Etching Patterns of Sodium Hypochlorite Pretreated Hypocalcified Amelogenesis Imperfecta Primary Molars: SEM Study

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Aim: *To investigate the etching patterns of hypocalcified amelogenesis imperfecta (AI) in primary molars pretreated with 5.25% NaOCl prior to phosphoric acid application using scanning electron microscopy (SEM).* ***Study design:*** *Ten hypocalcified AI primary molars were collected, sectioned longitudinally into 2 parts and allocated into two groups of ten specimens each. The enamel surface in the first group (control group) was etched using 37% phosphoric acid gel for 15 seconds; while in the second group (study group), it was pretreated using 5.25 sodium hypochlorite(NaOCl) for 60 seconds prior to acid etching. Each specimen was examined at 16 different sites, and evaluated for the etching pattern (types I, II, and III) distribution using SEM. A total of 320 microphotographs at 1,500 magnification were obtained using Auto-Cad 2007 software.* ***Results:*** *The etching pattern with phosphoric acid was not uniform with predominance of type III etching (65.63%), while the pretreated enamel surfaces showed a significant increase in type I and II(82.5%) etching patterns (P<0.001).* ***Conclusion:*** *Treatment of primary teeth affected by hypocalcified AI using 5.25% NaOCl prior to phosphoric acid etching significantly improves the etching pattern which is required for good resin bonding.*

Keywords: *amelogenesis imperfecta; sodium hypochlorite; etching patterns; primary molars.*

INTRODUCTION

Amelogenesis imperfecta (AI) exhibits multiple and complex problems that may have profound negative implications and psychological impacts on the affected children as well as their families.[1] Clinical consequences of AI include dental caries, tooth sensitivity, rapid tooth attrition, loss of vertical dimensions, gingivitis as well as poor esthetic.[2-4]

Based on the clinical features and inheritance pattern of enamel developmental defects, Witkop and Sauk[5] classified AI into 4 major groups and 14 subgroups; the hypoplastic, hypocalcified, hypomaturation forms, as well as hypomaturation hypoplastic with taurodontism. The hypocalcified variants are characterized by insufficiently mineralized soft enamel that affects both primary and permanent dentitions.[6,7]

Adhesives, as resin composites and resin-modified glass ionomers, are considered the treatment of choice for restoration of teeth affected by AI. Many studies proved that the adhesion lies in achieving a good etching quality by selective removal of the prismatic and inter-prismatic crystals causing surface roughness and micro-porosities. This produces sufficient monomer infiltration, better retention and bonding strength between the enamel surface and the restoration.[8,9]

SEM studies reported different etching patterns following acid treatment of enamel surfaces. Gwinnett[10] observed 4 etching characteristics. Etching of the prism cores with intact peripheries was the most common findings. Less frequently, the peripheral prisms were

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dissolved, while the cores were intact. The least common topographic changes displayed smooth or pitted surfaces with little distinct prism delineation. Silverstone et al[11] described 3 etching patterns. In type 1, the prism cores were preferentially removed. In type 2, the prism cores remained relatively intact, while the prism peripheries were lost. Type 3 showed a more random pattern, with areas corresponding to types 1 and 2 together with areas of surface porosities without a distinct prism morphology. Silverstone [12] considered type I and II the most retentive acid etching patterns for adhesive restorations.

It has been reported that the quality of enamel etching is affected by several parameters. It depends on the type and concentration of the acids, etching and rinsing time, composition and condition of the enamel surface as well as the amount of organic matter removal.[12-14] Studies have been carried out to assess the effect of these parameters on the etching pattern of abnormal enamel structure. Ultrastructural studies showed that hypocalcified AI affected enamel is more porous with higher organic content per volume compared to normal enamel. This higher amount of organic matrix negatively affects the quality of acid etching. It acts as a barrier preventing the proper contact between the acid and the enamel crystals. This results in clinically unfavorable bonding between the restoration and the hypocalcified enamel, hence contributes to the remarkable failure rates of the restorations.[15-17]

NaOCl is a non-specific proteolytic agent that is effective in removing organic compounds without damaging healthy tissue or tooth structure.[18] The concept of enamel deproteinization using NaOCL before acid etching of AI teeth was first adopted by Venezie et al 1994.[19] He found that enamel pretreatment with 5% NaOCL for one minute enhanced the bonding of an orthodontic bracket to a permanent tooth affected with hypocalcified AI.

Several studies have been performed to investigate the effect of enamel deproteinization with 5.25% NaOCl, before or after phosphoric acid etching application, on the etching pattern and bond strength of adhesive systems to sound permanent teeth, but their results had been controversial.[20-23]

To the best of our knowledge, the literature is lacking publications assessing the pretreatment effect of NaOCl on the etching quality of primary teeth affected by hypocalcified AI. To this end, the aim of present study is to investigate the etching patterns of hypocalcified AI primary molars pretreated with 5.25% NaOCl prior to phosphoric acid application using SEM. We hypothesize that pretreatment of hypocalcified AI enamel surfaces with 5.25% NaOCl improves the quality of surface etching.

MATERIALS AND METHOD

The present study was carried out in the Department of Pediatric Dentistry and Dental Public Health, Faculty of Dentistry, Alexandria University, while the SEM was performed at the Unit of Electron Microscopy, Faculty of Science, Alexandria University. Ethical Research Approval was obtained from the Ethical Committee of Faculty of Dentistry, Alexandria University. Indeed, an informed consent from patients/parents was taken to use the exfoliated primary teeth in the research.

Ten primary molars were collected from children affected with hypocalcified AI near their time of shedding. Teeth with cracked, fractured or missing enamel as well as carious or restored teeth were excluded from the study. Teeth were immediately washed under running water to remove any blood or adherent debris.

The selected teeth were then preserved in distilled water at room temperature. Each tooth was sectioned buccolingually into two halves using high-speed double-sided diamond disc. The 20 specimens obtained were encoded for identification. To get comparable samples, each tooth was sectioned into 2 parts, where one half of the crown acted as a control to the other one

Group I **(Control Group)**: where enamel of the buccal surfaces were etched using 37% phosphoric acid gel, applied with a microbrush for 15 seconds, washed with water and then dried with oil-free compressed air for 10 seconds.

Group II **(Study Group):** where enamel of the buccal surfaces were treated with 5.25% NaOCl, applied with a sterile cotton pellet for 60 seconds, rinsed with water for 20 seconds, and dried with oil-free compressed air for 10 seconds. Then the treated surfaces were etched following the same protocol as in group I.

Specimen preparation for SEM:

The specimens were fixed in 25% glutaraldehyde in phosphate buffer, rinsed, and dehydrated in ascending grades of alcohol till critical point of dryness was achieved. Specimens were then placed on special stubs and exposed to gold sputter coating, then examined for their surface topography by SEM.

The buccal surface of each specimen was examined at 16 different sites, and evaluated for types I, II and III etching patterns over the entire enamel surface. A total of 320 microphotographs (160 images per group) were taken at 1,500 magnification using Auto-Cad 2007 software (Microsoft Corporation, Macrovision Corp).

Frequencies and percentages were reported for the 3 different etching patterns among the study groups. Chi-square test was used to compare the etching patterns between groups. P value of <0.05 was considered significant. JMP version 12 was used for statistical analysis.

RESULTS

The control (I) and study (II) group samples were examined under SEM for identification of the different etching patterns (I,II, and III).

When the different etching patterns were analyzed separately (table 2), group I showed predominantly type III etching (105) followed by type I (40) and type II (15). Group II on the other hand displayed predominantly type I etching (75) followed by type II (57) compared to only (28) with type III etching when pretreated with sodium hypochlorite ($P<0.001$).

When the favorable etching patterns (types I and II) were analyzed together (table 3), the control group demonstrated predominance of the unfavorable type III etching (105) compared to (55) with the favorable etching patterns. On the contrary, group II showed predominance of the favorable etching (132) compared to (28) with type III etching ($P<0.001$).

SEM findings:

In-group I, the hypocalcified enamel surfaces revealed the three different etching patterns; type I, II and III with predominance of type III. Type I etching pattern was found in some specimens showing demineralized enamel prism core and intact inter-prismatic substance (fig.1). Type II etching pattern with selective demineralization of enamel prism periphery was seen in limited areas, and

Table 1: Distribution of different etching patterns at 16 different sites from each specimen in both study groups

Specimen number	Type I etching		Type II etching		Type III etching	
	Group I Control N %	Group II Study N %	Group I Control N %	Group II Study N %	Group I Control N %	Group II Study N %
1	0 (0)	8 (50.00)	4 (25)	0 (0)	12 (75)	8 (50.00)
2	7 (43.75)	0 (0)	0 (0)	15 (93.75)	9 (56.25)	1 (6.25)
3	4 (25.00)	15 (93.75)	0 (0)	0 (0)	12 (75.00)	1 (6.25)
4	0 (0)	4 (25.00)	4 (25.00)	6 (37.50)	12 (75.00)	6 (37.50)
5	3 (18.75)	0 (0)	0 (0)	10 (62.50)	13 (81.25)	6 (37.50)
6	9 (56.25)	3 (18.75)	0 (0)	13 (81.25)	7 (43.75)	0 (0)
7	3 (18.75)	14 (87.50)	0 (0)	0 (0)	13 (81.25)	2 (12.50)
8	8 (50.00)	12 (75.00)	0 (0)	0 (0)	8 (50.00)	4 (25.00)
9	4 (25.00)	10 (62.50)	3 (18.75)	6 (37.50)	9 (56.25)	0 (0)
10	2 (12.50)	9 (56.25)	4 (25.00)	7 (43.75)	10 (62.50)	0 (0)
Total	**40 (25.00)**	**75 (46.87)**	**15 (9.37)**	**57 (35.63)**	**105 (65.63)**	**28 (17.50)**

Table 2: Chi square test comparing the 3 different etching patterns among the study groups

	Type I N (%)	Type II N (%)	Type III N (%)	Total
Group I	40 (25.00)	15 (9.37)	105 (65.63)	**160**
Group II	75 (46.87)	57 (35.63)	28 (17.50)	**160**
Total	**115 (35.94)**	**72 (22.5)**	**133 (41.56)**	**320**
P value	<0.001			

Table 3: Chi square test comparing between the favorable etching patterns and type III among the two study groups.

	Favorable etching patterns Types I & II N (%)	Unfavorable etching pattern Type III N (%)	Total
Group I	55 (34.37)	105 (65.63)	160
Group II	132 (82.50)	28 (17.50)	**160**
Total	**187 (58.44)**	**133 (41.56)**	**320**
P value	<0.001		

was intermingled with few areas of amorphous etching pattern (fig. 2). Type III was widely spread in most of the specimens without a distinct prism morphology together with areas corresponding to type I and II etching (fig.3).

In-group II, the pre-treated hypocalcified surfaces showed marked surface roughness with predominance of types I and II etching patterns. Type I etching pattern was markedly found in most of the specimens either alone or intermingled with some areas of type II etching patterns (fig. 4). Type II etching pattern was also diffusely observed in some specimens (fig. 5), while the type III was interspersed in some areas between the other patterns (fig. 6).

DISCUSSION

High failure rates in resin bonding to hypocalcified AI teeth have been attributed to alterations in enamel surface and its high protein contents. Since bonding between enamel and adhesive restorations is greatly dependent on enamel retentive capacity, the application of NaOCL as a deproteinizing agent was suggested as a possible strategy to achieve optimum adhesion by removing the excess organic matter and acquired pellicle prior to acid etching.[21,24,25]

In the present study, the hypocalcified AI primary enamel surfaces were treated with 5.25% NaOCl, as a protein denaturant, prior to phosphoric acid application. It was claimed that enamel deproteinization might improve the etching quality, which would result in clinically more favorable bond strength of adhesives to the hypocalcified enamel.

The hypocalcified AI specimens were obtained from young patients at their exfoliation time to avoid fracture or cracking of the porous weak enamel surface, if forced extraction was performed.

Specimens in the control group showed the three patterns of acid etching with predominance of type III on most of the etched enamel surfaces. This finding can be attributed to variation in the structural organic content and micromorphological irregularities related to severity of hypocalcified AI affected enamel [26,27]

According to Wright *et al* [15] hypocalcified AI enamel are more porous and has a lower mineral content compared to sound enamel.

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Fig. 1: (a) SEM of group I (control group) showing areas of type I etching pattern (white arrow) with dissoluted prism cores, intermingled with irregularly etched pattern (blue arrow) (x 1.500). (b) Higher magnification of the previous micrograph showing type I etching with its characteristic intact prism peripheries (x5000).

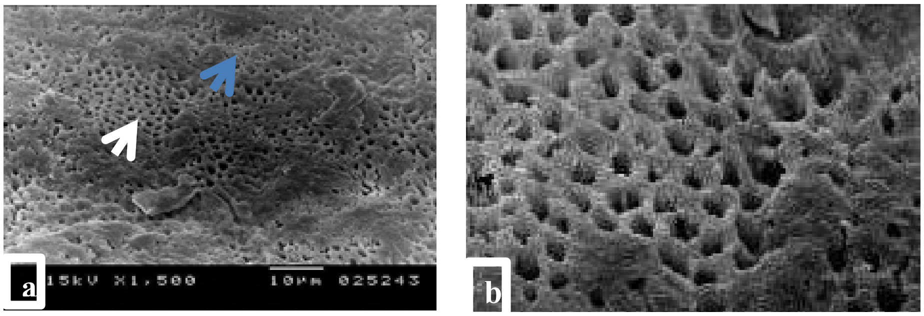


Fig. 2: (a) SEM of group I showing areas of type II etching pattern (yellow arrow) with dissoluted prism periphery but intact cores, adjacent to areas of irregular non uniform enamel surface (blue arrow) (x1.500). (b) Higher magnification of the previous micrograph showing type II etching pattern (x5000).

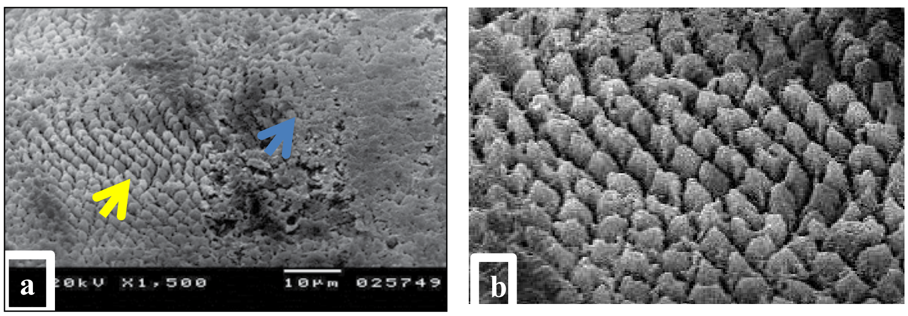


Fig.3: (a) SEM of group I displaying type III etching pattern with irregular roughness (blue arrow) together with areas corresponding to type I (white arrow) and II (yellow arrow) etching patterns (x1.500). (b) Higher magnification of the previous micrograph showing a combination between different etching patterns (x5000).

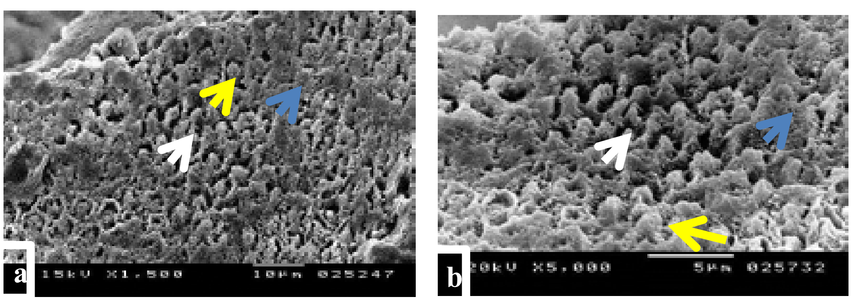


Fig. 4: (a) SEM of group II (study group) showing predominance of type I etching pattern (x 1.500). (b) Higher magnification of the previous micrograph showing type I etching with preferential loss of prism cores (x5000).

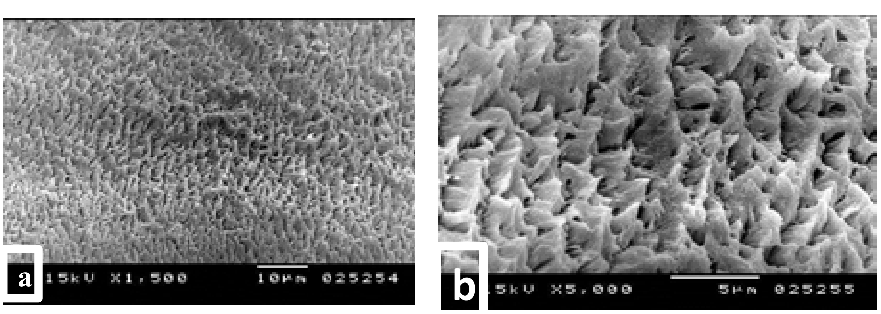


Fig.5: (a) SEM of group II showing large areas of type II etching pattern (yellow arrow) intermingled with areas of type I pattern (white arrow) (x 1.500). (b) Higher magnification of the previous micrograph showing type II etching with loss of prism peripheries (x5000).

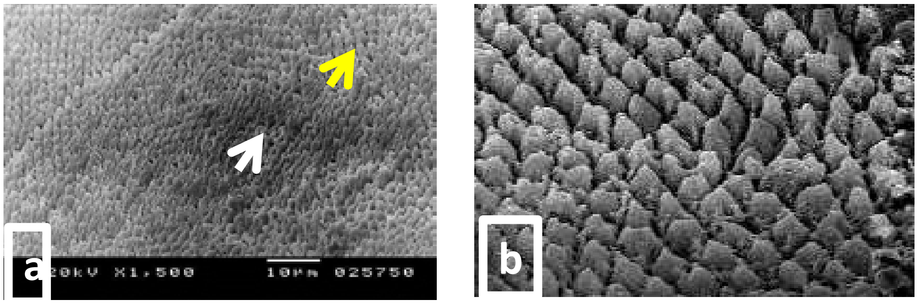
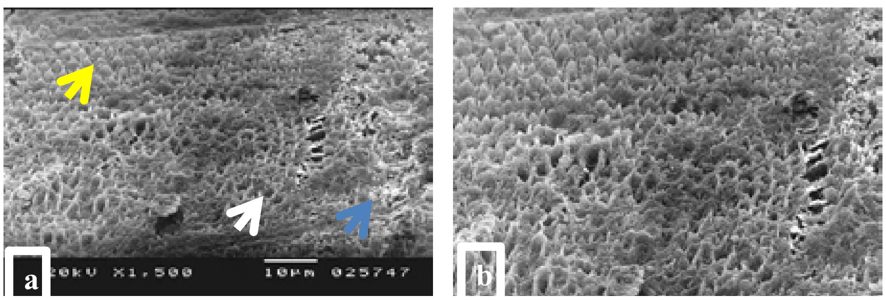


Fig.6: (a) SEM of group II showing type III etching pattern without a distinct prism morphology (blue arrow) together with areas corresponding to types I (white arrow) and II (yellow arrow) (x 1,500). (b) Higher magnification of the previous micrograph showing type III etching pattern (x5000).



This difference, in addition to the fact that phosphoric acid acts mainly on the mineralized hard tissues, may explain the inconsistent etching pattern observed in the control group in this study.

The results of the present study are in line with Seow and Amaratunge [13] who observed the three types of etching patterns after phosphoric acid application on both AI primary and permanent teeth. It is also consistent with previous studies of Espinosa *et al*, [20] Hobson *et al* [28,29] and Agarwal *et al* [30] done on sound permanent molars and premolars. They found that phosphoric acid application produced less than 50% of type I and II etching. This finding could also explain the presence of amorphous non-uniform eching pattern observed in the present study with the hypocalcified AI primary teeth.

On the other hand, the surface topography of group II demonstrated a significant increase in enamel surface roughness with predominance of types I (46.87%) and II (35.63%) etching patterns which are essentials for good bonding. Type III etching pattern was either absent or observed only in limited areas (17.5%). The process of deproteinization occurs when NaOCl solution come in contact with the organic material. Hence, specific reactions take place including saponification and neutralization leading to liquefaction of the organic matter in both enamel and acquired pellicle.[18]

The detection of types I and II etching patterns in group II is consistent with the findings of Espinosa *et al* [21] and Christopher *et al* [31] who found that treatment of enamel by NaOCl prior to application of acid etchant improved the quality of etching through the removal of excess enamel proteins. This action may explain the predominance of type I-II etching patterns in the present study. The limited occurrence of type III etching pattern in this group compared to group I provides further evidence for the effectiveness of NaOCl application on the hypocalcified AI primary teeth prior to acid etching.

On the other hand, the results of the present study are in contrast with Harleen *et al* [23] and Ramakrishna *et al* [32] who noted that enamel deproteinization prior to acid etching did not alter the surface topography of sound permanent teeth. They also suggested that the use of 37% phosphoric acid is still the best method for enamel etching. Furthermore, Harleen *et al* [23] found that enamel deproteinization using NaOCl did not enhance bonding to composite resins in sound permanent molars.

Since NaOCl enamel deproteinization prior to acid etching significantly increased the type I and II etching pattern, we conclude that enamel pretreatment of hypocalcified AI in primary teeth using 5.25% NaOCl produces a good quality of etching pattern.

One of the study limitations is the fact that the cases with hypocalcified AI teeth are not commonly encountered and thereby we could not enroll more cases. The study was also conducted in-vitro, where different oral environmental parameters such as oral pH and temperature could not be verified.

Further studies are recommended to evaluate the effect of NaOCl deproteinization, in-vitro and in-vivo, on the bond strength of different adhesive restorations to hypocalcified AI primary teeth.

CONCLUSION

Within the limitations of the present study, it can be concluded that treatment of primary teeth affected by hypocalcified AI using 5.25% NaOCl prior to phosphoric acid etching significantly improves the etching pattern which is required for good resin bonding.

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