Effect of Enamel Deproteinization in Primary Teeth

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Objective: *To evaluate and compare the topographical features of enamel surface, etched with different materials.* ***Study Design:*** *10 extracted human primary molars were randomly selected and cut and trimmed to 1 mm^2. Each group comprised of 10 blocks and the enamel was treated as follows:* ***Group I–****35% H_3PO_4;* ***Group II–****5.25% NaOCl + 35% H_3PO_4 ;****Group III–****5.25% NaOCl;* ***Group IV*** *no treatment was carried out. All the samples were prepared for Scanning electron microscope analysis. The images were obtained and evaluated for the quality type I-II etching of the enamel surface using Auto-CAD 2011 software.* ***Statistical Analysis Used:*** *Wilcoxon Signed Ranks Test ($p<0.001$).* ***Results:*** *The mean surface area of type I and II etching pattern values for Group- I was 39608.18 μm^2 and Group- II was 45051.34 μm^2.* ***Conclusion:*** *Deproteinization with 5.25% Sodium hypochlorite prior to acid etching could be used to increase the surface area of adhesion of composite material with the tooth surface.*

Key words: *Enamel, Deproteinization, Etching.*

INTRODUCTION

Anterior composite restorations are appropriate in a growing child as definite restorations are contraindicated until eruption of the clinical crown is complete. This may solve the problem of sensitivity and esthetics.[1]

Inspired by the industrial use of 85% phosphoric acid to facilitate adhesion of paints and resins to metallic surfaces, Buonocore envisioned the use of acids to etch enamel for sealing pits and fissures. Acid etching transforms the smooth enamel surface into an irregular surface and increases its free energy. When a fluid resin-based material is applied to the irregular etched surface, the resin penetrates into the surface, aided by capillary action. Monomers in the material polymerize, and the material becomes interlocked with the enamel surface. The formation of resin microtags within the enamel surface is the fundamental mechanism of resin-enamel adhesion. The acid-etch technique has changed the practice of restorative dentistry significantly.

Enamel etching results in three different micro morphologic patterns. The Type I pattern involves the dissolution of prism cores without dissolution of prism peripheries. The Type II etching pattern is the opposite of type I: The peripheral enamel is dissolved, but the cores are left intact. Type III etching is less distinct than the other two patterns. It includes areas that resemble the other patterns and areas whose topography is not related to enamel prism morphology.

Beginning with Buonocore's use of 85% phosphoric acid (H_3PO_4), various concentrations of phosphoric acid have been used to etch enamel. Gwinnett and Buonocore suggested the use of lower acid concentrations to prevent the formation of precipitates that could interfere with adhesion. Application of 50% phosphoric acid for 60 seconds result in formation of a monocalcium phosphate monohydrate precipitate that can be rinsed off. Concentrations less than 27% may create a dicalcium phosphate monohydrate precipitate, however which cannot be removed easily and consequently may interfere with adhesion. Silverstone *et al* found that the application of 30% to 40% phosphoric acid resulted in retentive enamel surfaces. Concentration greater than 40% seem to dissolve less calcium and result in etch patterns with poorer definition than when lesser than 40% are used. Consequently, most current phosphoric gels have concentration of 30% to 40%, although some studies using lower concentrations have reported similar to adhesive values.

An etching time of 60 seconds originally was recommended for permanent enamel using 30% to 40% phosphoric acid. Although

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one study concluded that shorter etch times resulted in lower bond strengths, other studies using scanning electron microscopy showed that a 15- second etch resulted in a similar roughness as that provided by a 60 second etch. Other *in vitro* studies have shown similar bond strengths and microleakage for etching times of 15 and 60 seconds. Clinically, reduced etching times do not seem to diminish the retention of pit-and-fissure sealants.[2]

Enamel consists of approximately 96% inorganic material, constituting biological hydroxyapatite crystals. Remnants of proteins from the period of development and water are also found in the enamel. The remaining components, by weight, are organic matter and water.

Enamel has an inert cellular tissue and a thickness of approximately 1- 2mm in permanent teeth and 0.5-1mm in primary teeth**.** The outer surface zone of enamel appears as a structure of prismless tissue, the aprismatic layer, in morphological analyses. The aprismatic layer is more frequently seen in primary teeth, where the width of the zone is larger compared to permanent teeth.[3]

The relationship between human saliva and dental enamel is a complex one, including that of the formation of the acquired enamel pellicle (AEP). AEP is a thin layer that is formed predominantly from salivary proteins and their products by selective adsorption onto the enamel surface. AEP consists predominantly of salivary proteins and peptides, but also includes non-salivary derived proteins, carbohydrates and lipids. This creates a protective interface between the tooth surface and the oral environment, and acts as a selectively permeable barrier that regulates the demineralization and remineralization processes of enamel. Furthermore, AEP influences the composition of the micro flora that inhabits the tooth surface.

There are also differences in amino acid composition between the AEPs of permanent and deciduous teeth. Although a generally similar pattern in the amount of the amino acids has been seen, the amino acids glycine, serine, and tyrosine were reported in statistically significantly different quantities in the two types of AEP. This suggests that the pellicles may have different protein compositions.[4]

Elimination of organic substances from the enamel surface before acid etching increases the resistance to orthodontic traction by providing a better acid etching pattern on enamel.[5]

Sodium hypochlorite (NaOCl) is known to be an excellent protein denaturant.[1] NaOCl is a non-specific proteolytic agent that effectively removes organic compounds at room temperature.[6]

The topographic quality of enamel etching with H_3PO_4 was not achieved over the entire adhesion surface. The NaOCl as a deproteinizing agent might be a possible strategy to optimize adhesion by removing organic elements of both the enamel structure and the acquired pellicle before acid etching.[7] Because of these reasons the present study was designed.

MATERIALS AND METHOD

This study was carried out in the Department of Pedodontics and Preventive Dentistry, Rajah Muthiah Dental College and Hospital, Annamalai University, Chidambaram, Tamil Nadu, India.

Ten human primary molars were extracted due to pain in pre shedding mobility from the patients attending the Department of Pedodontics and Preventive Dentistry. The teeth with enamel cracks or fractures along their buccal aspect, malformations, carious lesions, restorations or erosions were excluded.

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. To obtain enamel samples comparable among themselves and with uniform physical and chemical characteristics, the buccal surface of the crowns were marked with horizontal line at the middle third, then three vertical lines were marked equidistant to each other and were cut with high speed double sided diamond disk and trimmed to 1 mm^2. Thus 40 enamel blocks of 1mm^2 were obtained. The methodology was similar to that stated by Espinosa R. *et al***.**[7]

Each enamel block was encoded for identification purpose and was treated as per the following protocol.

Group I comprised of 10 blocks. The enamel surface was etched with 35% H_3PO_4 gel for 15 seconds, washed with sterile water and air sprayed for 10 seconds, then dried with oil free compressed air.[8]

Group II comprised of 10 blocks. The enamel surface was first treated with 5.25% NaOCl applied with sterile cotton pellet for 60 seconds, washed with sterile water and air sprayed for 10 seconds, then dried with oil free compressed air and then etched with 35% H_3PO_4 gel for 15 seconds, washed with sterile water and air sprayed for 10 seconds, then dried with oil free compressed air.[7]

Group III comprised of 10 blocks. The enamel surface was first treated with 5.25% NaOCl applied with sterile cotton pellet for 60 seconds, washed with sterile water and air sprayed for 10 seconds, then dried with oil free compressed air.[7]

Group IV comprised of 10 enamel blocks in which no treatment was carried out. (control group)

All the samples were prepared for Scanning Electron Microscope (SEM) analysis. The samples were coated with gold electrodepositing, using a Sputtering Effacoater (JEOL JFC-1600 AUTO FINE COATER) and prepared for surface SEM analysis using Scanning Electron Microscope (JEOL JSM 5610LV, Japan).

The observation zone for all the samples was standardized at the middle upper section of the tooth between the apex and the equator of the clinical crown. 5 microphotographs at 500x magnification were obtained from each enamel specimen. A total of 20 microphotographs for each molar were obtained in a consecutive order generating a total of 200 images or 50 images per group for its analysis.

To maintain uniform standard between samples (as each tooth was divided into 4 sections, which formed 3 treatment groups and one control group), each tooth was subjected to 3 different treatments ensuring that this handling was applied to teeth with the same enamel quality.

The images were obtained and evaluated for the quality type I-II etching of the enamel surface using Auto-CAD 2011 software.[7]

The data obtained were subjected to statistical analysis using Wilcoxon Signed Ranks Test.

RESULTS

The surface area of type I and type II etching patterns were determined of each image.

Tables 1 and 2 show the data for the total etched surface area displaying a type 1-2 pattern. The utmost pattern was found in **Group II** that is 45051.34μm^2.

Table 3 and graph 1 shows the mean and standard deviation values for surface area using Wilcoxon signed ranks test.

Table 1: Distribution of surface area of type I and II etching patterns after treating with 35% H_3PO_4.

S. No.	Surface area μm²	Frequency	Percentage%
1	37,000-37,999	9	18
2	38,000-38,999	9	18
3	39,000-39,999	10	20
4	40,000-40,999	11	22
5	41,000-41,999	10	20
6	42,000-42,999	1	2
Total		50	100

Table 1 showing the distribution of type I and II etching pattern treated with 35% H_3PO_4. Most of the samples with the value were between 37,000 μm² – 42, 999μm².

Table 2: Distribution of surface area of type I and II etching patterns after treating with 5.25% NaOCl and 35% H_3PO_4.

S No	Surface area μm²	Frequency	Percentage%
1	44,000-44,999	18	36
2	45,000-45,212	32	64
	Total	50	100

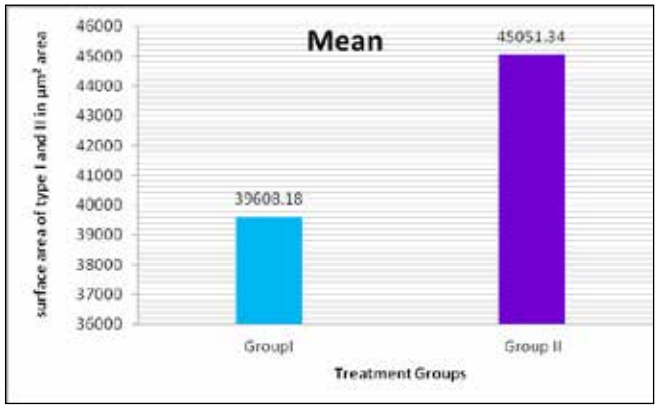
Table 2 showing the distribution of type I and II etching pattern treated with 5.25% NaOCl and 35% H_3PO_4. Most of the samples with the value were between 44,000 μm² – 45, 212μm².

Table 3: Mean and Standard deviation values for surface area using Wilcoxon signed ranks test

Variance	Mean μm²	SD	Wilcoxon signed ranks test	p-value
Group 1	39608.18	1484.723	6.154	< 0.001
Group II	45051.34	229.451		

Table 3 showing the mean surface area of type I and II etching pattern values for **Group- I, Group- II** which were 39608.18 μm² and 45051.34 μm², respectively. **Group –II** (5.25% NaOCl and 35% H_3PO_4) showed maximum type I and type II etched surface followed by **Group – I** (35% H_3PO_4).

Graph 1- Mean values for surface area using Wilcoxon signed ranks test



The Wilcoxon signed test had been applied to compare the two mean values. The surface area of type I and type II etching patterns were compared; p value < 0.001 was obtained which indicated statistically significant difference between tested materials. Hence it proved that the **Group II** has higher value that is 99.63%.

Group- III (35% NaOCl) and **Group IV** (no treatment) were not included for comparison as the results were zero at this level.

DISCUSSION

Composite resin is the most esthetic restorative material currently available for restoring anterior teeth. A review of the durability of the restorations in primary molars demonstrated a very high failure rate (62%) of composite resin restorations when clinically assessed over a period of 6 years. The most frequently recorded failures associated with all resin restorations was the presence of secondary caries around the margins, fractured restorations and loss of fillings.[9]

In general, the bond strength of the primary teeth was lower than that of the permanent teeth. The primary teeth have different micromechanical and histological characteristics from permanent teeth. The differences in the amount of mineral components, morphology and structure between primary and permanent were thought to be responsible for such low bond strengths.[10]

The technique of acid etching was introduced with the purpose of creating micro porosities on the surface of the enamel, thus increasing the adherence of composites to that surface.[11]

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.[7]

Etching involved selective removal of component(s) from a solid surface.[12]

Some authors reported that in enamel adhesion of resin composite, the Type-3 patterns showed high rates of failure due to lower bond strengths, but Type-1 and Type-2 patterns showed no significant difference in failure rate.[13]

Retentive morphology should be homogeneous over the entire treated surface.[7]

The etching time of 15 seconds, using 35% Phosphoric acid, was advocated by Costa LRRS. *et al* (1998).[8]

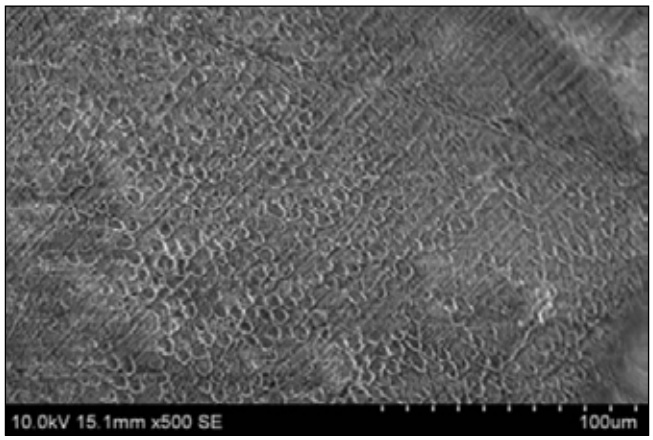
Silverstone showed that the most retentive etching patterns were types 1 and 2, because the porous surface offered retentive areas of greater size and depth. The topographic quality of enamel etching with H_3PO_4 was not achieved over the entire adhesion surface. The NaOCl as a deproteinizing agent might be a possible strategy to optimize adhesion by removing organic elements of both the enamel structure and the acquired pellicle before acid etching. 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.

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.[7]

Sodium hypochlorite (NaOCl) is known to be an excellent protein denaturant.[1]

The **Group I** specimens were etched with 35% H_3PO_4 gel for 15 seconds, washed with sterile water and air spray for 10 seconds, then dried with oil free compressed air. The present study showed that the mean surface area of type I and II etching pattern obtained by this group was 39608µm^2 which was 87.59%. Polishing the enamel surface was intended to eliminate the organic components that hinder effective enamel etching. Why some of the organic material was not removed by cleaning and acid etching was still difficult to explain. It was important to realize that the action of H_3PO_4 on the enamel surface occurs mostly on mineralized tissues (inorganic matter). Unfortunately, this acid did not eliminate the organic matter. Proof of this was the "collagen network" resulting from demineralization of dentin by H_3PO_4 where the collagen fibers were left intact. In a study by Espinosa R *et al* the surface area with type 1 and 2 etching pattern was determined as 48.3%.[7] Ahuja B *et al* in their study found that the surface area with type 1 and 2 etching pattern was 55.76%.[14]

Figure 1: SEM picture of tooth sample treated with Group I

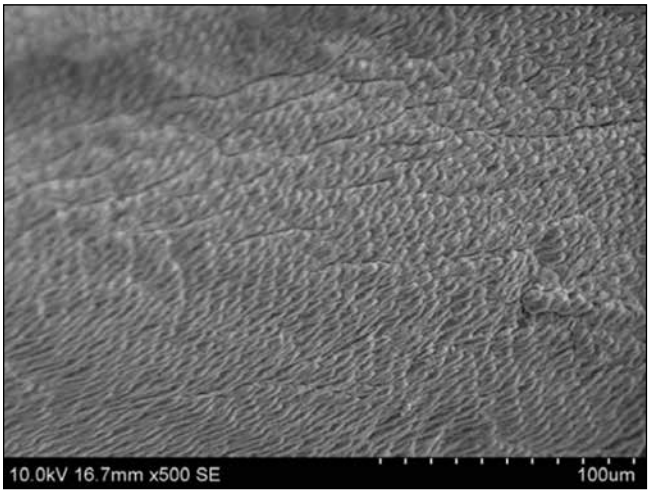


The **Group II** specimens were first treated with 5.25% NaOCl applied with sterile cotton pellet for 60 seconds. Washed with sterile water and air spray for 10 seconds, then dried with oil free compressed air and then etched with 35% H_3PO_4 gel for 15 seconds, washed with sterile water and air spray for 10 seconds, then dried with oil free compressed air.[7] In the present study the mean surface area of type I and II etching pattern obtained by **Group II** was 45051µm^2 which was 99.63% and was comparable to the results obtained by Espinosa R *et al* 94.47%.[7] This might be due to the following reasons. The organic components hinder effective enamel etching. The action of H_3PO_4 on the enamel surface occurs mostly on mineralized tissues (inorganic matter) and the collagen fibers are left intact.[7] But Sodium hypochlorite (NaOCl) is a protein denaturant[13] by the following mechanism shown by Solera and Silva-Herzog 2006[7] and Mohammadi Z.[15]

- pH similar to calcium Hydroxide ($CaOH_2$).
- NaOCl + H_2O (water) →NaOH (Sodium Hydroxide) + HOCl (Hypochlorous acid). NaOH acts on fatty acids forming soap (saponification) which reduces surface tension. The Hypochlorous acid (HClO) etches and neutralizes aminoacids.
- The Chlorine (Cl) ion acts on cell metabolism inhibiting its enzymatic action.
- The Hydroxyl ion (OH^-) binds to Calcium (Ca) ions denaturalizing proteins formation of ($CaOH_2$).[7]

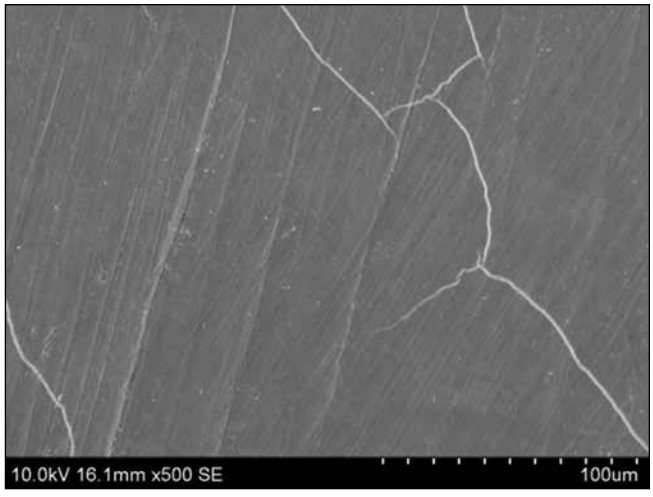
Ahuja B *et al* in their study found that the surface area with type 1 and 2 etching pattern was 53.58%. The results might be different because of the fact that the number of images has been curtailed to 150 and the total surface area of type I-II etching pattern was not calculated for all the specimens as per their study. [14]

Figure 2: SEM picture of tooth sample treated with Group II



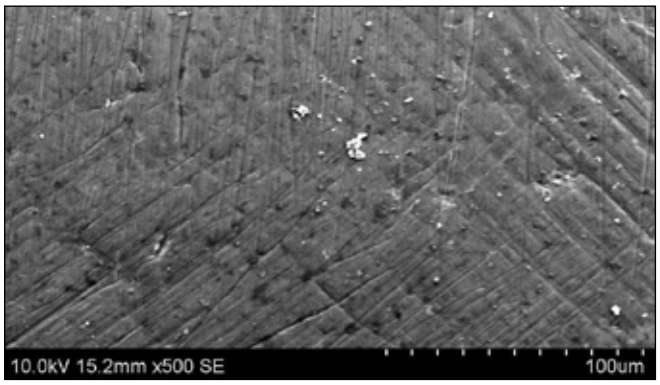
The **Group III** comprised of 10 specimens treated with 5.25% NaOCl applied with sterile cotton pellet for 60 seconds, washed with sterile water and air spray for 10 seconds, then dried with oil free compressed air.[7] The results of the present study showed that the mean surface area of type I and II etching pattern obtained by this group was 0µm^2 but a clean protein free surface can be seen because Sodium hypochlorite (NaOCl) is only protein denaturant.[1] and phosphoric acid etched hard tough tissues by selectively demineralization to expose patterns.[16]

Figure 3: SEM picture of tooth sample treated with Group III



The **Group IV** specimens comprised of 10 enamel blocks which were included in the control group in which no treatment was carried out.

Figure 4: SEM picture of tooth sample without any treatment Group IV (control group)



5% NaOCl enhanced enamel bonding in Hypocalcified Amelogenesis Imperfecta by removing excess protein that interfered with establishing a clinically successful acid etch pattern.[1]

Espinosa R *et al* showed that removing the organic content from the enamel surface with 5.25% sodium hypochlorite 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.[7]

In the present study, **Group –II** (5.25% NaOCl and 35% H_3PO_4) showed maximum type I and type II etched surface of 45051.34 μm^2 followed by **Group – I** (35% H_3PO_4) 39608.34 μm^2 which was significant ($p<0.001$).

Espinosa R. *et al* in their study stated that when the enamel was deproteinized with 5.25% NaOCl for 1 minute prior H_3PO_4, the surface and topographical features of the replica resin penetration surface increases significantly.[17]

Justus R *et al* in their study concluded that significantly greater shear bond strength could be obtained with Fuji ortho LC if the enamel surface was wetted for 1 minute with 5.25% NaOCl, before etching and applying 5.25% NaOCl to the enamel surface eliminated the organic elements. This effect allowed the acid etchant to penetrate more effectively into the enamel, creating type 1 and 2 etching patterns.[18]

Ahuja B *et al* stated that enamel deproteinization by 5.25% NaOCl did not grossly alter the surface topographic features of enamel before acid etching in their study.[14]

Narula H *et al* in their study concluded that there was no significant effect of sodium hypochlorite enamel deproteinization by 5.25% NaOCl on shear bond strength of composite restoration.[16]

Some possible concerns of NaOCl were the taste, tolerance by young children and possible soft tissue reactions. NaOCl has a chlorinated odor and has no taste.[7]

CONCLUSION

Thus, within the limitations of the present study, it could be concluded that deproteinization with 5.25% Sodium hypochlorite prior to acid etching could be used to increase the surface area of adhesion of composite material with the tooth surface.

More laboratory and clinical studies are necessary before unlimited clinical use of this alternative technique.

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