Deproteinizing Agents as an Effective Enamel Bond Enhancer- An *in Vitro* Study

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Objective: *The aim of this study was to compare the effect of different deproteinizing agents on shear bond strength of composite to primary teeth enamel.* ***Study Design:*** *Forty sound primary molars divided in 4 groups of 10 teeth each. In control group 1, enamel was etched for 60 seconds with 37% phosphoric acid and rinsed with water. Group 2: after acid etching deproteinizing agent 5 % sodium hypochlorite was applied for 60 seconds and rinsed. Group 3: after acid etching deproteinizing agent papain gel was applied for 60 seconds and rinsed. Group 4: after acid etching deproteinizing agent bromelain gel applied for 60 seconds and rinsed. Following this, bonding agent was applied to treated enamel surface and composite resin disc were build. Samples were then tested for shear bond strength using Universal Testing Machine.* ***Results:*** *Mean SBS was highest for group 4 and lowest for group 1. No statistically significant difference (p value >0.05) was found between all the four groups.* ***Conclusion:*** *Among deproteinizing agents, deproteinization when carried out with bromelain gel and sodium hypochlorite showed effective bond strength as compared to papain.*

Key words: *Sodium Hypochlorite, enamel deproteinization, phosphoric acid, acid etching, shear bond strength*

INTRODUCTION

In today's era, esthetic is the foremost concern of patients undergoing dental treatment. As a result restorative treatment has shown a paradigm shift from dental amalgams to composites. But composites have their own demerits like adhesion failure leading to microcracks, post operative sensitivity and discoloration. This led researchers in the direction of enhancing the bond between enamel surface and the restorative materials.

Bonding to enamel depends upon the type of etching agent, duration of etching, concentration of acid being used and composition of the enamel surface. Since there are structural differences between primary and mature tooth enamel, it is logical to expect differences in etching quality and bond strength of resins.[1,2] Various studies have 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 teeth. [3-5] Therefore there was a need to modify the organic content of enamel in primary teeth . Deproteinization is one such method.

Deproteinization of enamel involves the removal of organic content i.e. proteins from the enamel. Sodium hypochlorite (NaOCl) has been used successfully in endodontics as an irrigating solution to disinfect, remove debris, as well as organic materials from the canals.[6,7] 5% sodium hypochlorite as enamel deproteinizing agent had been first studied by Venezie *et al*,[8] in teeth affected by amelogenesis imperfecta. Aras *et al*,[9] studied the influence of 5% sodium hypochlorite deproteinization treatment on bond strength before and after acid conditioning in primary, mature and immature permanent tooth enamel. They reported that deprotinization after acid etching enhanced the bond strength in primary and immature teeth.

Literature today shows various other deproteinizing agents like papain gel from papaya plant, bromelain enzyme from stem of pineapple plant and calcium hypochlorite[10]. Pithon *et al*,[11] used 10% papain gel as an enamel deproteinizing agent before bonding procedure and achieved good results with respect to the shear bond strength of orthodontic brackets bonded with resin-modified glass ionomer cement. Dayem and Tameesh,[12] assessed the deproteinizing effect of bromelain enzyme that led to decreased leakage scores of adhesive system.

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Therefore this study was undertaken to

1. Compare the effect of different deproteinizing agents on shear bond strength of composite to enamel
2. Establish the effectiveness of acid etching and deproteinization over conventional acid etching.

MATERIALS AND METHOD

This *in vitro* study was undertaken in the Department of Pedodontics & Preventive dentistry and Department of Pharmacology , I.T.S Dental College and Hospital, Muradnagar, Uttar Pradesh. Institutional Ethical committee clearance was obtained prior to start of this study. Also informed consent was taken from patients who participated in this study.

Forty sound primary molars with intact coronal portion of teeth that were extracted because of physiological root resorption were used in the study. Teeth with enamel cracks or fractures along the buccal aspect, dental pathology, malformations, carious lesions, restorations or erosions were excluded.

The extracted teeth were thoroughly washed in running water to remove blood and adherent tissue using hand scalers. All teeth were stored in sterile water until used for study. Each tooth was then mounted on steel mold of height 3 inches and diameter 2.5 cm containing self cure acrylic resin. The teeth were aligned within the molds so that the buccal surface of crowns were exposed such that the enamel surface could be delineated for etching and bonding using an adhesive tape with a 3 mm diameter hole. The delineated area was positioned such that the composite disc was 1 mm above the mounting surface and paralled the force during shear bond testing.

Forty primary molars were equally divided into four groups of ten teeth each.

Group 1(control group): Enamel surfaces were etched with 37% phosphoric acid gel (Eco-Etch etching gel ,Ivoclar Vivadent) for 60 seconds, washed with sterile water and air dried before bonding application (Prime & Bond NT, Dentsply)

Group 2: Group 1+ 5% sodium hypochlorite (Novo Dental Products Pvt Ltd, Mumbai) applied with sterile cotton pellet for 60 seconds, rinsed with water and dried before bonding application (Prime & Bond NT, Dentsply)

Group 3: Group 1 + papain gel (86.9% in Carie Care, Eco-works solutions Pvt Ltd) applied for 60 seconds, rinsed with water and dried before bonding application (Prime & Bond NT, Dentsply)

Group 4 : Group 1+ bromelain gel (60% indigenously prepared) applied with sterile cotton pellet for 60 seconds, rinsed with water and dried before bonding application (Prime & Bond NT, Dentsply)

After following the enamel treatment for each group, composite resin (3M ESPE, Filtek™ Z350 XT Universal Restorative nanocomposite), disc were build on the buccal surface of teeth using a hollow ring with internal diameter 4 mm.

The shear bond strength of all samples were measured on Universal Testing Machine using an indenter. The specimens were tested using a cross head displacement rate of 1mm/minute. The minimum load required to produce the bond failure was determined from the first load drop on load deflection plot. Shear bond strength values were recorded as Newton (N) initially and then they were calculated as megapascals (MPa).

Megapascals(MPa) = shear bond strength/area= Newton (N) /mm^2

Statistical analysis of the shear bond strength values were evaluated using one-way analysis of variance (ANOVA) and Post hoc LSD (Least Significant Difference) Multiple Comparison Test

RESULTS

Table 1 shows the mean and standard deviation of shear bond strength for all groups.

The shear bond strength of Group 1, Group 2, Group 3 and Group 4 were 12.16 ±2.40, 13.94 ±4.04, 12.83 ±2.86 and 14.51 ±2.87 respectively.

Group 4 (14.51 ±2.87) exhibited the highest shear bond strength while Group 1 (12.16 ±2.40) showed the lowest shear bond strength. (Table 1)

On applying one-way ANOVA, we found the shear bond strength has no significant difference in all four groups (p value > 0.05) (Table 1)

The shear bond strength of Group 3 (12.83 ±2.86**)** were comparable to group 1 (12.16 ±2.40), but were statistically non significant. The shear bond strength of Group 2 (13.94 ±4.04) were comparable to group 4 (14.51 ±2.87), but were not statistically significant.

Intergroup comparison using Post hoc LSD test showed, that the difference between the four groups was not significant. (Table 2)

Table 1: Mean values of shear bond strength of the test groups (MPa)

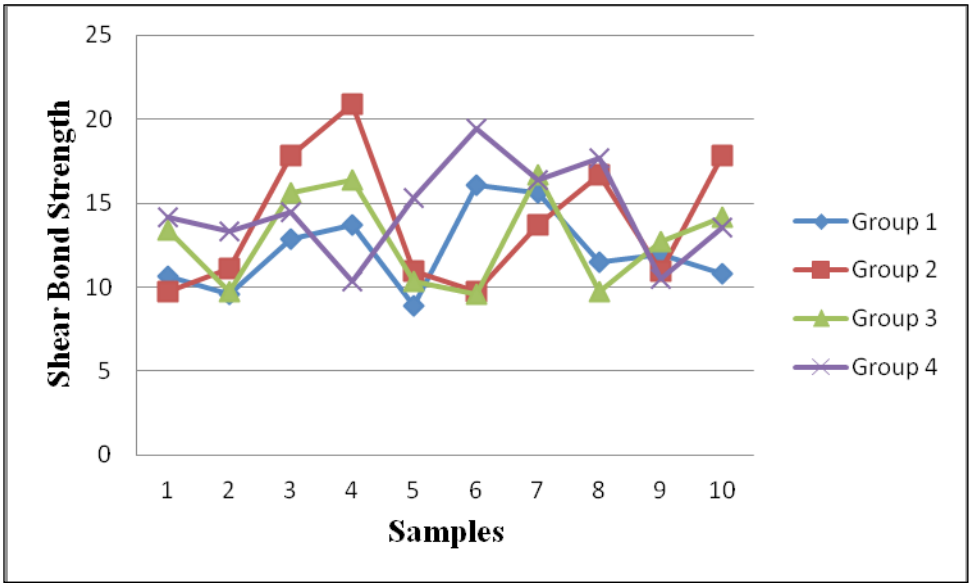
GROUP	No. of Samples	Mean ± Std. Deviation	p value
1	10	12.16 ±2.40	0.336*
2	10	13.94 ±4.04	
3	10	12.83 ±2.86	
4	10	2.87	

*Not significant (p value >0.05)

Table 2: Post hoc LSD Multiple Comparison Test between test groups

LSD Comparison between groups				
	(I) GRP	(J) GRP	Mean Difference (I-J)	Sig.
LSD	1	2	-1.78	.207
		3	**-.66**	**.633**
		4	**-2.34**	**.099**
	2	**3**	**1.11**	**.427**
		4	**-.56**	**.686**
	3	**4**	**-1.67**	**.234**

Figure 1: Line diagram representation of shear bond strength of all samples in each group



DISCUSSION

Composites are considered to be the material of choice for esthetic pediatric dentistry, but have shown higher failure rates in pediatric patients due to low bond strength to enamel as well as dentin. Lower bond strength in primary teeth may be attributed to chemical, physiological and micromorphological differences of primary teeth such as aprismatic enamel, small tooth size and less number of dentinal tubules with decreased permeability.[13] Various studies previously has carried out enamel deproteinization procedure in permanent teeth to enhance the bond between enamel and composite.[9,11,14-16] The concept of sodium hypochlorite as enamel deproteinizing agent has been extensively studied by various authors in permanent teeth.[14-18]

Currently other agents like papain gel and bromelain enzyme, are gaining interest as deproteinizing agents. Papain is a proteolytic enzyme extracted from the latex of leaves and fruits of adult green papaya, Carica papaya.[19] Therefore in the present study we used caries solvent 'Carie Care' containing 86.9% papain gel as deproteinizing agent. It is a chemomechanical caries removal agent that has as its main active ingredient from papaya extract – an endoprotein, chloramines and dye.[20] Its use in removal of partially degraded collagen in carious dentin makes us think of its applicability in enamel deproteinization.[19] Pithon et al,[11] has shown that papain 10 % gel has promising effect on shear bond strength of orthodontic brackets bonded with resin modified glass ionomer cement on bovine incisors.

Bromelain is also a proteolytic enzyme, obtained from stem of the pineapple plant (Ananas comosus).[21] Dayem and Tanmeesh,[12] tested bromelain enzyme for reducing the nanoleakage in dentin of permanent teeth . They established that application of bromelain enzyme after acid etching, eventually led to better bondability of adhesive system on permanent maxillary molars. In our study, we prepared bromelain solution by dissolving 10 mg of bromelain powder in 10 ml of saline as vehicle. Few freshly prepared concentration of this solution were again diluted with saline in the ratio of 1: 2, 2:2, 3:2 were tested for assessing an effective concentration to achieve good bond strength. Accordingly, in the present study we used 60% concentration (3:2) of indigenously prepared bromelain gel.[22] (thickening agent methylcellulose was added to bromelain solution)

The results of our study show that incorporating a step of deproteinization after acid etching has definitely affected the mean shear bond strength but not appreciably. Group 4 (acid etching+bromelain gel) shows slightly better bond strength as compared to other groups, but the difference is statistically non significant with group 1(acid etching), group 2(acid etching +sodium hypochlorite), group 3 (acid etching + papain). However, studies carried out with 5% sodium hypochlorite as a deproteinizing agent after acid etching in permanent teeth has shown effective bond strength as compared to conventional acid etching.[9, 16] The results of our study are in accordance with study done by Aras *et al*,[9] where the shear bond strength was tested in primary enamel treated with acid etching followed by deproteinization using 5% sodium hypochlorite. The reason for lowest bond strength in group 3 (acid etching + papain gel) might be due to presence of clove oil component in Carie Care, that negatively affected the bond strength.[23,24]

CONCLUSION

An addition of deproteinization step after acid etching in a restorative procedure would increase the chair side time and would require co-operation from pediatric patients. However, in order to achieve good bond strength of restorations to enamel surface in pediatric patients the addition of a deproteinizing step after acid etching is justified as it increases the bond strength.

The present study did observe slightly effective enhancement of the bond strength using deproteinizing agents after acid etching. Among deproteinizing agents, deproteinization when carried out with bromelain gel and sodium hypochlorite showed effective bond strength as compared to papain.

Keeping in mind the limitations of the present study in terms of random sampling, sample size, enamel structural changes on storage, anatomic variation of primary teeth, study needs to be further carried out to prove the role of newer agent bromelain gel in enamel deproteinization.

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