ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 12, Issue, 03, pp.10541-10545, March, 2020

DOI: https://doi.org/10.24941/ijcr.38127.03.2020

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

COMPARATIVE EVALUATION OF STRUCTURAL CHANGES IN ACID ETCHED PRIMARY ENAMEL PRETREATED BY 5.25 % SODIUM HYPOCHLORITE GEL AND SOLUTION – AN SEM STUDY

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ARTICLE INFO

Article History:
Received 14th December, 2019
Received in revised form
10th January, 2020
Accepted 18th February, 2020
Published online 28th March, 2020

Key Words:

Enamel Deproteinization, Acid Etching, Sodium Hypochlorite, Phosphoric Acid.

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ABSTRACT

Background: Resin based composite materials are widely used for aesthetically restoring primary teeth. Fundamental mechanism for resin - enamel adhesion relies on resin - micro tag formation. This is achieved by acid etching, where the smooth enamel surface is transformed into an irregular surface with increased surface energy. Primary enamel has increased organic content than permanent teeth which stand as a barrier in the way for achieving generalised retentive surface over the entire acid treated surface. Deproteinization of enamel is proposed as a non- invasive way by which organic protein content of enamel can be removed. 5.25 % sodium hypochlorite has been used as an endodontic irrigant due to excellent protein denaturing action, which might also be effective in removing organic content of primary enamel structure. **Objectives:** To evaluate and compare the effect of sodium hypochlorite conditioning on the surface micro morphological features of acid etched primary enamel. **Methods:** Buccal enamel surface of 10 extracted human primary molars were cut and into trimmed to forty ^{2} mm^{2} blocks. Each group comprises of 10 enamel blocks (1mm^{2} size).

Group 1: No treatment group
Group 2: Enamel surface etched with 37 % H3PO4 for 15 sec, washed and dried for 10sec
Group 3: Enamel surface pre-treated with 5.25% NaOCl solution for 120 seconds, washed, dried and etched as for Group1.
Group 4: Enamel surface pre-treated with 5.25% NaOCl gel for 120 seconds, washed, dried and etched as for Group1.

Samples subjected to SEM analysis and 5 microphotograhs of each sample were obtained at 500x magnification and evaluated for quality of etching pattern of enamel in percentage using Auto –CAD 2014 software.

Statistical analysis used: Anova
Result: The mean value of acid etching pattern was similar between Group 2 and Group 3(p = 0.78), but the mean value of acid etching was significantly lower in the Group 4 (p=0.04) than in the control group.
Conclusion: Acid etching with 37% phosphoric acid for 15 seconds still remains the best method of pretreatment in primary enamel. There is no significant effect of deproteinization with 5.25% NaOCl on acid etched enamel surface.

Citation: ***Dr. Darsana Krishnan, Dr. K. Korath Abraham, Dr. Ektah Khosla et al.,*** **2020.** "Comparative evaluation of structural changes in acid etched primary enamel pretreated by 5.25 % sodium hypochlorite gel and solution – an sem study", *International Journal of Current Research*, 12, (03), 10541-10545.

INTRODUCTION

Enamel is the hardest and highly mineralised tissue in the body composed of 96% inorganic material and the remaining components, by weight, are organic matter (0.6%) and water (3.5%). Primary and permanent enamel shows tremendous variation in morphology and composition.

The thickness of enamel is approximately 1-2mm in permanent teeth and 0.5- 1mm in primaryteeth. Aesthetic restorations are gaining popularity in primary dentition which is achieved by composite resins. The success of adhesive restorations require aproper retentive surface. Intact enamel as such does not support bonding. Buonocore in 1955, proposed the concept of acid etching enamel with orthophosphoric acid to attain satisfactory bonding of resin with tooth enamel (Silverstone,

1975). Acid etching thus changes the enamel topography from a low reactive to a surface that is more susceptible to adhesion. Hence, acid etching, removes approximately 10µ of enamel surface and creates a morphologically porous layer (5µ to 50µ deep) which increase the surface energy and thus fluid resin contact surface (Hoffman, 1969). Prismless enamel is usually distributed throughout the surface of human enamelwhichacts as a barrier for proper acid etching. The prismless enamel contain indistinct and abnormal prism structures, or no prism structures. The primary enamel also shows an increase in organic content when compared with permanent enamel. Thickness of prismless enamel in primary teeth is 16- 45µm, while in permanent teeth prismless enamel has a thickness of approximately 15 -20 µm.

The etching pattern on permanent teeth was first described by Pool and Johnson (1967) (Galil, 1979), which was further classified by Silverstone et al (1975). Three patterns of enamel surface etching were noticed.

- Type I etching pattern: hollowing of prism centers with relatively intactperipheries.
- Type II etching pattern: prism peripheries were removed and prism cores were projecting towards the original enamelsurfaces.
- Type III etching pattern: Some etched region showed neither type I nor type II etching patterns exclusively but instead area appeared as generalized surfaceroughening (Shinohara, 2006).

Of these types, type I and type II etching patterns shows greatest retention with adhesive materials (Carstensen, 1992). Bunocore in 1955, used 85 % phosphoric acid for 30 seconds to achieve satisfactory bonding in permanent teeth. Acid etching time for primary teeth is bounded with controversies. Early studies suggested that 2 minutes of etching time were necessary in primary enamel to ensure favourable enamel etch patterns. It has been firmly established that essence of adhesion lies in achieving the best acid etching with a generalized retentive morphological condition over the entire acid etched enamel surfaces (Carstensen, 1992). However, the studies have shown that topographic quality of enamel etching with phosphoric acid is not achieved over the entire adhesion surfaces. More than 69% of treated surface had no etching.7% presented tenuous etching; only 2% was ideally etched (Cerci, 2012). This was the main reason for increased failure rate of sealants and adhesive restorations. Quality of etched enamel surface depends mainly on etching agent, acid concentration, etching time and composition of enamel surface. Organic deposits such as surface cuticle and stained pellicle cover the enamel surface (Nordenvall, 1980). These remnants might interfere with etching process, resulting in lower resin adhesion. To counteract these limitations, invasive technics like slight grinding or abrading of enamel was suggested, leading to superficial enamel loss (Buonocore, 1955). Deproteinization of enamel is a noninvasive way by which organic content/protein content of enamel can be removed (Pithon et al., 2013). Since 1920, sodium hypochlorite solution has been used as an endodontic irrigant due to its excellent protein denaturing and anti-bacterial action. Hand et al observed that dilution more than 5.25 % greatly reduced the tissue dissolving property of sodium hypochlorite (Espinosa, 2008). Venezia *et al* first proposed enamel deproteinization with 5 % sodium hypochlorite after acid etching which resulted in improved bonding of orthodontic bracket to hypo mineralized enamel. Removal of excess proteins may provide an advantage on bonding of restoration (Ahuja et al., 2010). Gordon et al observed that most of the tissue dissolving activity of Sodium hypochlorite was lost after two minutes of contact with organic tissue (Harleen, 2011). Espinosa et al, showed that enamel deproteinization with 5.25% sodium hypochlorite for 1 minute prior to phosphoric acid etching doubles enamels retentive surface to 94.47% and there was an increase in type 1 & type 2 etching pattern (Ramakrishna, 2014). Other deproteinizing agents like papain gel, bromelain enzyme and chlorine dioxide are included in the literature. Pithon et al evaluated the effect of bromelain in association with10% papain gel as deproteinizing agent on orthodontic bracket bonding and found an increase in the shear bond strength of bracket bonded with RMGIC (Pithon, 2013). Hasija et al compared the effect of different deproteinizing agents on shear bond strength of composite to primary teeth enamel and observed that deproteinization with bromelain gel showed effective bond strength (Hasija, 2017). However, the aim of this study was therefore to evaluate the effect of pretreatment with 5.25% NaOCl on acid etched primary enamel surface .

MATERIALS AND METHODOS

This study was carried out in the Department of Pediatric and Preventive Dentistry, Mar Baselios Dental College, Kothamangalam, Kerala, India. Ten primary molars were extracted due to preshedding mobility from the patients attending the Department of Pediatric and Preventive Dentistry. The teeth with enamel cracks or fractures along their buccal aspect, malformations, carious lesions, restorations or erosions were excluded. Samples were stored in saline solution at 37^{o}C, after extraction and were polished with pumice and rinsed with distilled water for 10 seconds. Roots were amputated and separated with a low speed double sided diamond disk (Shofu, Japan) under continuous water spray irrigation.

To obtain enamel samples comparable among themselves and with uniform physical and chemical characteristics, the buccal surface of each crown was marked with 2 horizontal lines dividing the crown portion into 3 parts and the middle section was taken as the study specimen .In that middle section, 3 vertical lines were marked equidistant to each other and was cut with the same disc and trimmed to 1mm^2 giving 4blocks per sample .Thus, 40 enamel blocks of 1mm^2 was obtained from 10 teeth. These blocks were the divided into their respective groups depending on the treatment given. To maintain uniform standard between samples, each tooth was divided into four sections, which formed three treatment groups and one control group. Each tooth was subjected to 3 different treatments ensuring that the surface treatments were applied to teeth with the same enamelquality (Ramakrishna, 2014).

PREPARATION OF THE SAMPLE FOR STUDY

Group 1 (No treatment group): 10 enamel blocks of 1mm^2 obtained from middle portion of all teeth were utilized for SEM analysis. All the blocks were observed under a Scanning Electron Microscope for any defects or cracks produced during sectioning process.

Group 2 (control -Acid etch group) : the specimens were etched with 37 % H3PO4 gel , applied with a micro brush for 15 seconds, washed with sterile water and air sprayed for 10 seconds ,then dried with oil free compressed air.

Group 3 (NaOCl solution followed by H3PO4): The enamel surfaces were pretreated with 25% NaOCl solution, applied with sterile cotton pellet for 120 seconds, washed with sterile water for 10 seconds, then dried and treated as group 2

Group 4 (NaOCl gel followed by H3PO4): The enamel surfaces were treated with 5.25% NaOCl gel, applied with sterile cotton pellet for 120 seconds, washed with sterile water for 10 seconds, then dried and treated as group 2. After the assigned treatment, all the specimens were prepared for SEM analysis.

Preparation of specimen for sem analysis: 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 samples was standardized at middle upper section of tooth, 5 microphotographs at 500x magnification were obtained from each enamel specimen covering the entire treated sample surface. A total of 20 microphotographs for each molar were obtained in a consecutive order. Thus, a total of 200 images or 50 images per group was generated for its analysis. The acquired images were subjected to descriptive analysis. The SEM photographs were interpreted by two separate examiners who were blinded to the treatment rendered and well trained to analyse the SEM views. To obtain quantitative results, the sample were evaluated using Auto CAD 2014 software to grade each of the image.

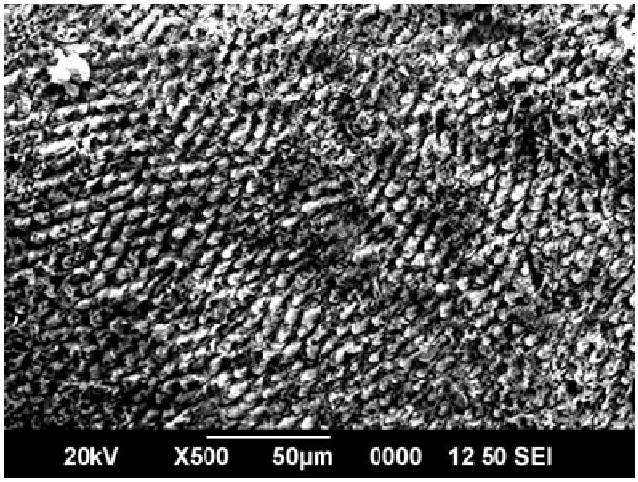
Statistical Analysis: The data thus obtained were subjected to statistical analysis which was performed using SPSS (Statistical package for Social Sciences) version 12 for Windows to find the difference variation between the groups with ANOVA

RESULTS

The surface area of Type 1 and Type 2 etching patterns were determined for each image. The highest percentage of Type 1-Type II acid etch pattern was noticed in group II as shown in figure1. Descriptive statistics are presented in Table 1.Tukey's HSD showed significant difference between Group 2 /Acid etch group and Group 4 /5.25% NaOCl Gel + H3PO4 (p =0.04). No significant difference between Group 2 /Acid etch group and Group 3/5.25% NaOCl solution + H3PO4.(p =0.73).No significant difference between Group 3 /5.25% NaOCl solution + H3PO4. and Group 4 / 5.25% NaOCl gel + H3PO4.(p =0.18)

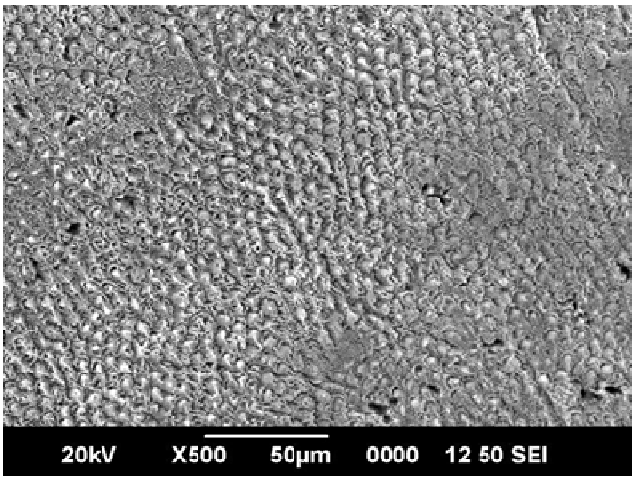
DISCUSSION

The micromechanical and histological characteristics of primary teeth differ from that of permanent teeth. The differences in the amount of mineral components, morphology and structure between primary and permanent were thought to be responsible for the low bond strengths in primary enamel compared to permanent enamel. The enamel surface is highly resistant to acid dissolution due to the presence of prismless enamel. The surface of enamel generally contains indistinct, abnormal or no prism structures (Robinson, 2000).



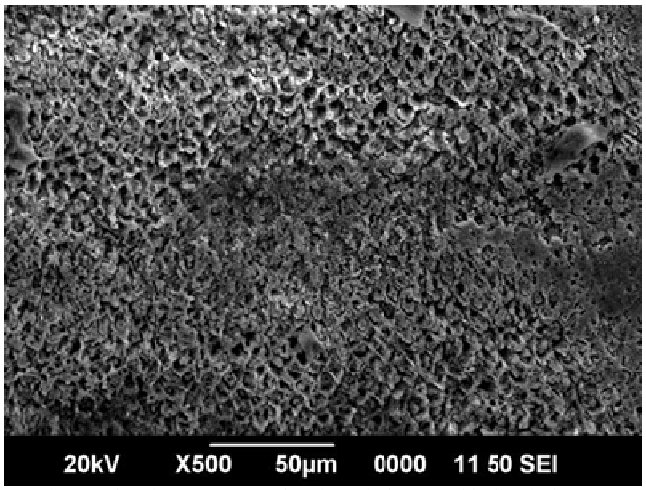
SEM image of showing uniform acid etched surface.

Figure 1. enamel surface after acid etching with 37 % H3PO4 for 15 seconds



SEM image shows porous non uniform etching of enamel prisms

Figure 2. Pretreatment with 5.25 % sodium hypochlorite solution followed by acid etching



SEM image showing remnants of aprismatic layer and indiscriminate pattern of etching

Figure 3. 5.25% sodium hypochlorite Gel followed by acid etching

The enamel surface is usually in a low energy, weakly reactive, and hydrophobic state. When exposed to acid, it becomes a high energy, low reactive and hydrophilic surface. This high energy provides a favorable environment for bonding of resin to tooth structure (Ripa, 1966; Richard, 1995).

Table 1 Comparison between acid etched surface patterns (in Group 2, Group 3 and Group 4) and type of surface treatment (in percentage)

Descriptive statistics for Type 1-2 total etched surface patterns (μm^2)								
					95% confidence interval for mean			
Groups	N	Mean	Std deviation	Std error	Lower bound	Upper bound	Min	Max
Group 2	10	41.54	22.03	6.97	25.78	57.3	12.00	67.00
Group 3	10	34.8	24.59	7.78	17.21	52.39	2.40	78.00
Group 4	10	18.54	10.04	3.18	11.36	25.72	4.20	33.40

(Group 2 – acid etch only; Group3 – NaOCl solution followed by H3PO4; Group 4 - NaOCl gel followed by H3PO4)
Statistical analysis showed that:

- The mean of percentage of type 1-type 2 etch pattern in Group 2 is 41.54%
- The mean of percentage of type 1-type 2 etch pattern in Group 3 is34.8%
- The mean of percentage of type 1-type 2 etch pattern in Group 4 is18.54%

Table 2. Comparison between acid etched patterns (in Group 2, 3 ,4) and type of surface treatment (in percentage %) – statistical analysis by ANOVA(p<0.05)

ANOVA					
percentage					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	2796.05	2.00	1398.03	3.52	0.04

The mean area of ideal etch pattern in percentage obtained in table 1 were analyzed for significance by subjecting to Analysis Of Variance (ANOVA).

INFERENCE

There is statistically significant difference in values among different acid etching treatment methods as p value is 0.04 (p value < 0.05)

Table 3. Post hoc test for comparing acid etched surface patterns in 3 different groups: - Group 2 (Acid etch only), Group 3 (NaOCl solution followed by H3PO4), Group 4 (NaOCl gel followed by H3PO4)

Multiple Comparisons						
Type 1 Type 2 acid etched surface patterns in micrometer Square						
Tukey HSD						
					95% Confidence Interval	
(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Group 2 Acid Etch Group	5.25% NaOCl Solution followed by H3PO4	6.74	8.91	0.73	-15.35	28.83
	5.25% NaOCl Gel followed by H3PO4	23.00*	8.91	0.04	0.91	45.09
Group 3 5.25% NaOCl Solution + H3PO4	Group acid etch	-6.74	8.91	0.73	-28.83	15.35
	5.25% NaOCl Gel + H3PO4	16.26	8.91	0.18	-5.83	38.35
Group 4 5.25% NaOCl Gel +H3PO4	Group acid etch	-23.00*	8.91	0.04	-45.09	-0.91
	5.25% NaOCl Solution + H3PO4	-16.26	8.91	0.18	-38.35	5.83

*The mean difference significant at p value < 0.05 level.

INFERENCE Specific Post Hoc test is in line with ANOVA test and there is significant difference among each group.
Turesky's HSD showed :

- Significant difference between Group 2 /Acid etch group and Group 4 /5.25% NaOCl Gel + H3PO4.(p =0.04)
- No significant difference between Group 2 /Acid etch group and Group 3/5.25% NaOCl solution + H3PO4.(p=0.73)
- No significant difference between Group 3 /5.25% NaOCl solution + H3PO4. and Group 4/
- 5.25% NaOCl gel + H3PO4.(p =0.18)

The technique of acid etching was introduced with the purpose of creating micro porosities on the surface of the enamel, thus enhancing the adherence of composites to tooth surface. 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.Type-1 and Type-2 patterns showed a significant increase in bond strength. Retentive morphology should be homogeneous over the entire treated surface. The Group II specimens were etched with 35% H3PO4 gel for 15 seconds. The present study showed that the mean surface area of type I and II etching pattern obtained by this group was 17,800 µm2 which was 41.5%.

The Group III specimens were pretreated with 5.25% NaOCl solution for 120 seconds and then etched with 35% H3PO4 gel for 15 seconds, The mean surface area of type I and II etching pattern obtained by in this was 15220µm2 which was 34.8 % and was against the results obtained by Espinosa R et al 94.47%. As evident in Figure2 indiscriminate etching patterns or clogging of etched surface was noticed in most of the specimen. This might be due to accumulation of organic debris in the etched prism surface. Bhoomika *et al*. stated that enamel deproteinization prior to acid etching, did not grossly alter the surface topographic features. Similar results were obtained in the present study. there was no statistically significant increase in the retentive

surface area of acid etched primary enamel pretreated with 5.25 % sodium hypochlorite solution. On the contrary, Espinosa *et al*. and Christopher *et al.* observed that enamel surface pretreated with 5.25% sodium hypochlorite prior to acid etching dramatically increased the retentive surface area. The increase in retentive surface area might be due to removal of organic smear layer from surface of enamel by deproteinization which cannot be achieved by acid etching alone. Ramakrishna *et al*. observed in their study that there was no enhancive effect of enamel deproteinization, after acid etching on the topographic quality of enamel, rather indiscriminate pattern of etching was predominantly seen in the total etched surface area. present study was also in accordance with the above mentioned study, with mean surface area of type I and II etching pattern obtained in Group 1V (H3PO4 gel followed by NaOCl). was 7532µm^2 where a mean percentage of 18.4% of total area was ideally etched.

In almost all specimens of group IV as evident in Figure 3, indiscriminate etching patterns or clogging of etched surface was noticed. This might be due to clogging of etched surface by the methylcellulose present along with sodium hypochlorite in the gel. One of the limitations of the present study is that in vitro setting may not simulate the effect of deproteinization on acid etching in-vivo. In addition, possible concerns of sodium hypochlorite are the taste, chlorinated odour, tolerance by young children and possible soft tissue reactions. Finally, the clinical implications of these findings are important. Based on the results of the present study, it can be inferred that, acid etching is adequate to produce retentive area as the differences in total etched area after the three different surface treatment regimen were not statistically significant.

Conclusion

Thus within the limits of the present study, following conclusions can be drawn:

- Enamel deproteinization could not grossly alter the surface micromorphological features of acid etched primaryenamel.
- 37% phosphoric acid treated for 15 seconds, exhibited increased ability in inducing ideal etching pattern compared to deproteinized and acid etchedgroup.
- Deproteinization with 5.25 % NaOCl gel caused clogging of etched surface and thus resulted in poor enamel etchingquality.

Enamel deproteinization with 37 % phosphoric acid is adequate to produce retentive areas for resin bonding.

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