Enamel Deproteinization Before Acid Etching and its Effect on the Shear Bond Strength – An *in vitro* Study

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Objective: *This study was undertaken to assess the effect of enamel deproteinization with 5.25% sodium hypochlorite (NaOCl) before phosphoric acid (H_3PO_4) etching on the shear bond strength of Adper™ Single Bond 2 adhesive and Filtek™ Z- 350 XT composite resin .* ***Study design:*** *Forty human sound permanent molars which were extracted for periodontal reasons were used in the experimental protocol as under: Group-A (20 teeth):– A 5X4 mm window of the enamel surface was etched with 37% H_3PO_4 gel for 15 seconds, washed with distilled water and air dried. A single coat of Adper™ Single Bond 2 adhesive was applied and photo polymerized for 20 seconds and Filtek™ Z- 350 XT composite resin block of length 5mm, width 4mm and height 5mm respectively was built and photo polymerized in increments for 20 seconds each. Group-B (20 teeth):- Similarly the enamel surface was treated with 5.25% NaOCl for 60 seconds, washed with distilled water and then etched with 37% H_3PO_4 gel for 15 seconds, washed with distilled water and air dried. A single coat of Adper™ Single Bond 2 adhesive was applied and photo polymerized for 20 seconds and Filtek™ Z- 350 XT composite resin block of length 5mm, width 4mm and height 5mm respectively was built and photo polymerized in increments for 20 seconds each. The shear bond strength of all the samples were measured (in MPa) on Instron Mechanical Testing Machine.* ***Results:*** *The mean shear bond strength value for Group-A was 13.51 ± 5.726 MPa and for Group-B being 15.06 ± 6.220 MPa. No statistically significant difference in the shear bond strengths was observed between the two groups.* ***Conclusions:*** *No significant effect of sodium hypochlorite enamel deproteinization on the shear bond strength of Adper™ Single Bond 2 adhesive and Filtek™ Z- 350 XT composite resin before acid etching was observed in this study.*

Keywords: *Sodium hypochlorite, Enamel Deproteinization, Phosphoric acid, Etching, Adhesive, Composite resin, Shear bond strength*

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INTRODUCTION

The goals of acid etching enamel are to clean the dental structure, remove the smear layer, microscopically increase the roughness by removing the prismatic and interprismatic crystals and to increase the free surface energy to produce sufficient monomer infiltration, seal the surface with adhesive and contribute to the retention of the resin composite restorations.[1] Phosphoric acid (H_3PO_4) ranging from 30% to 40% have been used for decades to bond resin – based materials to the enamel.[2,3] The micromechanical retention of resin materials into the enamel porosities, resulting from acid etching, thus creates a strong and durable bond.

However, recent studies have shown that the topographic quality of the enamel etching with 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] 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. Originally four enamel etching patterns have been described by Silverstone *et al* in 1975[6] and Type-I and Type-II patterns are considered to be ideal for a good bond. Espinosa *et al*[7] in 2008 showed that removing organic content from the enamel surface with 5.25% sodium hypochlorite (NaOCl) as a deproteinizing agent prior to H_3PO_4 etching, doubles the enamel's retentive surface significantly

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from 48.8% to 94.47% and increased the Type-I and Type-II etching patterns. This technique could optimize adhesion significantly by removing organic elements of both the enamel structure and acquired pellicle. In support to their preceding study[7] Espinosa *et al*[8] again in 2010 evaluated the qualitative and quantitative resin tag penetration with a resin replica model and concluded that enamel deproteinization with 5.25% NaOCl for 60 seconds prior to H_3PO_4 etching almost doubled the enamel's retentive surface from 46% to 73% and the topographical features of the replica resin penetration surface increased significantly with Type-I and Type-II etching patterns. On the contrary, Bhoomika *et al*[9] in 2010 concluded that 5.25% NaOCl enamel deproteinization did not grossly alter the surface topographic features of enamel surface before acid etching in relation to Type-I and Type-II etching patterns and the use of 37% H_3PO_4 for 15 seconds alone has been proved to be the best method for pre-treatment of enamel. One more significant finding observed in our previous study[9] was the clogging of the etched surface in deproteinized enamel samples because of accumulation of organic debris which might hamper the best possible clinical outcome i.e. adhesion quality or the bond strength.

Low shear bond strength can be associated with inadequate bonding and wider gaps between the restoration and the tooth. These gaps may allow: 1) bacterial infiltration 2) post - operative pulpal inflammation and 3) microleakage.[10] Two key factors encountered for adhesive failure reside in the quantity of the etched surface as well as in the quality of the etched pattern. It may be possible that if 5.25% NaOCl deproteinization of enamel surface enhances the Type-I and Type-II etching patterns as proved in the previous studies,[7,8] then at the same time it should also enhance the effective bond strength between the enamel surface and adhesive and composite resin complex. Roberto *et al*[11] in 2010 concluded from their *in vitro* study that enamel deproteinization with 5.25% NaOCl before acid etching significantly increased the bracket bond strength with respect to Fuji Ortho LC and Transbond XT as well.

Hence this *in vitro* study was undertaken to support the results of our preceding study,[9] if any, by assessing the effect of 5.25% NaOCl enamel deproteinization before acid etching on the shear bond strength of adhesive and composite resin complex.

MATERIALS AND METHOD

This study was partially carried out in the Department of Pedodontics and Preventive Dentistry, K.D. Dental College and Hospital, Mathura, Uttar Pradesh, INDIA and at Central Institute of Plastics Engineering and Technology (CIPET), Amritsar, Punjab, INDIA. Institutional Ethical Committee clearance was obtained prior to the start of this study. Informed written consent was also obtained from the patients who participated in this study.

Fifty seven human sound permanent molars extracted for periodontal reasons were chosen from the patients attending the Department of Oral and Maxillofacial Surgery of K.D. Dental College and Hospital. The teeth with enamel cracks or fractures along their buccal aspect, malformations, carious lesions, restorations or erosions were excluded from the study.[7,9] Immediately after extraction all the teeth were thoroughly washed in the running water to remove blood and any adherent tissue was removed using hand scalers. All the teeth were collected, and utilized for the study within a span of four months.

The extracted teeth were then stored in distilled water with thymol crystals added to it for a maximum of one week. Thereafter, they are stored in distilled water in a refrigerator until their use. The distilled water was changed weekly to minimize deterioration.[12]

Forty permanent molars from the collected specimens were randomly divided into 2 groups of 20 teeth each. The apices of all the teeth were sealed with sticky wax. The teeth in each group were separately color coded for the purpose of differentiation.

Any visible water was removed by a light/brief stream of oil-free compressed air. An adhesive tape with a 5mm/4mm rectangular window was applied to the buccal surface of the crown to delineate an area for etching and bonding. The middle of the middle third of the buccal surface was utilized for pre-treatment as per the protocol followed in the earlier study[9] for standardization and comparison if any. The delineated buccal surface of all the teeth were treated as per the following protocol for each group:

Group-A*:* The delineated enamel surface was etched with 37% H_3PO_4 gel (Dentsply International, USA) applied with a micro brush (3M ESPE, USA) for 15 seconds, washed with distilled water and then dried with oil free compressed air for 20 seconds. A single coat of Adper™ Single Bond 2 adhesive (3M ESPE, St. Paul, MN, USA) was then applied to the etched enamel surface according to the manufacturer's instructions and was photo polymerized for 20 seconds. Filtek™ Z- 350 XT composite resin block of length 5mm, width 4mm and height 5mm respectively was built on the bonded surface and photo polymerized in increments for 20 seconds each. The same procedure was followed for all the specimens.

Group-B*:* The delineated enamel surface was first treated with 5.25% NaOCl applied with sterile cotton pellet for 60 seconds, washed with distilled water and dried with oil free compressed air for 10 seconds and then etched and bonded as per the protocol followed in Group-A.

Each tooth was then mounted in stainless steel molds of height 1½ inches and diameter of 2.5 cm containing self cure acrylic resin (DPI, Mumbai, India). The teeth were aligned with in the molds so that their buccal surface of the crowns was exposed such that the composite block was 1mm above the mounting surface[12] and paralled the force during shear bond strength testing. The mounted teeth were then placed immediately in water to reduce the heat generated by polymerization reaction.

All the test samples were stored in water for 24 hrs at 37ºC prior to testing (Test type-1[12]). The shear bond strength of all the samples were measured on Instron Mechanical Testing Machine (LR – 100K, Lloyd Instruments, U.K.)

using a stainless steel shear probe. The specimens were tested using a cross head displacement rate of 0.5 mm/ minute.[12] The minimum load required to produce the bond failure was determined from the first load drop on the load deflection plot. Shear bond strength values were calculated in megapascals (MPa).[12]

Statistical Analysis

The data thus obtained was subjected to statistical analysis which was performed using SPSS (Statistical package for social sciences) version 15.0 for Windows with Independent - 't' test to compare the shear bond strength values of both the groups and the level of significance was set at 0.05 (5%).

RESULTS

The mean values of the shear bond strength obtained for Group - A were 13.51 ± 5.726 MPa and for Group - B were 15.06 ± 6.220 MPa (Table 1, Table 2 and Bar diagram 1). The independent -'t' test revealed no statistically significant difference between the two groups ($p > 0.05$) (Table 3).

Table 1. Mean values of shear bond strength in Group-A and Group-B

Test Sample No.	Group- A (MPa)	Group- B (MPa)
1	13.12	21.02
2	11.94	13.12
3	10.88	19.09
4	9.12	15.71
5	12.06	35.05
6	14.96	10.36
7	17.24	8.09
8	11.76	15.66
9	6.2	7.48
10	15.69	24.13
11	16.56	11.29
12	31.92	11.33
13	20.46	13.34
14	6.83	10.06
15	14.72	12.46
16	16.8	13.37
17	7.35	15.12
18	12.06	14.68
19	10.89	16.21
20	9.69	13.78
Mean	**13.51**	**15.06**

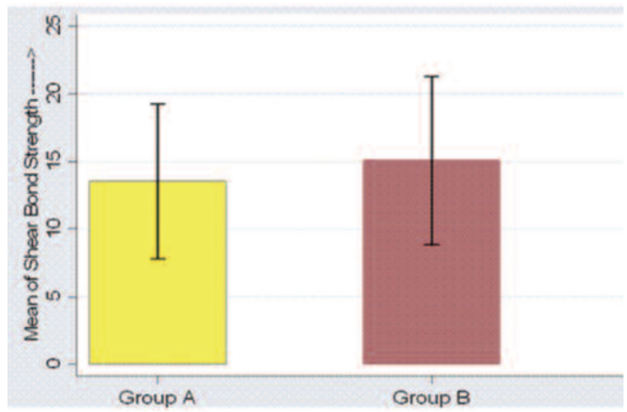
Table 2. Description of the results regarding shear bond strength values (MPa)

Statistics	Group-A	Group-B
Mean	13.51	15.06
Standard Deviation	5.72678	6.22012
Standard Error	1.28055	1.39086
Coefficient Variation	40%	40%
Minimum value	6.20	7.48
Maximum value	31.92	35.05

Table 3. Independent "t" test (comparison between the two Groups)

	N	Mean ± S.D.	t value	p value
Group - A	20	13.51 ± 5.726		
Group - B	20	15.06 ± 6.220	-0.8225	0.4159 *
Total	40	14.29 ± 5.953		

$p > 0.05$ – * Not significant



Bar Diagram 1. Box chart display of mean shear bond strength values

DISCUSSION

In spite of enormous advances in adhesive technology since the introduction of enamel etching by Buonocore[13,14] five decades ago, the interface of dental hard substance and composite, luting agent or adhesive remains the Achilles heel of adhesive restorations.[15,16] Phosphoric acid is commonly used to etch hard tough tissues in an attempt to improve adhesive infiltration and retention. They selectively demineralize to expose patterns of prismatic and interprismatic enamel. But the topographic quality of enamel etching is not achieved over the entire adhesion surface.[4,5] To counteract these limitations various invasive and non-invasive techniques including NaOCl deproteinization[7-9,11] have been investigated.

Adhesive performance on enamel and dentin may be quantified using several methodologically distinct approaches, roughly divided into macro or micro setups, depending upon the size of the bonded area.[17] The macro bond strength, i.e. with a bonded area larger than 3mm^2, can be measured in shear or tensile mode, or using a push-or pull-out protocol. For its unrivalled ease and speed of use, shear bond strength technique remains the most commonly used macrobonding test method.[17]

Although in vitro tests do not completely predict how dental materials will behave in the oral cavity, these tests are valuable. In 1994 International Organization for Standardization (ISO) presented guidance on adhesive testing[18] and modified in 2003[12] in order to standardize adhesion tests so that in vitro studies could provide similar, relevant and reproducible results that would support in vivo testing.[19] The generally observed wide variation of bond strength of given adhesives was attributed to the challenging number of influencing factors in specimen preparation and testing. Pashley *et al*[20] had summarized the bonding variables for etch-and-rinse adhesives, subdividing them into substrate, etching, priming, bonding, storage and testing.

In the present study the enamel surface was not altered[12] prior to etching and deproteinization respectively for both the groups, thus allowed us to correlate the results with our preceding study.[9] Also the shear bond strength in this study was tested after storing the test samples for 24 hrs in water at 37ºC (Test type-1)[12] only and thermocycling was not employed. Thermocycling can lower bond strength values, since hot water accelerates hydrolysis,[21] although this is a matter of discussion in the literature. Many researchers concluded that there is no significant difference in the mean bond strengths after thermal stress.[22-31] Bishara *et al*[32] concluded that in most of the bond strength studies, conclusions are drawn from the thermocycled samples only and they recommended that the thermocycled samples should be compared with non-thermocycled samples. Hence further studies are also needed to evaluate the effect of thermal stress on the bond strength. Moreover the template[12] required for building the composite resin block was also not employed in this study keeping the unaltered curved buccal surface of all the test specimens into consideration.

The mean shear bond strength values obtained in this study for Group – A was 13.51 ± 5.726 MPa and for Group-B were 15.06 ± 6.220 MPa respectively. No statistical significant difference in the mean shear bond strength values was observed between the two groups. As observed by Espinosa *et al*[7] in 2008 and further supported by their excellent study using resin replica model[8] in 2010 regarding the effect of enamel deproteinization before acid etching in significantly enhancing the Type-I and Type-II etching patterns, we did not observe an enhancement of the effective bond strength of the these deproteinized enamel samples in our study. Roberto *et al*[11] concluded that enamel deproteinization has a significant effect on the bracket bond strength with respect to Fuji LC and Transbond XT as well. The results of the present study supports our earlier study[9] and we also feel that enamel deproteinization with 5.25% NaOCl has no enhancive effect on the shear bond strength of Adper™ Single Bond 2 adhesive and Filtek™ Z- 350 XT composite resin. Hence, further studies are needed to prove the role of enamel deproteinization before acid etching and its effect on the bond strength.

CONCLUSIONS

Within the limitations as in any other *in vitro* study, the following conclusions can be drawn from this study:

The shear bond strength was not enhanced after enamel deproteinization with 5.25% NaOCl. It is logical to believe that more extensive research is needed to derive a final conclusion about the effect of enamel deproteinization. The use of 37% phosphoric acid for 15 seconds still remains the best method for pre-treatment of enamel.

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