

Comparing The Effect Of Primary Enamel Deproteinization Before And After Acid Etching On The Shear Bond Strength - An In-Vitro Study

Research Article

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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 shear bond strength when primary enamel is bonded to composite resin.

Materials and Methods: Forty-five enamel specimens were randomly distributed into 3 groups (15 each) according to the surface treatment, in Group I: enamel is acid etched only, in Group II: specimens are acid etched then exposed to 1 minute NaOCl deproteinization, and in Group III enamel specimens were deproteinized then acid etched. An adhesive was applied (Adper Single Bond universal) was applied, nanohybrid composite (Z350) was placed using Tygon catheter. All the samples were then subjected to the SBS test using a universal testing machine. Data analysis was performed using a one-way ANOVA test followed by the Tukey test. P-values less than 0.05 were considered significant.

Results: Comparison of the mean SBS between the groups showed a statistically significant difference between all groups, Group II displayed the highest mean value followed by Group III, while Group I where enamel was only etched with phosphoric acid showed the least mean value.

Conclusions: Deproteinizing the enamel of primary teeth with NaOCl before or after acid etching showed a significant increase in the SBS compared to the application of acid etch alone, moreover, deproteinization after acid etching yielded the highest SBS values.

Keywords: Shear Bond Strength; Primary Enamel; Deproteinization; Sodium Hypochlorite.

Introduction

In order to achieve adequate bonding to enamel, appropriate preparation of the surface is required, which involves removing the outer pellicle and surface roughening, in a process called conditioning. Acid etching with phosphoric acid is one of the conditioning techniques that uses acid gel to create micro-porosities on the surface, that can render it more receptive to resin penetration and thus better adhesion through micromechanical interlocking [1].

The advancement of enamel pretreatment with orthophosphoric acid by Buonocore in 1955 [2] is a benchmark in adhesive and cosmetic dentistry. The breakthrough concerned enhancing the adhesion of acrylic resins to enamel set the stage for abundant research work to reach to a better understanding and improvements in the quality of adhesive bonds to the tooth structure through

endeavors involving various materials and techniques.

The success of adhesive restorative materials and their long-term clinical performance is challenged by the continuous exposure to conditions that may affect their bond strength; as these materials have to withstand high mechanical forces during mastication and hence, a strong bond to the tooth structure is required [3].

It has been established that factors influencing bonding to enamel include the type of etching agent, duration of etching, concentration of acid being used, composition of the enamel surface and removal of organic material [4]. And hence, higher amount of protein content reported in defective enamel structures has been recognized by earlier studies to adversely affect the quality of the bond [5, 6].

Given the structural differences between primary and permanent

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enamel regards the less mineral content, the presence of a thicker outer layer of less organized primeless enamel and for most, the higher amount of organic content. All these features compromise the etching capacity, bonding mechanism, and bond efficacy of the resinous material to primary enamel [7].

Enamel deproteinization has been proposed as a non-invasive technique that aim to achieve a clinically successful etching pattern and improve the bond strength through effectively removing the organic content from the enamel surface [8-10].

Sodium hypochlorite (NaOCl) is recognized as potent protein denaturant, it has been used efficiently to eliminate organic material from the root canal spaces, and consequently it was thought of as a possible maneuver to optimize adhesion to the tooth structure through ridding enamel from the organic elements investing the outer layer the enamel structure and the acquired pellicle [8].

The pioneer attempt by Espinosa et al. back in 2008 [8], showed that enamel pretreatment with 5.25% NaOCl for 60 seconds before acid etching significantly improves both the quantity and the quality of the etching namely duplicating the area of etched enamel in addition to significantly increasing the proportion of type I and II etching patterns that have greater retentive capacities compared to type III. Thus, this modality was dubbed with high potential to optimize adhesion and improve bond strengths. The same researchers further affirmed their results with a following study two years later using resin replica models showing a high proportion of resin tag penetration equivalent to type I and II etching in the samples that were subjected to deproteinization prior to etching on larger areas of the etched enamel specimens [12]. Moreover, adhesive resin showed a significant penetration in artificial enamel carious lesions specimens evaluated by Gómez et al., when the conventional technique was complemented with NaOCl deproteinization [12].

Although there is almost a unanimity that deproteinization improves the quality of the bond of the tooth substrates to resin, however, there is not a consensus regarding applying the agent before or after acid etching, as other researchers further substantiate that deproteinization granted better results when conducted on acid etched enamel specimens with relatively high protein content as those of primary and immature permanent teeth [13-15].

Taking in consideration the uncertain sequence of application and the paucity in literature investigating the bond strength of composites bonded to deproteinized primary enamel rather than exploring only the topographic changes, this study's main objectives is to test the null hypothesis that there is no difference concerning the effect of primary enamel deproteinization before or after acid etching on composite resin shear bond strength in addition to validating the effect of that extra step on the bonding outcome.

Materials and Methods

This study is an experimental in-vitro study, comparing the shear bond strength values of three groups of primary enamel specimens bonded to composite resin where the specimens are subjected to different conditioning methods prior to bonding.

Forty five (N=45) sound primary molars that were extracted due

to looseness caused by physiologic root resorption from patients visiting the outpatient clinic of the Pediatric Dentistry and Dental Public Health Department, Ain-Shams University, Cairo, Egypt, were included in the study. The teeth with intact coronal portion only were used in the study while those with enamel cracks or fractures along the buccal aspect, malformations, carious lesions, restorations or erosions were excluded. Teeth were washed under running water and cleaned from any debris and attached soft tissue, and immersed in saline solution which was daily renewed until being tested [16].

Each tooth was cut 2mm below cement-enamel junction and sectioned mesiodistally into two halves under copious air-water coolant spray using a diamond disc mounted on a low speed straight hand piece, the sectioned buccal surfaces were totally embedded in chemical cured acrylic resin placed in polyvinyl ring such that the dentin side was embedded within the acrylic and the buccal enamel surfaces were exposed for bonding in order to allow for standardized and secured placement during SBS testing and later the specimens. [17].

A 320 grit Sand paper was used for flattening & a 400 and 600 grit sand paper were used for smoothening of the enamel surface under water coolant in order to obtain a smooth flat surface. The specimens were cleaned with running water and ultrasonic cleaner to ensure absence of any debris [15].

The enamel specimens were assigned numbers then randomly allocated in the following groups as follows:

Group I: (control) The enamel surface was etched with 37% H₃PO₄, applied for 15 s with a microbrush, washed with sterile water for 20 s, and then dried with compressed air for 15 s [15].

Group II: (acid etching followed by deproteinization) the samples were etched as in group I, after achieving dryness, the surfaces were treated with 5.25% NaOCl (Clorox®) applied with a sterile cotton swab for 60 s, washed with sterile water, then dried for 10 s [16].

Group III: (deproteinization followed with 5.25% NaOCl) 5.25% NaOCl was applied with a sterile cotton swab for 60 s, washed with sterile water, then dried for 10 s, then the samples are etched as in group I.

Later, all the conditioned enamel surfaces were bonded to composite as follows, A disposable micro brush was used to apply the adhesive on the tooth structure for 20 seconds, followed by gentle air drying for 5 seconds till complete evaporation of the solvent which was assessed by the absence of motion of the adhesive layer on the tooth upon application of air then it was light cured for 20 seconds with Elipar™ light cure with a light intensity of 1200 Mw/cm². Rubber Tygon catheter of 2 mm internal diameter and 2 mm height was placed on the etched enamel surface to act as a mould for building composite buttons on enamel surfaces. The catheters were cut off using a sharp lancet, and the specimens were stored in normal saline at 37°C for 24 hours before testing [17]. The SBS was done using universal testing machine with constant cross head speed of 1 mm/min using a chisel driving the load onto the specimen at the enamel-composite interface till debonding. Shear bond strength values were recorded as Newton (N) initially and then they were calculated as megapascals (MPa).

Results

Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows. Data normality was checked using Kolmogorov-Smirnov test and Shapiro-Wilk test. One-way ANOVA was used for comparison between groups followed by Tukey post hoc test. p values less than 0.05 were considered significant.

Comparing the mean SBSs among the study groups showed that the highest mean value was obtained in Group II, followed by Group III, while the least value was obtained in Group I.

One-way ANOVA test performed to compare the mean shear bond strength values among the study groups showed significant difference between studied groups and Tukey post hoc test showed statistical difference between all pairs.

Discussion

The increased preference for composites application among clinicians is credited to the conservative tooth preparation, good physical properties, esthetics and also their adhesive capability to tooth tissues [18]. A satisfactory bond strength between the tooth structure and the restorative material is crucial for the clinical success of the restoration, otherwise failure in form of recurrent caries, tooth sensitivity, and defective restorations might result from stresses created at the interface by resin contraction forces [19].

During its development, enamel is initially composed of a protein rich matrix, and as enamel matures, the protein matrix is degraded, and the preliminary hydroxyapatite crystals act as growth centers around which minerals are deposited during enamel maturation. In primary teeth however, the outermost enamel surface exhibits an excess of protein content in addition to a layer of disorganized aprismatic enamel which in turn negatively affect the etching procedure and hence the bond strength [20]. Phosphoric acid etch can demineralize the inorganic components of enamel, nevertheless it does not eliminate the organic matter on the enamel surface and from this point, deproteinizing agents came in interplay to augment the bond strength to enamel surface [21].

In this study, shear bond strength test (SBS) was chosen to assess the bond strength of composite resin to the preconditioned enamel surfaces. This test is very popular because of its relative simplicity as specimens do not need further processing following bonding, moreover specimens in other tests are difficult to be aligned in the testing machine without creating deleterious stresses. Furthermore, a good correlation coefficient is documented between annual failure rates of composite restoration and shear bond strength [22, 23].

Although there is a multitude of studies investigating the effect of different deproteinizing agents incorporated in the enamel conditioning protocol prior to bonding, yet the results of which are inconclusive and sometimes contradictory, which questions the validity of adding another step to the already technique sensitive procedure, furthermore, incorporating such step whether before or after acid etching resulted in perplexing outcomes, that even makes the sequence of application not a clear cut regimen.

The results of the current study showed that sodium hypochlorite enamel conditioning for one minute prior to bonding significantly improves the bond strength of composite to primary enamel, whether this step was conducted prior to acid etching or following it. This was found to go in harmony with the results of Aras et al. [13], as the authors also concluded from their research conducted on 3 different enamel types, that the deproteinization of both primary and immature permanent enamel improved the shear bond strength values to composite resin more than permanent enamel, the later only showed in substantial improvement with deproteinization. Their results could be explained through assuming that the effect of NaOCl is only detectable if applied on substrates higher in protein content.

And hence when this protein has been eliminated, the resultant enamel surface would display detectable bond enhancement, which is the case in primary and immature permanent enamels compared to the permanent enamel which has comparatively lower protein content.

Furthermore, a later study [14] on immature permanent enamel also found a favorable outcome of deproteinization regardless the sequence of application which was demonstrated as increased

Table 1. Comparing Mean, standard deviation, standard error and range of shear bond for all study groups.

Enamel pretreatment group	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum	P value
Group I	15	13.479	0.945	0.244	12.45	15.31	<0.001*
Group II	15	20.105	1.975	0.510	17.54	23.22	
Group III	15	18.719	1.272	0.329	16.32	20.9	

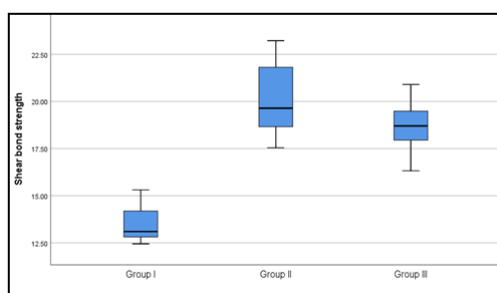


Figure 1. Box plot representation of the SBS values among the study groups.

surface roughness of the pretreated enamel, even with lower concentration of NaOCl (2.5% applied for one minute before or after acid etching) and on top off all, the researcher utilized non-polished and uncut enamel specimens which presumably retain thicker prismless enamel layer and organic pellicle at the surface, yet the results still validated the process of deproteinization to enhance the topographic features of enamel and make it more receptive to composite bonding.

On contrary, the results of Ahuja et al. [10] and Harleen et al. showed that enamel deproteinization before acid etching did not favor a stronger interface between the substrate and composite resin compared to specimens that are etched only, these two studies depicted no significant change in the etching patterns observed by scanning electron microscopy or the shear bond strength values respectively. It is worth mentioning here that the aforementioned researches utilized permanent enamel specimens which inherently display less organic content and maybe more abraded surfaces with less thickness of the aprismatic enamel layer, these two factors could have rendered deproteinization as a step with least value in the bonding procedure.

Although, the contamination of the etched enamel surface can jeopardize the bond between the conditioned enamel and composite resin. The highest shear bond strength values in this research were obtained in group II in which NaOCl deproteinization was applied on etched enamel surface just before bonding, in addition, these values were significantly higher than in the acid etch group (control) and group III in which deproteinization was initiated before acid etching. Therefore, it is evident that after acid etching there might be a better chance for NaOCl to work on the organic content and eliminate it from the etched surfaces, rather than acting before hand on unetched surfaces with higher inorganic content.

Regarding comparing the effect of different sequences of application of the deproteinizing agent whether before or after acid etching, Aras et al. [13] reached to the conclusion that NaOCl deproteinization following acid etching conceded the highest SBS values in primary enamel and immature permanent teeth specimens, compared to the reversed sequence (NaOCl/ acid etch). The authors further promoted this technique claiming that the SBS values of immature permanent enamel specimens treated with this exact sequence, approached those of permanent enamel that were only acid etched. This inference would encourage clinicians to advocate such protocol of enamel pretreatment specially in immature permanent teeth which are known for their porous, less mineralized and high in organic content enamel surfaces.

Later, Hasija et al. [15] demonstrated comparable results related to the improvement in the bond strength to primary enamel adopting the same sequence of application (acid etching/deproteinization sequence). The researchers however tested the effect of other deproteinizing agents (papain and bromelain proteolytic enzymes) in addition to the NaOCl, and all the study groups showed an enhancement in the mean values of SBS compared to the control group in which no deproteinization was done. That is why the authors advocated deproteinization after acid etching to achieve better clinical outcomes though they did not investigate the effect of applying the deproteinizing agent before etching.

Although deproteinization adds an additional step in the al-

ready technique sensitive procedure of applying composite restorations, which in turn increases the chair side time that could be problematic in young patients, yet the benefit of significantly enhancing the bond to primary enamel could encourage practitioners to incorporate this procedure routinely when considering primary enamel conditioning.

Conclusions

Considering the results of the current research the following conclusions could be withdrawn.

- NaOCl deproteinization could be considered as a complementing step to enhance the mechanical outcome of primary enamel conditioning prior to bonding to composite resin.
- The shear bond strength values significantly increases when deproteinization was preformed after acid etching compared to before acid etching or when no deproteinization was done.

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