Enamel Deproteinization before Acid Etching – A Scanning Electron Microscopic Observation

Bhoomika Ahuja * / Ramakrishna Yeluri ** / Sudhindra Baliga M *** / AK Munshi ****

Objectives: *This study was undertaken to evaluate the topographical features of enamel surface deproteinized with sodium hypochlorite (NaOCl) and etched with phosphoric acid (H_3PO_4) compared to phosphoric acid alone using Scanning Electron Microscopic (SEM) Analysis.* ***Study Design:*** *30 enamel blocks of 1mm^2 from ten human sound extracted permanent molars were obtained and treated as under:* ***Group 1*** *(10 blocks): Enamel surface was etched with 37% H_3PO_4 gel for 15 seconds.* ***Group 2*** *(10 blocks): Enamel surface was treated with 5.25% NaOCl for 60 seconds and then etched with 37% H_3PO_4 gel for 15 seconds. 10 enamel blocks were included in the control group where no treatment was carried out. The samples were subjected to SEM analysis and 5 microphotographs of each sample were obtained at 500X magnification and evaluated for the quality of etching pattern of the enamel in percentage (%) using Auto-CAD 2007 software.* ***Results:*** *Mean values of etching pattern in Group 1 being 55.76% and Group 2 being 53.58%. No significant difference was observed between the two groups.* ***Conclusion:*** *The use of 37% phosphoric acid for 15 seconds still remains the best method for pretreatment of enamel.*

Keywords: *Enamel deproteinization, Acid etching, Sodium hypochlorite, Phosphoric acid*
J Clin Pediatr Dent 35(2): 169–172, 2010

INTRODUCTION

The aim of the etching of enamel is to improve the retentive properties of the enamel for the best possible adhesion of the restorative material. The acid-etch technique involves discrete etching of enamel in order to remove contaminants, increase the surface energy of enamel and create selective dissolution of prism cores or peripheries with resultant micro-porosity into which resin can flow and can be polymerized to form a mechanical bond with enamel.[1] It has been firmly established that the essence of adhesion lies in the best acid etching, with a generalized retentive morphological condition over the enamel surface.[2] The quality of etching depends on the etching agent, acid concentration, etching time and composition of the enamel surface.[3] However, recent studies have shown that the topographic quality of enamel etching with phosphoric acid (H_3PO_4) is not achieved over the entire adhesion surface, that more than 69% of the treated surface had no etching whatsoever, while 7% presented tenuous etching and only 2% was ideally etched.[4,5] Thus to counteract these limitations, various invasive and non-invasive techniques such as grinding or abrading enamel, air abrasion and laser, were used but no good results were obtained.

Since sodium hypochlorite (NaOCl) has been used to remove the organic smear layer from the root canal space, its role in removing the organic content from the enamel surface may prove to be substantial, thus the use of 5.25% 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.[6] Thus this study was undertaken to evaluate the effect of NaOCl enamel deproteinization before acid etching using Scanning Electron Microscope (SEM) Analysis.

MATERIALS AND METHOD

This study was partially carried out in the Department of Pedodontics and Preventive Dentistry, K.D. Dental College and Hospital, Mathura, India and Ethical committee clearance was obtained before the beginning of this study. The SEM Analysis was carried out at Sophisticated Analytical

* Bhoomika Ahuja, BDS, Post graduate student, Department of Pedodontics and Preventive Dentistry, K.D. Dental College and Hospital, Uttar Pradesh, India.
** Ramakrishna Yeluri, MDS, Associate Professor, Department of Pedodontics and Preventive Dentistry, K.D. Dental College and Hospital, Uttar Pradesh, India.
*** Sudhindra Baliga M, MDS, Associate Professor, Department of Pedodontics and Preventive Dentistry, K.D. Dental College and Hospital, Uttar Pradesh, India.
**** AK Munshi, MDS, Dean, Director and Principal, Senior Professor and Head, Department of Pedodontics and Preventive Dentistry, K.D. Dental College and Hospital, Uttar Pradesh, India.

Send all correspondence to: Dr. Ramakrishna Yeluri, Associate Professor, Department of Pedodontics and Preventive Dentistry, K.D. Dental College and Hospital, Mathura-281001, Uttar Pradesh, India.

Phone:- +919997951558
Fax+915652825050

E mail: drramakrishnay@indiatimes.com
kittypedo@yahoo.com

Instrument Facility for Electron Microscopy, Department of Anatomy, All India Institute of Medical Sciences (AIIMS), New Delhi, India.

The methodology used was similar to as suggested by Espinosa *et al*.[6] Ten human sound permanent molars extracted for periodontal reasons were chosen from the patients attending the Department of Oral and Maxillofacial Surgery. 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 37ºC. 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 (Shofu, Japan) and trimmed to 1 mm^2. Thus 30 enamel blocks of 1 mm^2 were obtained.

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

Group 1: This group comprised of 10 blocks. The enamel surface was etched with 37% H_3PO_4 gel (Dentsply Int., India) for 15 seconds, washed with sterile water and air spray for 10 seconds, then dried with oil free compressed air.

Group 2: This group also included 10 blocks. The enamel surface was treated with 5.25% NaOCl (Prime Dent, India) 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 as per the protocol followed for Group 1.

10 enamel blocks were included in the control group in which no treatment was carried out. To maintain a uniform standard between the samples (as each tooth was divided into three sections, which formed two treatment groups and one control group), each tooth was subjected to two different treatments ensuring that this handling was applied to teeth with the same enamel quality. All samples were then placed on stubs for gold sputtering and were coated with gold electrodepositing, using a Sputtering Effacoater (Agar Sputter Coater) and prepared for surface SEM analysis (LEO 435VP, Variable Pressure Scanning Electron Microscope, Zeiss, Leica). The observation zone for all the samples were randomly selected at 5 different sites for each sample. Five microphotographs at 500X magnification were obtained from each enamel specimen covering the entire treated sample surface. A total of 150 images or 50 images *per* group (Figures 1 to 3) were obtained and evaluated for the quality of Type I-II etching of the enamel surface using Auto-CAD 2007 software (Microsoft Corporation, Macrovision Corp). All the evaluations were tabulated as percentage of etching for different groups.

The data thus obtained were subjected to statistical analysis which was performed using SPSS (Statistical package for Social Sciences) version 12 for Windows to find the extent of relation between the groups with Pearson's co-relation

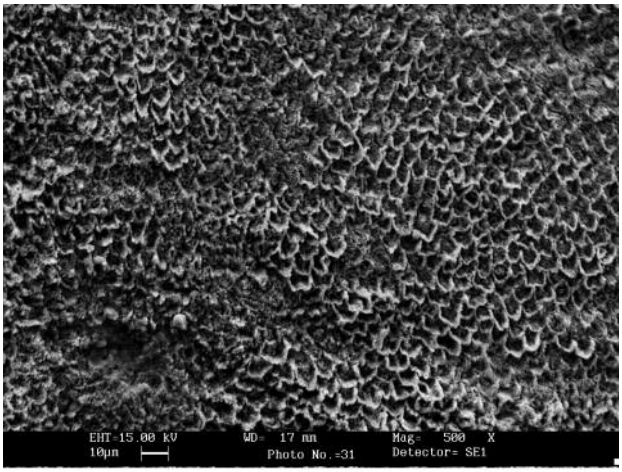

Figure 1. SEM Microphotograph of Group 1(mag. 500X). No uniform pattern of etching is observed on the enamel surface treated with phosphoric acid alone

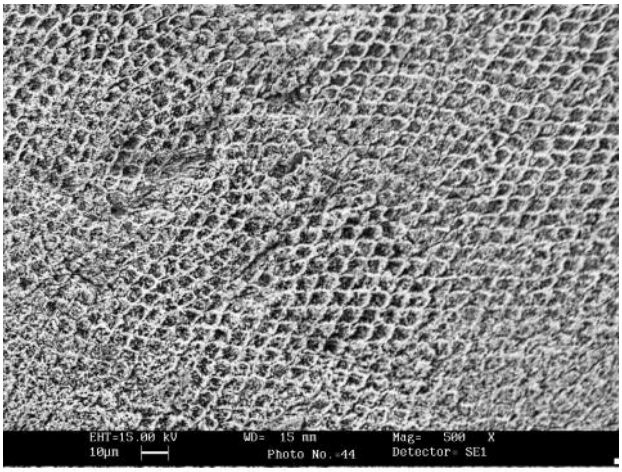

Figure 2. SEM Microphotograph of Group 2 (mag. 500X) showing clogging of etched enamel surface because of accumulation of organic debris

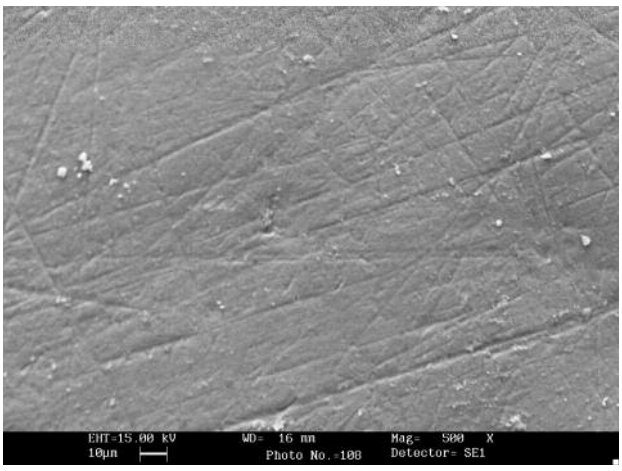

Figure 3. SEM Microphotograph of Control Group (mag. 500X). Normal enamel surface characteristic and remnants of organic pellicle can be seen.

test and Paired t test was used to compare the two groups; the level of significance was set at 0.05(5%).

RESULTS

Each sample of Group 1 and Group 2 (10 samples each) were evaluated for Type I-II etching pattern and the mean values were obtained (Table I) for Group 1 being 55.76 ± 25.47% and Group 2 being 53.58 ± 22.47%. It was seen that Pearson's correlation (Table II) revealed no statistically significant correlation between the groups (P>0.05). According to Paired t test (Table III), no significant difference was observed between the two groups in which only etching with 37% phosphoric acid was done and in other, first deproteinization with 5.25% NaOCl was done and then etching was performed.

Table 1: Mean values of Type I-II etching pattern in Group 1 and Group 2

	Group 1	Group 2
Block 1	56.8%	60.4%
Block 2	44.2%	28%
Block 3	66.4%	56%
Block 4	48.8%	49%
Block 5	66.8%	81%
Block 6	52%	68.8%
Block 7	76.6%	48.8%
Block 8	65%	49.8%
Block 9	30%	49.6%
Block 10	51%	44.4%
Mean	**55.76%**	**53.58**

Table 2. Pearson's Correlation test between the two Groups

		GROUP 1	GROUP 2
GROUP 1	Pearson's Correlation	1	0.212
	P value		0.139
GROUP 2	Pearson's Correlation	0.212	1
	P value	0.139	

Table 3. Paired t- Test (comparison between the two Groups)

	T	df	P value
Group1 – Group 2	.49	49	.62

DISCUSSION

Buonocore in 1955[7] came up with a procedure to improve and enhance the adhesion of the restorative material to enamel through the process of acid etching. Further research into the underlying mechanism of the bond suggested that tag like resin extensions were formed and micromechanically interlocked with the enamel microporosities created by etching.[8, 9]

Generally, the use of a phosphoric acid concentration between 30% and 40%, an etching time of not less than 15 seconds, and washing times of 5 to 10 seconds have been recommended to achieve the most receptive enamel surface for bonding.[10,11] Historically, some controversy existed about the concentration of phosphoric acid that would provide optimal etching efficacy, because some acids have been reported to form precipitates on the surface that might interfere with resin bonding.[11,12] Chow *et al*[13] showed that 50% phosphoric acid applied for 60 seconds on enamel produces a precipitate of monocalcium phosphate monohydrate that can be rinsed off. A precipitate of dicalcium phosphate dihydrate produced by etching with less than 27% phosphoric acid was found not to be easily removable. Calcium dissolution and etching depth increases as the concentration of phosphoric acid increases until the concentration reaches 40%, at higher concentrations thereafter, a reverse effect is obtained. The etching time has also been reduced from the traditional 60-second application with 30% to 40 % phosphoric acid to etching times as brief as 15 seconds. Several laboratory and clinical studies have demonstrated bonding effectiveness to be equivalent with etching times from 15 to 60 seconds[14–16] respectively.

Acid etching removes about 10µm of the enamel surface and creates a micro-porous layer from 5µm to 50µm deep. Originally four enamel etching patterns have been described by Silverstone *et al* in 1975.[17] These include Type I, in which there is predominant dissolution of the prism cores; Type II, in which there is predominant dissolution of the prism peripheries; Type III, in which no prism structure is evident and indiscriminate erosion is seen and Type IV which is junction between Type I and Type II etching patterns. The effect of acid etching on the enamel depends on several parameters.[18] The kind of the acid used, the acid concentration, the etching time, the form of the etchant (gel, semi gel, or aqueous solution), the rinsing time, the way in which etching is activated (rubbing, agitation, and/or repeated application of fresh acid), whether enamel is instrumented before etching, the chemical composition and condition of enamel, whether the enamel surface belongs to the primary or permanent teeth or whether enamel is fluoridated, demineralized, or stained.

Sodium Hypochlorite solutions have been used as wound irrigants since 1915[19] and as an endodontic irrigant as early as 1920[20] due to its bactericidal and proteolytic properties. Irrigation of the root canals with sodium hypochlorite solutions (in the concentrations ranging from 1% to 5.25%) is now widely accepted. As an endodontic irrigant, sodium hypochlorite solution is relatively cheap, is bactericidal and virucidal, it dissolves proteins, has a low viscosity, and it has a reasonable shelf life.[21] It has been seen that sodium hypochlorite exhibits a dynamic balance as is shown by the reaction:

$$NaOCl + H_2O \leftrightarrow NaOH + HOCl \leftrightarrow Na^+ + OH^- + H^+ + OCl^-$$

When sodium hypochlorite comes in contact with organic material, several chemical reactions take place, i.e. fatty acids react with sodium hydroxide creating soap and

glycerol (saponification reaction), amino acids react with sodium hydroxide creating salt and water (neutralization reaction) and also reacts with hypochlorous acid creating chloramines and water. These reactions occur simultaneously and synergistically leading to liquefaction of organic tissues.[22]

The action of phosphoric acid on the enamel surface occurs mostly on its mineralized part i.e. its inorganic matter. Unfortunately this acid does not eliminate the organic matter on the enamel surface which comprises of less than 1%, but can be effective in enhancing the etching pattern. Since NaOCl has been used to remove the organic tissues from the root canal space, its role in removing the organic content from the enamel surface may give fruitful results. So, it was used as the deproteinizing agent in our study to investigate better etching pattern if at all.

Only Type I–II patterns were observed, as they are the predominant patterns seen on etched enamel surface. In Group 1, the Type I-II pattern of etching observed in our study was 55.76% while Espinosa *et al* (2008)[6] reported it to be less than 50% of the total enamel surface. They also reported that pretreatment with NaOCl almost doubled the Type I-II pattern to 94.47%, but in our study it was found to be only 53.58%. The results might be different from Espinosa *et al* (2008)[6] 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. One more significant finding observed in this study is that in almost all the specimens of Group-2 as evident in Figure 2, is the clogging of the etched surface in deproteinized enamel specimens because of accumulation of organic debris which might hamper the best possible final clinical outcome i.e. bond strength. As only one such study has been reported in the literature, further studies are needed to evaluate the effectiveness of deproteinization in enhancing the etching pattern of the enamel surface.

CONCLUSIONS

It was observed that enamel deproteinization did not grossly alter the surface topographic features of enamel before acid etching in our study. The use of 37% phosphoric acid for 15 seconds still remains the best method for pretreatment of enamel.

REFERENCES

1. Guba CJ, Cochran MA, Swartz ML. The effect of varied etching and etching solution viscosity on bond strength and enamel morphology. Operative Dent, 19: 146–153, 1993.
2. Ohsawa T. Studies on solubility and adhesion of the enamel in pretreatment for caries preventive sealing. Bull Tokyo Dent, 1: 65–82, 1972.
3. Silverstone LM. The acid etch technique, an in vitro studies with special reference to the enamel surface and the enamel resin interface in Proceeding of an International Symposium on Acid etch technique eds Silverstone LM and Dogon IL, St. Paul M N. North Central publishing; 1974: 13–39.
4. Hobson RS, Rugg -Gunn AJ, Booth TA. Acid –etch patterns on the buccal surface of human permanent teeth. Arch Oral Biol, 47: 407–412, 2002.
5. Hobson RS, Crotty T, Thomson JM, Jepson NJ. A quantitative study of enamel acid etch patterns on surfaces used for retention of resin bonded fixed prostheses. Eur J Prosthodont Restor Dent, Sep (13): 123–128, 2005.
6. Espinosa R, Valencia R, Uribe M, Ceja I, Saadia M. Enamel deproteinization and its effect on Acid Etching: An in vitro study. J Clin Pediatr Dent, 33(1): 13–20, 2008.
7. Buonocore MG. A simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. J Dent Res, 34: 849–853, 1955.
8. Gwinnett AJ, Matsui A. A study of enamel adhesives: The physical relationship between enamel and adhesive. Arch Oral Biol, 12: 1615–1620, 1967.
9. Buonocore MG, Matsui A, Gwinnett AJ. Penetration of resin dental materials into enamel surfaces with reference to bonding. Arch Oral Biol, 13: 61, 1968.
10. Asmussen E, de Araujo PA, Peutzfeldt A. In vitro bonding of resin to enamel and dentin: an update. Trans Acad Dent Mater, 2: 36, 1989.
11. Gwinnett AJ. Interactions of dental materials with enamel. Trans Acad Dent Mater, 3: 30, 1990.
12. Phillips RW. Skinner's science of dental materials, 8th ed. Philadelphia: Saunders, p. 25, 1982.
13. Chow LC, Brown W.E. Phosphoric acid conditioning of teeth for pit and fissure sealents. J Dent Res, 50: 1517–1525, 1973.
14. Barkmeier WW, Gwinnett AJ, Shaffer SE. Effect of enamel etching time on bond strength and morphology. J Clin Orthod, 19: 36–38, 1985.
15. Nordenvall KJ, Brannnstrom M, Malmergren O. Etching of deciduous teeth and young and old permanent teeth. A comparison between 15 and 60 seconds of etching. Am J Orthod, 78: 99, 1980.
16. Swift EJ, Perdiago J, Heymann HO. Bonding to enamel and dentin: a brief history and state of the art 1995. Quintessence Int, 26: 95, 1995.
17. Silverstone LM, Saxton CA, Dogon IL, Fejerskov O. Variation in the pattern of acid etching of human dental enamel examined by scanning electron microscopy. Caries Res, 9: 373–387, 1975.
18. Tagami J, Hosoa H, Fusayama T. Optimal technique of etching enamel. Oper Dent,13: 181, 1988.
19. Dakin HD. On the use of certain antiseptic substances in the treatment of infected wounds. Br Med J, 2: 318–320, 1915.
20. Crane AB. A predictable root canal technique. Philadelphia: Lea & Febiger, p. 69, 1920
21. Best M, Springthorpe VS, Sattar SA. Feasibility of a combined carrier test for disinfectants: studies with a mixture of five types of microorganisms. Am J Inf Cont, 22: 152–62, 1994.
22. Scott DB, Wyckoff RWG. Carbon surface replicas for electron microscopy and electron diffraction. JR Microsc Soc, 75: 217–222, 1955.