neutralizes aminoacids.
- The Chlorine (Cl) ion acts on cell metabolism inhibiting its enzymatic action.
- The Hydroxyl ion binds to Ca ions denaturalizing proteins formation of (CaOH\textsubscript{2}).

With all NaOCl advantages, an aspect not studied to date involves the effect of enamel surface deproteinization prior H\textsubscript{3}PO\textsubscript{4} etching. The use of 5.2% sodium hypochlorite (NaOCl) as a deproteinizing agent may be a possible strategy to optimize adhesion by removing organic elements of both the enamel structure and the acquired pellicle before acid etching. (Diagram 1).

The purpose of this \textit{in vitro} study was to identify the topographical enamel features of a deproteinized enamel with NaOCl prior of H\textsubscript{3}PO\textsubscript{4} etching.

**MATERIALS AND METHODS**

Ten human mandibular first and second permanent molars extracted for periodontal reasons were chosen, from patients ranging 44 to 60 years of age 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 37°C. Each tooth was polished with pumice and rinsed with distilled water for 10 seconds. Roots were amputated (diagram 2a) 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 (b) along the mid coronal buccal aspect of the molar using the same disk. This section was then divided vertically into 4 comparable 1 mm\textsuperscript{2} enamel blocks (c and d).

Each of the 40 fragments was encoded for identification purposes and prepared to receive one of the following 3 treatments:

\textbf{Group A }\textbf{(Acid):} The enamel surface was etched with 37% H\textsubscript{3}PO\textsubscript{4} 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.

Group AH1 (Acid + Sodium Hypochlorite + 30 seconds): The enamel surface was treated with 5.25% NaOCl applied with sterile cotton pellet for 30 seconds, washed, then dried with sterile water for 10 seconds, and etched as for Group A.

Group AH2 (Acid + Sodium Hypochlorite + 60 seconds): 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.

All samples were coated with gold electodepositing, using a Sputtering Efficocater (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 enamel specimen covering the entire treated sample surface. A total of 80 microphotographs for each molar were obtained in a consecutive order, generating a total of 800 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 4 sections, which formed the 3 groups), each tooth was subjected to the three 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 surface area of each image (µm²) was determined, defining them into type 1-2 patterns. The area with type 3 etching pattern was determined separately.

Tables 1 and 4 and Graph 1 show the data for the total etched surface displaying a type 1-2 pattern. The utmost pattern was found in group AH2. From a total surface of 76.6mm², 71.8 mm² (94.47%) produced a type 1-2 etched pattern, followed by group AH1, 55.9 mm² (74.1%), out of a total surface of 75.12 mm² and group A with only 36.8 mm² (48.83%) of an area of 72.7 mm².

Table 2 and Graph 2 shows the data for the total etched surface exhibiting a type 3 pattern. From a total surface area of 72.7 mm² group A displayed 35.8 mm² (49.3%). On the other hand, the same type 3 etching pattern was found in group AH2 with 4 mm² surface (5.2%) out of a total of 76.6 mm² (Graph 2).

Table AH2 produced the greatest etched surface, followed by group AH1.

Even if the different groups displayed some similarity in
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the type an etched area distribution Pearson’s correlation test showed no correlation between groups (Tables 1-3).

Table 3. Pearson Correlation Test

<table>
<thead>
<tr>
<th>Group</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>0.138</td>
<td>0.190</td>
</tr>
<tr>
<td>AH1</td>
<td>0.138</td>
<td>1</td>
<td>0.620</td>
</tr>
<tr>
<td>AH2</td>
<td>0.190</td>
<td>0.620</td>
<td>1</td>
</tr>
</tbody>
</table>

No relationship between any of the groups

Table 4. Percentage Distribution of Type 1-2 Etching in the Different Groups and Their Correlation with the Different Groups for Each Sample

<table>
<thead>
<tr>
<th>Sample</th>
<th>Group A</th>
<th>Group AH1</th>
<th>Group AH2</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>32.69*</td>
<td>95.03*</td>
<td>98.60*</td>
</tr>
<tr>
<td>02</td>
<td>43.67*</td>
<td>68.40*</td>
<td>93.98*</td>
</tr>
<tr>
<td>03</td>
<td>33.49*</td>
<td>84.38*</td>
<td>92.75*</td>
</tr>
<tr>
<td>04</td>
<td>57.24*</td>
<td>81.86*</td>
<td>80.46*</td>
</tr>
<tr>
<td>05</td>
<td>56.99*</td>
<td>80*</td>
<td>98.53*</td>
</tr>
<tr>
<td>06</td>
<td>11.51*</td>
<td>50.31*</td>
<td>96.44*</td>
</tr>
<tr>
<td>07</td>
<td>55.45*</td>
<td>77.52*</td>
<td>97.75*</td>
</tr>
<tr>
<td>08</td>
<td>84.67*</td>
<td>67.74*</td>
<td>94.92*</td>
</tr>
<tr>
<td>09</td>
<td>41.41*</td>
<td>65.57*</td>
<td>96.44*</td>
</tr>
<tr>
<td>10</td>
<td>71.19*</td>
<td>70.17*</td>
<td>94.80*</td>
</tr>
<tr>
<td>Average</td>
<td>48.83**</td>
<td>74.10**</td>
<td>94.47**</td>
</tr>
</tbody>
</table>

(*n = 20; **n = 200) p< 0.05 between groups

Graph 3.

DISCUSSION

It has been shown that proper enamel etching depends on the type and acid concentration, etching time, and composition of the enamel surface. What is proper? When after all these years we still discuss how long we should etch primary and permanent teeth. What is proper? If after all these years, with all the different techniques and materials we still face the burden of adhesive failures requiring to redo some of our earlier work. Even some insurance companies pose a restriction of re-application prior to 5 years of restoration’s initial placement.

Two key factors encountered for adhesive failure reside in the quantity of the etched surface as well as in the quality of the etching pattern.

Adhesion to enamel depends on achieving the maximum retentive capacity of the surface from the effect of acid etching. This retentive morphology should be homogeneous over the entire treated surface. Notwithstanding, the topographic quality of enamel etching with H₃PO₄ is not achieved over the entire adhesion surface. Our study showed more than 50% of the treated surface was not etched. This result is in agreement with the work of Hobson, where he found more than 69% of intact surface (Graph 3, Table 4).

Polishing the enamel surface is intended to eliminate the organic components that hinder effective enamel etching. Why some of the organic material is not removed by cleaning and acid etching is still difficult to explain. However, it is highly likely that, despite our best efforts, the organic layer cannot be entirely removed without considering the proteins immersed in the crystals forming the enamel (Figures 1 and 2).

It is important to realize that the action of H₃PO₄ on the enamel surface occurs mostly on mineralized tissues (inorganic matter). The morphological changes generated vary from tooth to tooth with a prevalence of a type 3 etching pattern, which decreases significantly the ability of materials to bond effectively to enamel.

Unfortunately, this acid does not eliminate the organic matter. Proof of this is the “collagen network” resulting from demineralization of dentin by H₃PO₄ where the collagen fibers are left intact.

This study showed that enamel deproteinization with 5.25% NaOCl for 60 seconds prior etching with H₃PO₄ exhibited the best results. Table 1 showed a 94.47% type 1-2 pattern (Table 1, Graph 1), compared to 49.3% of type 3 pattern produced by the action of H₃PO₄ (Table 2, Graph 2). [Figure 3 C–D].

Also, enamel deproteinization with Sodium Hypochlorite for 60 seconds doubled the type 1-2 etched surface from 48.8% to 94.47% (Table 4). In this sense increasing the type 1-2 etched surface could only increase significantly the retention of all adhesives restorations.

In terms of the time needed to achieve the best results after enamel deproteinization with 5.25% for 30 or 60 seconds, Tables 1, 2 and 4 show a significant difference regarding the total surface (Table 3) etched as well as the quality of
the etched surface. Enamel deproteinization for 30 seconds increased significantly compared with traditional etching but was not as effective as 60 seconds (Figure 3).

The type 2-3 etched pattern increases from 48.8% seen with phosphoric acid to 74.1% after enamel deproteinization with 5.25% NaOCl for 30 seconds to 94.4% with enamel

Figure 1. A. SEM x1000 microphotograph of the enamel surface polished with pumice and distilled water. One can see an organic pellicle (dark color) all over the enamel surface that could not be removed with polishing and pumice. B. SEM x1000 microphotograph from a different area of the same sample, been treated with pumice and distilled water and only deproteinized with 5.25% NaOCl for 60 seconds. A clean protein free surface and prism configuration can be seen.

Figure 2. A. Wear section, obtained with x200 light microscope. Labial surface of a healthy enamel. Observe the difference in proteic content in the enamel surface (dark color) between Retzius striae; B. SEM x150 microphotograph of a sample etched with 37% H₃PO₄ for 15 seconds. Observe the difference in proteic content (dark lines) of enamel surface between Retzius stria; C. Close up of Figure B, x500, etching has not occurred in grooves because of accumulation of proteins in those areas.
Figure 3. Sample 6, group A: A (X500) B (X1000). Enamel surface etched with phosphoric acid for 15 seconds, showing poor retention of entire surface (type 3 etching over 60% of its surface).

Figure 4. Sample 6, group AH1: A (X500) B (X1000). Enamel surface deproteinized with 5.25% NaOCl for 30 seconds and etched with 37% H₃PO₄ for 15 seconds, increasing retention of the enamel surface. Compare them with traditional etching (Figure 3).

Figure 5. A (X500) B (X1000). Sample 6, group AH2. Enamel surface deproteinized with 5.25% NaOCl for 1 minute and etched with 37% H₃PO₄ for 15 seconds. Retentive features of entire surface are achieved over the entire surface of the sample, increasing type 1-2 etched surface.
deproteinization with 5.25% NaOCl for 60 seconds (Figure 3).

Some possible concerns of NaOCl are the taste, tolerance by young children and possible soft tissue reactions. NaOCl does not react with soft tissues, has a chlorinated odor and has no taste.

In summary, the clinical observation of an etched surface as whitish, chalkish, dryish demineralized surface prior deproteinization with NaOCl could now guarantee the quality and retention of all adhesives materials. Hence, a new frontier opens in front of us and is ready to be tested.

CONCLUSIONS

- Conventional H3PO4 enamel etching has significant limitations, etching less than 50% of the total enamel’s surface.
- Enamel deproteinization with prior to phosphoric acid etching doubles enamel’s retentive surface to 94.47%.
- The topographical features of the etched enamel surface increases significantly the type 1-2 etching pattern when deproteinization with 5.25% NaOCl for 1 minute is used prior phosphoric acid etching.

REFERENCES

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