Deproteinization Treatment on Bond Strengths of Primary, Mature and Immature Permanent Tooth Enamel

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Objective: *The aim of this study was to examine the effect of pre-post deproteinization treatment with 5% sodium hypochloride on shear bond strength (sbs) of adhesive resin to primary, immature and mature permanent teeth enamel.* ***Method:*** *30 teeth were used for each of primary, immature and mature permanent teeth groups. (totally 90). In control groups, enamel was etched for 60s with 37% phosphoric acid (3M) and rinsed for 10s (Procedure A). In experimental groups, deproteinization was applied with 5% NaOCI solution for 120s before (Procedure D+A) and after acid-etching (Procedure A+D). Gluma Comfort Bond (Heraeus-Kulzer) and Charisma (Heraeus-Kulzer) composite resin were applied to etched enamel surfaces. Data were determined with Two-Way ANOVA and LSD Multiple Comparison Test ($p<0.05$).* ***Results:*** *SBS was significantly lower in primary and immature permanent teeth than mature permanent teeth ($p<0.05$). "Procedure A+D" statistically increased sbs values in primary and immature permanent teeth ($p<0.05$).*
Conclusion: *Deproteinization after acid etching significantly enhanced the shear bond strength values in primary and immature permanent teeth.*
Keywords: *Enamel maturation, deproteinization, bond strength, acid-etch technique.*

INTRODUCTION

Traditionally, etching enamel surfaces with orthophosphoric acid, a concept first proposed by Buonocore (1955), has been a usual clinical procedure to increase the bond strength between the composite resin and etched enamel.[1] Today we know that etching quality depends on the type of etching agent, acid concentration, etching time, and organic removal.[2,3] Nevertheless, the structural differences of enamel are important on demineralization of enamel as at least the properties of the acid-etching materials.[4,5] Since there are structural differences between primary, mature and immature tooth enamel, it is logical to expect differences in etching quality and bond strength of resins to mature versus immature and primary teeth enamel.[6,7] In this respect, up to date a definite agreement is not present on ideal acid-etching times of immature permanent tooth enamel.[5,8] Similarly, there is a controversy regarding its actual effectiveness in primary tooth enamel.[9,10]

In previous studies it has been shown that, etching procedure is affected negatively by higher amount of organic structure and presence of an aprismatic layer on the enamel surface of deciduous and immature permanent teeth.[5,11,12] Although there are many studies that evaluated the bond strength of the conventional etching systems, a few studies considered the possible effects of the level of enamel proteins on etching quality.[5,8,13-15]

Sodium hypochloride (NaOCL) is known to be an excellent protein denaturant that should be capable of removing excess enamel protein.[13,16] Venezie *et al*,[13] predicted that pretreating Amelogenesis Imperfecta (AI) affected enamel with NaOCl would make the enamel crystals more accessible to the etching solution, resulting in a clinically more favorable etched surface. In this regard, it was observed that deproteinization treatment with NaOCl after acid conditioning have enhanced the bond strength of composite resin to Amelogenesis Imperfecta affected [14] and fluorosed tooth enamel.[15] Espinosa *et al* [17] showed that removing the organic content from the enamel surface with 5.2% sodium hypochloride as a deproteinizing agent prior to phosphoric acid etching, doubles significantly enamel retentive surface to 94.47% and increased the type I and II etched enamel. In a recent study, they showed that, enamel deproteinization prior to phosphoric acid etching almost doubled enamel retentive surface to 73% with resin replica technique.[18] However, studies dealing with the effect of NaOCl pre or post treatment on the bond strength of composites in primary and immature permanent tooth enamel have not been reported yet.

Therefore, the purpose of this study was to evaluate the effect of deproteinization treatment, before and after acid conditioning of the primary, immature (unerupted) and mature (erupted) permanent teeth enamel shear bond strengths.

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Table1. Descriptives of all test groups (Mean MPa ± Standard Deviation (SD), Standard Error, Minimum and Maximum values).

Tooth Groups	Procedures	n	Mean Mpa	± SD	Standard Error	Minimum	Maximum
Primary teeth	A	10	14,5070	2,89468	,91538	11,11	18,62
	A+D	10	18,4480	2,30085	,72759	13,61	21,16
	D+A	10	17,0560	2,98986	,94548	12,93	21,11
Mature permanent teeth	A	10	28,1160	3,46670	1,09627	24,40	35,46
	A+D	10	30,4500	4,91923	1,55560	26,67	40,91
	D+A	10	29,0620	3,04061	,96152	25,85	35,60
Immature permanent teeth	A	10	22,8400	4,13751	1,30840	15,76	27,73
	A+D	10	26,0660	3,79130	1,19892	17,64	32,53
	D+A	10	25,0450	4,04892	1,28038	19,62	31,74

MATERIALS AND METHOD

Maxillary and mandibular human premolars extracted for orthodontic treatment were collected from adult patients older than 21 years and served as the mature permanent teeth group. Unerupted third molars having completely formed root development surgically extracted served as the immature permanent teeth group (post eruptive maturation is not finished). Sound primary molars extracted for physiological root resorption were used as primary teeth group.

30 noncarious teeth were used for each of primary, immature and mature permanent teeth groups (totally 90 teeth). Teeth with enamel cracks or fractures along their buccal aspect, dental pathology, malformations, carious lesions, restorations or erosions were excluded. Initially, the teeth were cleaned with a rubber prophy cup and nonfluoride pumice to eliminate any contaminants and stored in 0.1 thymol dissolved in distilled water until used for examination. A flat surface of 3 mm in diameter was prepared on the buccal surfaces of all teeth by moist grinding on 200-400 and 600-grid silicon paper. Then teeth were embedded in acyrilic resin blocks. Each of the primary, immature and mature teeth groups were randomly divided into three subgroups (resulting in 10 teeth each).

Group 1 (A); Enamel surfaces of primary, immature and mature teeth were etched for 60 s with 37 % phosphoric acid gel (3M Multipurpose Etching Gel, 3M Dental Product USA) washed with water and air spray for 20 s and then dried with oil free compressed air.

Group 2 (AD); Enamel surfaces of primary, immature and mature teeth were etched as Group 1 and then 5% NaOCI solution was applied for 60 seconds and rinsed with water for 20 seconds before bonding application.

Group 3 (DA); After deproteinization with 5% NaOCI solution for 60 seconds as group 2, enamel surfaces were washed and dried with water syringe and then similar etching procedures as in Group 1 was performed in all three types of teeth.

Two separate adhesive layers of Gluma Comfort Bond (Herause-Kulzer, Germany) were applied to enamel surface using an applicator tip and light cured for 20s (Polofil Lux Unit, Voco, Germany). A cylindrical split teflon mold 3mm in diameter and 2mm in height was filled with Charisma microfilled composite resin (Herause Kulzer, Germany) in one increment . Each cylinder was bonded at a 90° angle to the enamel surface and light cured for 40 seconds.

The specimens were stored in deionised water at 37° C for 24 hours. Then, all the samples were thermo cycled for 1000 cycles between 5 and 55° C with a dwell time of 30 s and a transfer time of 10 s in each bath. Shear bond strength was measured in a Lloyd Universal Testing Machine (Lloyd LRX;Lloyd, Foreham, Herts, UK) with a crosshead speed of 1 mm/min. Shear bond strength values were recorded as Newton (N) initially and then they were calculated as Megapascal "MPa" with formula in below.

Megapascal (Mpa) = N (Newton) (Strength) / mm^2 (Area)

Statistical analysis of the shear bond strength values was completed utilizing two-way analysis of variance (ANOVA) and followed by LSD Multiple Comparison Test to evaluate differences among the groups at a significance level of ($p<0.05$).

RESULTS

Table 1 and 2 shows the mean and standart deviations of shear bond strengths for each group. All significance levels between groups were summarized in Table 3.

The primary teeth group A (14,5 ± 2,89 MPa) exhibited the lowest shear bond strength while the mature permanent teeth Group AD (30,45 ± 4,91 MPa) exhibited the highest bond strength among the groups (Table 1).

When comparing the control groups (group A) of primary, immature and mature teeth, the difference between the three groups was significant (Table 3).

The effect of deproteinization treatment before and after conventional acid etching procedures were found to have no significant effect on the shear bond strength of mature permanent teeth enamel

Table 2. Statistical differences between test parameters in study according to Two-Way-ANOVA ($p<0.05$).

Source	df	Mean Square	F	Significance level (p<0.05*)
Intercept	1	49744,809	3860,804	,000*
Types of tooth	2	1208,457	93,791	,000*
Test procedures	2	76,226	5,916	,004*
Type of tooth * Test procedures	4	2,343	,182	,947

Table 3. Statistically differences between test groups according to LSD Multiple Comparison Test (α<0.05).

LSD-Comparisons between groups		Mean Difference (I-J)	Standard Error	Significance levels (α<0.05*)
Primary teeth / Procedure A	Primary teeth / Procedure A+D	-3,9410	1,60528	,016*
	Primary teeth / Procedure D+A	-2,5490	1,60528	,116
	Mature teeth / Procedure A	-13,6090	1,60528	,000*
	Mature teeth / Procedure A+D	-15,9430	1,60528	,000*
	Mature teeth / Procedure D+A	-14,5550	1,60528	,000*
	Immature teeth / Procedure A	-8,3330	1,60528	,000*
	Immature teeth / Procedure A+D	-11,5590	1,60528	,000*
	Immature teeth / Procedure D+A	-10,5380	1,60528	,000*
Primary teeth / Procedure A+D	Primary teeth / Procedure D+A	1,3920	1,60528	,388
	Mature teeth / Procedure A	-9,6680	1,60528	,000*
	Mature teeth / Procedure A+D	-12,0020	1,60528	,000*
	Mature teeth / Procedure D+A	-10,6140	1,60528	,000*
	Immature teeth / Procedure A	-4,3920	1,60528	,008
	Immature teeth / Procedure A+D	-7,6180	1,60528	,000*
	Immature teeth / Procedure D+A	-6,5970	1,60528	,000*
Primary teeth / Procedure D+A	Mature teeth / Procedure A	-11,0600	1,60528	,000*
	Mature teeth / Procedure A+D	-13,3940	1,60528	,000*
	Mature teeth / Procedure D+A	-12,0060	1,60528	,000*
	Immature teeth / Procedure A	-5,7840	1,60528	,001*
	Immature teeth / Procedure A+D	-9,0100	1,60528	,000*
	Immature teeth / Procedure D+A	-7,9890	1,60528	,000*
Mature permanent teeth / Procedure A	Mature teeth / Procedure A+D	-2,3340	1,60528	,150
	Mature teeth / Procedure D+A	-,9460	1,60528	,557
	Immature teeth / Procedure A	5,2760	1,60528	,002*
	Immature teeth / Procedure A+D	2,0500	1,60528	,205
	Immature teeth / Procedure D+A	3,0710	1,60528	,059
Mature permanent teeth / Procedure A+D	Mature teeth / Procedure D+A	1,3880	1,60528	,390
	Immature teeth / Procedure A	7,6100	1,60528	,000*
	Immature teeth / Procedure A+D	4,3840	1,60528	,008
	Immature teeth / Procedure D+A	5,4050	1,60528	,001*
Mature permanent teeth / Procedure D+A	Immature teeth / Procedure A	6,2220	1,60528	,000*
	Immature teeth / Procedure A+D	2,9960	1,60528	,066*
	Immature teeth / Procedure D+A	4,0170	1,60528	,014*
Immature permanent teeth / Procedure A	Immature teeth / Procedure A+D	-3,2260	1,60528	,048*
	Immature teeth / Procedure D+A	-2,2050	1,60528	,173
Immature permanent teeth / Procedure A+D	Immature teeth / Procedure D+A	1,0210	1,60528	,527

(p >0,05). Similarly, deproteinization treatment did not affect the shear bond strength to enamel when employed before acid etching in either the primary or immature permanent teeth groups (p>0.05). However, bond strengths to primary and immature permanent tooth enamel were enhanced significantly when the deproteinization treatment was employed after acid etching procedure (p< 0, 05).

DISCUSSION

The initial stage of enamel development is characterized by the secretion of a protein-rich, partially mineralized matrix. During maturation, this matrix is removed by proteases with associated growth of hydroxyapatite crystals until the enamel reaches its final hardened stage.[19] Thus enamel surfaces of immature permanent teeth are more porous, contain more protein and less mineral than

mature permanent teeth.[6,8,20] Similarly, there is an aprismatic zone and excess proteins on the enamel surface of primary teeth.[21]

The action of phosphoric acid on the enamel surface occurs mostly on its mineralized part and this acid does not eliminate the organic matter on the enamel surface.[22] Previous studies have shown that acid etching patterns and bond strength values of primary and immature permanent teeth differ from mature permanent teeth.[8,11,23,24] As the bond between enamel and restoration is highly dependent on the enamel surface alterations, removal of the excess proteins in primary and immature permanent teeth may provide an advantage on the bonding of the restoration.[13,25]

Sodium hypochlorite is a non-specific proteolytic agent that effectively removes organic components at room temperature. Since NaOCl has been used to remove the organic tissues from the root canal space, it was thougth that its role in removing the organic content from the enamel surface may give fruitful results.[22] So, it was used as the deproteinizing agent in previous studies. These studies were conducted in amelogenesis imperfecta affected teeth,[14] fluorosed teeth[15] and mature permanent teeth.[17,18] The present study investigated the effect of removal of the excess organic matter by NaOCl pre or post treatment, on the bond strength of composites in primary and immature permanent teeth enamel in comparison with mature permanent teeth.

There are a lot of in-vitro researches on bonding materials in literature. It was reported that, adhesion of "Gluma" to enamel is performed by mediations of glutaraldehyde and HEMA. Glutaraldehyde adheres to organic structure and HEMA adheres to inorganic structure.[26,27] It was suggested that, changes in organic structure of teeth may affect the bond strength of Gluma positively or negatively.[28] Thus, Gluma was particulary chosen as bonding material in this study.

The results of the present study have shown that the shear bond strengths of primary and immature permanent teeth were significantly lower than the mature permanent teeth group. These findings are in accordance with previous studies[11,20,23,24] and shows that organic content of enamel plays crucial role on the mechanism of adhesion between resin material and enamel.

When comparing the effect of deproteinization it was found that deproteinizing after acid etching was more efficient than deproteinizing before acid etching in all groups. Although the shear bond strengths were enhanced after pre and post deproteinization, the difference between the groups was not significant in mature permanent enamel. There are a few studies reporting the effect of NaOCl deproteinization on mature permanent enamel. However, the studies generally dealed with the difference in topographic features after deproteinization. Ahuja *et al,*[22] who evaluated the effect of NaOCl enamel deproteinization before acid etching, reported that enamel deporteinization did not grossly alter the surface topographic features of enamel. However, Espinosa *et al*[17,18] investigated the topographical enamel features of a deproteinized enamel with NaOCl prior of acid etching and concluded that conventional acid etching of enamel has significant limitations, etching less than 50% of the total enamel's surface. They reported that enamel deproteinization prior to phosphoric acid etching doubles enamel's retentive surface. In the only study investigating the effect of deproteinization on shear bond strentgh of mature enamel, Justus *et al* [29] have reported that deproteinization with NaOCl prior to phosphoric acid etching enhanced the bracket bond strengths of adhesives. No research to our knowledge has been published evaluating whether deproteinization of primary and immature permanent enamel surfaces increases shear bond strength.

Bond strength values in deproteinization before acid etching groups were greater than only acid etching groups in all three types of teeth, but the differences were not significant. However, in primary and immature permanent teeth groups deproteinization after acid etching significantly enhanced bond strength values when compared with only acid etching and deproteinization before acid etching. 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.

CONCLUSION

Deproteinization after acid etching significantly enhanced the shear bond strength values in primary and immature permanent teeth. Further studies are needed to evaluate the real clinical effectiveness of deproteinization in primary and immature permanent teeth.

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