Original Article

Effect of Deproteinization Before and after Acid Etching on the Surface Roughness of Immature Permanent Enamel

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Abstract

Objective: The purpose of this *in vitro* investigation was to assess the effect of deproteinization before and after acid etching on the surface roughness of immature human enamel of permanent teeth compared to acid etching alone using noncontact three-dimensional (3D) optical profilometer. **Materials and Methods:** Forty-eight enamel blocks were randomly distributed into 4 groups (12 each) according to the surface treatment in the form of deproteinized with 2.5% sodium hypochlorite (NaOCl) before and after acid etching with 32% phosphoric acid (H3PO4) compared to application of H3PO4 alone. The surface roughness (Sa) was measured using a 3D optical noncontact surface profiler. Two specimens from each group were selected and prepared for scanning electron microscopic (SEM) analysis. Shapiro–Wilk test, one-way analysis of variance, and Tukey's honest significance difference test were used. All statistical analyses were established with a significance level of $P < 0.05$. **Results:** The highest surface roughness (Sa) was recorded for Group 3/NaOCl ± H3PO4 and the lowest Sa was recorded for Group 1 (control). All surface treatments applied showed significantly greater values of surface roughness (Sa) than the enamel surfaces with no surface treatment (control). There was significant difference between control group and Group 2/H3PO4 ($P = 0.002$), Group 3/NaOCl ± H3PO4 ($P = 0.0001$), and Group 4/H3PO4 ± NaOCl ($P = 0.017$). There was no significant difference between Group 2/H3PO4 and Group 4/H3PO4 ± NaOCl. SEM evaluation showed different topographical features of deproteinized enamel surface. **Conclusions:** Deproteinizing the enamel of immature permanent teeth with 2.5% NaOCl before and after acid etching with 32% H3PO4 increased surface roughness compared to the application of H3PO4 alone.

Keywords: *Enamel deproteinization, enamel etching, phosphoric acid, sodium hypochlorite*

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Introduction

Sodium hypochlorite (NaOCl) is recognized as a potent solution for denaturing protein.[1,2] To address the restrictions of using acid only to etch the surface of the enamel, numerous noninvasive and invasive methods were used to improve the bond effectiveness between restorative materials and enamel.[3] The idea of deproteinizing enamel with 5.25% NaOCl before etching with phosphoric acid (H_3PO_4) and its influence on the patterns of etching and bonding of the adhesives to tooth structure has been investigated.[3,4] Enamel deproteinization with 5% NaOCl resulted in enhancing bonding of orthodontic brackets to the hypocalcified enamel.[5,6] This improvement of bond strength to hypocalcified enamel using restorative materials is extremely reliant on the alterations produced to the enamel surface and elimination of proteins.[6,7]

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Previous investigations aimed to increase the properties of the enamel to have better retention of the adhesive materials.[8,9] The use of NaOCl for deproteinization of enamel eliminates the organic elements of the acquired pellicle.[10] A study identified the topographical appearance of enamel deproteinized with 5.25% NaOCl and etched with H_3PO_4 concluded that the topographical features of enamel have more Types 1 and 2 etching pattern.[10] The degree of penetration of an adhesive resin in artificial enamel carious lesions was evaluated after using NaOCl as deproteinization agent showed a significant penetration of the sealing resin when the conventional technique is complemented with the application of 5.25% NaOCl for 1 min.[11] Other studies evaluated 10% papain gel for deproteinizing enamel with acid etching to enhance the quality of the bond between enamel surface and composite resin and resin-modified glass ionomer cement concluded that 10% papain gel can be used to deproteinize the surface of enamel to enhance bonding of orthodontic brackets.[12,13]

Surface roughness of enamel could be evaluated directly on extracted human teeth[14] or indirectly using epoxy replicas[15,16] or silicone impressions[17] for examination of enamel surface using scanning electron microscope (SEM). Indirect approaches do not permit an accurate measurement of surface roughness.[14] Roughness of enamel could be measured quantitatively using linear or optical three-dimensional (3D) tools and qualitatively using electron microscopy.[18]

The clinical application of the objectives of the study is reflected on the importance of increasing the properties of the enamel such as surface roughness to have better retention and to improve the bonding of the adhesive materials as deproteinization and acid etching could be essential aspects of clinical dentistry. This is generally seen in clinical practices where sealants, adhesive restorations, and orthodontic brackets are failing, which results in repeated dental visits, thus prolonging the dental treatment and increasing its cost. Deproteinization removes the organic content which could increase the enamel surface area and thus makes bonding more efficient. There is minimal research on whether acid etching before or after deproteinization of immature permanent enamel affects surface roughness. Therefore, the purpose of this *in vitro* investigation was to assess quantitatively the surface roughness of enamel of immature permanent teeth deproteinized with 2.5% NaOCl before and after acid etching with 32% H_3PO_4 compared to application of 32% H_3PO_4 alone using noncontact 3D optical profilometer. In addition, the topographical features of the enamel were qualitatively evaluated using the SEM. The null hypothesis tested was there is no difference in the effects of different applied surface treatments on surface roughness of the enamel.

Materials and Methods

This study was recognized by the Ethical Committee, College of Dentistry Research Center, King Saud University. Twenty-four human permanent third molars from adults 20–25 years with intact buccal surfaces were collected after extractions and stored in 0.1% thymol solution. The power sample size was 0.81 and level of significant $\sigma = 0.05$ with estimated standard deviation (SD) = 0.9, the sample size should be at least 10 in each group. Teeth with enamel malformations/defects, fractures/cracks of the buccal surface were excluded. Each tooth was rinsed with distilled water for 10 s, embedded in acrylic resin (Ortho-Jet, Lang Dental MFG. Co., Inc., IL, United States), and then sectioned vertically in the middle third of the buccal surface and roots were amputated using a low-speed diamond saw mounted under water spray (IsoMet-2000 Precision Saw, Buehler, Lake Bluff, IL, United States).

Forty-eight half enamel buccal surfaces were obtained and reembedded in the acrylic resin with the buccal surface flat and randomly distributed into 4 groups (12 each) according to the control (no treatment) and different surface treatments used. The enamel surface in Group 1 was not treated and acted as control. The dry enamel surface in Group 2 was etched with 32% H_3PO_4, Scotchbond universal etchant gel (3M ESPE, St Paul, MN, USA) which was applied with a microbrush for 15 s, rinsed for 15 s with water and dried with a cotton pellet. The enamel surface in Group 3 was treated with 2.5% NaOCl solution applied with sterile cotton pellet for 60 s, washed, then dried with water for 10 s, and etched as for Group 2. The enamel surface in Group 4 was etched with 32% H_3PO_4 gel similar to Group 2 and then treated with 2.5% NaOCl applied with sterile cotton pellet for 60 s, washed, then dried with water for 10 s. All the specimens were stored in distilled water at room temperature (approximately 25°C) for 24 h, rinsed with distilled water for 1 min, and blotted dry with tissue paper before measurement of surface roughness.

Optical profiler analysis

The surface roughness (Sa = Arithmetic mean height; in micrometer [μm]) of each specimen was analyzed with a 3D optical noncontact surface profiler (Contour Gt-K1 optical profiler, Bruker Nano, Inc., Tucson, AZ, USA) based on noncontact scanning interferometry. The objective standard camera ×1.0 has

a magnification ×5. The profile meter-scanned area (3 measurements in different directions) was approximately 1.3 mm × 1.0 mm and was situated at the center of each surface. Multi-Core Processor with Vision64 Software for accelerated 3D surface measurement and analyses were used for image transfer (Bruker Nano Surface Division, Inc., Tucson, AZ, USA).

Shapiro–Wilk test, one-way analysis of variance (ANOVA), and Tukey's honest significance difference test (HSD) were applied to compare and evaluate interactions between different surface treatments/groups. All statistical analyses were established with a significance level of $P < 0.05$. The statistical analysis was performed with SPSS Version 16.0 (SPSS Inc. Released 2007. SPSS for Windows, SPSS Inc., Ill Chicago, USA).

Two specimens from each group were prepared for SEM analysis of the topographical features of the enamel after surface treatment. The surface of the enamel was examined under a scanning electron microscope (Jeol JSM-T 330 A, Tokyo, Japan) and representative photomicrographs of the surfaces were taken at ×2000 magnification.

Results

The Shapiro–Wilk test for normality indicated that the data are normally distributed as $P < 0.05$.

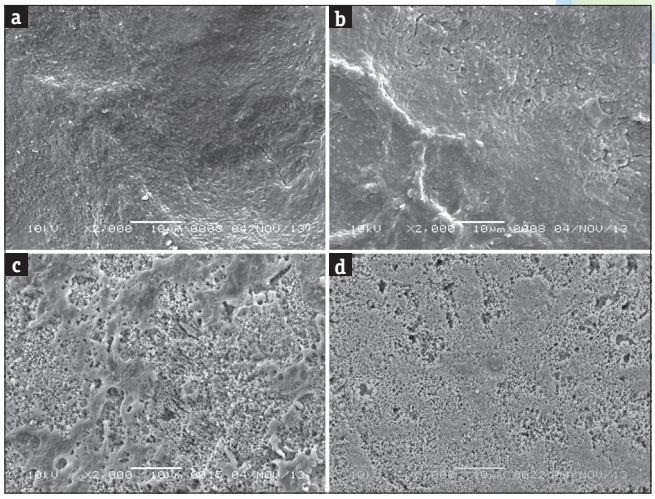

Figure 1: (a) Scanning electron microscope of the surface of the control group showing superficial layer of unground aprismatic enamel (2KX). (b) Scanning electron microscope of unground enamel surface etched with phosphoric acid showing remnants of aprismatic layer and indiscriminate type of etching pattern (2KX). (c) Scanning electron microscope of unground enamel surface treated with sodium hypochlorite then etched with phosphoric acid showing a porous nonuniform etching of the enamel prisms with pits and small areas of remnants aprismatic layer (2KX). (d) Scanning electron microscope of unground enamel surface treated with phosphoric acid then sodium hypochlorite showing an irregular, porous nonuniform prismatic structure with pits and amorphous areas of remnants aprismatic layer (2KX)

Descriptive statistics are presented in Table 1. The highest surface roughness (Sa) was recorded for Group 3 (Mean ± SD) (0.528 ± 0.056) in which the enamel surface was treated with 2.5% NaOCl solution and then etched with 32% H_3PO_4. The lowest Sa was recorded for Group 1 (0.270 ± 0.039) where enamel surface was not treated and acted as control.

All surface treatments applied showed significantly greater values of surface roughness (Sa) than the enamel surfaces with no surface treatment, which acted as control. One-way ANOVA revealed significant differences between the groups ($P < 0.0001$). Tukey's HSD showed significant difference between control group and Group 2/H_3PO_4 ($P = 0.002$), Group 3/NaOCl + H_3PO_4 ($P = 0.0001$), and Group 4/H_3PO_4 + NaOCl ($P = 0.017$). There was a significant difference between Group 2/H_3PO_4 and Group 3/NaOCl + H_3PO_4 ($P = 0.0001$). There was a significant difference between Group 3/NaOCl + H_3PO_4 and Group 4/H_3PO_4 + NaOCl ($P = 0.0001$). There was no significant difference between Group 2/H_3PO_4 and Group 4/H_3PO_4 + NaOCl.

SEM evaluation showed variations of the topographical features of enamel surface. SEM of the surface of the control group showed superficial layer of unground aprismatic enamel [Figure 1a] which lacking etching pattern. Figure 1b of unground enamel surface etched with H_3PO_4 shows remnants of aprismatic layer and indiscriminate type of etching pattern, which was mild. SEM of unground enamel surface treated with NaOCL then etched with H_3PO_4 showed a porous nonuniform etching of the enamel prisms with pits and small areas of remnants aprismatic layer [Figure 1c]. Increasing retention of enamel surface was observed compared to Figure 1b and Types 1 and 2 etching patterns were observed. SEM of unground enamel surface treated with H_3PO_4 then NaOCL showed an irregular, porous nonuniform prismatic structure with pits and amorphous areas of remnants aprismatic layer [Figure 1d]. Etching pattern was similar to that of Figure 1c, and Types 1 and 2 etching patterns were observed.

Table 1: Mean, standard deviation, standard error and range for all groups

Groups	Mean±SD	SE	Minimum	Maximum
Group 1	0.270±0.039	0.011	0.187	0.323
Group 2	0.362±0.065	0.019	0.257	0.454
Group 3	0.528±0.056	0.016	0.433	0.607
Group 4	0.343±0.066	0.019	0.236	0.435

SD=Standard deviation; SE=Standard

Discussion

The null hypothesis of the present study was rejected as there was a difference in the surface roughness of the enamel subjected to deproteinization with NaOCl before or after acid etching with H_3PO_4 compared to application of H_3PO_4 alone. The present study showed that deproteinization with 2.5% NaOCl before or after acid etching with 32% H_3PO_4 as well as the application of 32% H_3PO_4 alone increased surface roughness of immature enamel compared to no treatment in the control group. However, no difference was found between Group 2 in which H_3PO_4 was used and Group 4 in which H_3PO_4 was used followed by NaOCl. In contrast, previous studies concluded that the use of 37% H_3PO_4 alone is better than application of 5.25% NaOCl before acid etching.[4-6] In the present study, unground enamel surface etched with H_3PO_4 showed remnants of aprismatic layer and indiscriminate type of etching pattern which was mild. However, other investigations have demonstrated that the topographic features of enamel etched with H_3PO_4 are not accomplished over the whole surface as only about 30% of the treated surface had etching, 2% was perfectly etched, and 7% showed tenuous etching.[19,20] This is was also similar to the SEM results of this study where unground immature enamel surface treated with H_3PO_4 followed by NaOCL or vice versa showed an irregular, porous nonuniform prismatic structure with pits and amorphous areas of remnants aprismatic layer and Types 1 and 2 etching patterns. These consequences may be seen clinically with failure of the adhesive restorations, orthodontic brackets, or sealants.[21,22] Therefore, grinding the enamel to increase surface retention and eliminate part of the organic material present was suggested.[23] Compared to the aforementioned invasive method, NaOCl is a noninvasive method which acts on fatty acids to reduce surface tension, acts on cell metabolism to inhibit its enzymatic action, neutralizes amino acids, and denaturalizes proteins formation.[24] All surface application treatments completed in this study showed significantly more values of roughness than unground enamel with no surface treatment (control) which indicates differential enamel loss due to different treatments.[25] This is reflected on the measurements of surface roughness, causing increased peak-to-valley heights of noncontact 3D optical profilometer used in this study.[25]

One study utilized 5.25% NaOCl before H_3PO_4 etching demonstrated that prior deproteinization by NaOCl doubled retentive surface of the enamel to 94.47%.[3] On the other hand, a study examined the effect of pre-post deproteinization treatment with 5% NaOCl on bonding adhesive resin to enamel of primary, immature, and mature permanent teeth concluded that deproteinization after acid etching significantly enhanced bonding to primary and immature permanent teeth.[26] The enamel surfaces used in this study were unground and only buccal surface was used. In other studies, enamel was ground and also self-etching as well as H_3PO_4 with different concentrations was used for etching. In our study, enamel in the middle third of buccal surface was used to have comparable zone from different teeth with possible similar physical and chemical characteristics. The same area of enamel was used to avoid influence of enamel structural such as dissimilarities in the alignment of enamel prisms and sheath on the roughness.[27,28] Furthermore, to anisotropic structure of enamel, the chemistry of the surface influences the properties such as more mineralized surface (9%) than inner enamel after eruption.[27-29] The presence of natural roughness of enamel might be due to Retzius grooves, pits, small defects, and mineral deposits.[30] However, in our study, teeth were not polished with pumice as a previous study indicated slightly increase in the roughness after acid etching and pumice prophylaxis.[30]

In the present study, we used immature permanent teeth. Enamel surfaces of immature permanent teeth are more porous, contain more protein and less mineral than mature permanent teeth.[31,32] The action of H_3PO_4 on the surface of enamel occurs generally on its mineralized part and this acid does not eliminate the organic matter.[4] Previous studies have shown that acid etching patterns and bond strength values of primary and immature permanent teeth differ from mature permanent teeth.[32-34] Bond strength values in primary and immature permanent teeth deproteinized after acid etching significantly enhanced bond strength values when compared with only acid etching and deproteinization before acid etching.[26] In immature permanent teeth, deproteinization after acid etching increased the shear bond strength significantly so that it nearly reaches the mature teeth control group and the difference in between the groups becomes nonsignificant.[26] As in orthodontic bonding, acid etching of uncut enamel surfaces produces a tough profile, caused by the aprismatic layer of enamel which is extremely packed with apatite crystals creating an acid-resistant substrate.[35] In the present study, SEM evaluation revealed the differences made on enamel after different surface treatment and showed different patterns of etching between different groups compared to control group which showed superficial layer of unground aprismatic enamel lacking etching pattern. These differences may be due to separate and combined effect of the material used for different surface treatments.

The linear contact profilometer is a linear measurement for roughness, but it produces lower roughness (Ra) values than the optical profilometer due to the inadequacy

of the spatial dimensions of its tip in detecting microcracks.[36] Furthermore, the contact profilometer may injury enamel because of its contact with the surface. Optical profilers measure roughness (Sa) of a selected microarea at a high spatial resolution with no contact with the specimen. In addition, preparation of the specimen is not required.[14] Sa is a surface roughness, and for technical surfaces, the relationship between Ra and Sa is 1.25; however, this rule does not have to apply to biological specimen.[14] We used a noncontact 3D optical profilometer in this study.

The most commonly mentioned limit of surface roughness (Ra) is below 0.2 μm for adherence of dental biofilm and increase of roughness above this value lead to accumulation of bacteria.[37,38] However, the aforementioned investigations were not performed on enamel surface, but on artificial materials such as cellulose acetate. The surface of enamel is extremely complex with different irregularities which permits bacterial colonization.[39] The greater the level of magnification during measurement of roughness, the lower Ra or Sa values measured for the same surface. Thus, the results from different studies cannot be simply compared. No study reporting human enamel 3D roughness measured at a similar magnification has been published for comparisons.

Effect of saliva and pellicle *in vivo*

The results of this investigation should consider the limitations of the study, including its *in vitro* setting, which may not simulate the effect of deproteinization and acid etching *in vivo*. In addition, the clinical condition in the mouth is not easy to mimic in the laboratory.[40] It is also difficult to maintain a standardization during processing of the samples and application of different surface treatment in the laboratory. The enamel specimens in our study might not have the same quality despite the fact that the same areas of enamel were used in this study to have comparable zone from different teeth with possible similar physical and chemical characteristics. Nevertheless, in this *in vitro* study, standardization of experimental conditions was followed whenever possible and the results demonstrated a clear effect between surface roughnesses of the tested surface treatment.

Conclusions

Within the methodology of this investigation, it can be concluded that deproteinizing the enamel of immature permanent teeth with 2.5% NaOCl before and after acid etching with 32% H_3PO_4 increased surface roughness compared to application of 32% H_3PO_4 alone. The lowest surface roughness was recorded for untreated enamel surface. The highest surface roughness was recorded for the enamel surface treated with 2.5% NaOCl solution followed by etching with 32% H_3PO_4.

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Conflicts of interest

There are no conflicts of interest.

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