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Assessment of Pit and Fissure Sealants Following Enamel Deproteinization Using Three Different Methods for Shear Bond Strength and Microleakage- A Comparative In Vitro Study

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Rohan Bhatt,[1] Mehta Miral,[2] Megha Patel,[3] Foram Patel,[4] Disha Makwani [5]

1Professor, Department of Pediatric and Preventive Dentistry, Karnavati School of Dentistry, Karnavati University, Gandhinagar, Gujarat, India.
Email: additionaldirectorksd@karnavatiuniversity.edu.in

2Postgraduate Student, Department of Pediatric and Preventive Dentistry, Karnavati School of Dentistry, Karnavati University, Gandhinagar, Gujarat, India.
Email: miral9829@gmail.com

3Professor and Head, Department of Pediatric and Preventive Dentistry, Karnavati School of Dentistry, Karnavati University, Gandhinagar, Gujarat, India.
Email: meghapatel@karnavatiuniversity.edu.in

4Senior lecturer, Department of Pediatric and Preventive Dentistry, Karnavati School of Dentistry, Karnavati University, Gandhinagar, Gujarat, India.
Email: foram@karnavatiuniversity.edu.in

5Senior lecturer, Department of Pediatric and Preventive Dentistry, Karnavati School of Dentistry, Karnavati University, Gandhinagar, Gujarat, India.
Email: dishamakwani@karnavatiuniversity.edu.in

Corresponding Author: Dr. Miral Mehta

Contact: 9427704737
Email: miral9829@gmail.com

Keywords:

Deproteinization, Papain, Bromelain, Microleakage, Shear bond strength

Abstract

Background: Occlusal caries is thought to be most effectively prevented by sealing pits and fissures. The deproteinization may have an impact on the adhesiveness and retentive ability of pit and fissure sealant, among other factors.

Aim: To assess the impact of three deproteinizing agents applied before and after acid etching on the microleakage and shear bond strength of pit and fissure sealants.

Methodology: Included were one hundred fifty-four extracted, healthy human maxillary and mandibular molars. 6.25% sodium hypochlorite (NaOCl), 10% papain gel, and 6% bromelain gel were the deproteinizing agents that were used. Group A (deproteinization done after etchant application), Group B (deproteinization done before etching), and Group C (control group) were the three groups into which the samples were split. Based on the type of deproteinization agent used, Group A and Group B were further divided into three groups. After that, sealant (3M ESPE Clinpro) was applied and given a 20-second light cure. Using a stereomicroscope magnified 40 times to evaluate microleakage and a universal testing machine to measure shear bond strength (SBS), respectively.

Results: For microleakage, scores were lowest for Bromelain group, followed by Papain group and NaOCl group and highest for control group. Statistically significant difference was found between control and bromelain group (P=0.039). Mean values for shear bond strength was highest for Bromelain group (18.65±3.56), followed by Papain group (12.00±2.46), NaOCl group (9.77±2.31) and control group (7.71±1.58). Statistically significant differences were found for Papain group (P=0.047) and Bromelain group (P=0.047). Also, deproteinization before acid etching showed superior results both for SBS and microleakage.

Conclusion: When deproteinization is performed before acid etching, the SBS values increase and the sealant microleakage score decreases. As an alternative to 5.25% sodium hypochlorite, 10% papain enzyme and 6% bromelain enzyme can be used successfully. The bromelain group, however, demonstrated superior results because it effectively removed the organic materials from tooth surface.

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1. Introduction:

When bacteria in biofilms are exposed to fermentable carbohydrates for long duration, the balance between demineralization and remineralization is upset, leading to the development of dental caries, a pathogenic microbial infectious disease. [1] Pit and fissure sealants are frequently applied to the occlusal surfaces of posterior teeth to prevent caries, especially in young children who are more susceptible to the condition. Permanent molars are more susceptible to caries in their eruption phase. This is because of the presence of immature enamel and poor oral hygiene in children. Acting as a physical barrier, sealants stop the enamel from demineralizing and stop fissure microorganisms from interacting with their food sources. [2]

The effectiveness of a pit and fissure sealant depends on a number of factors, including the ability of the fissure sealant to adhere to the enamel, retention, and resistance to microleakage. [2] Phosphoric acid is used to create micro-porosities for the retention of sealant. [3] Organic residues and the aprismatic structure of the enamel reduces the effectiveness of etching hence hinder proper adherence. Because phosphoric acid cannot dissolve organic material, it is insufficient for effective etching. [4]

Several tooth preparation methods, including bonding agents, pumice prophylaxis, air abrasion, lasers, and deproteinization, have been recommended to increase sealant retention. Deproteinization, one of the potential methods for improving material adhesion and retention, produces the ideal type I or II pattern of etching by removing the organic material. [5]

Sodium hypochlorite (NaOCl) has antibacterial qualities and the potential to dissolve the organic smear layer. It eliminates organic material from surface of enamel which could have beneficial effects. [6] However, sodium hypochlorite is a strong oxidizer, it should be used with caution, especially in children, to avoid damaging the oral soft tissues. Researchers have been looking for more natural deproteinizing agents due to its unappealing flavour and odour as well as the potential for adverse soft tissue reactions. [7]

The enzyme papain is obtained from the papaya latex, which has antibacterial and antiinflammatory qualities. Without endangering the vital tissue, it dissolves the fibrin coating created by the inflammatory process and cleaves partially degraded collagen fibrils. [8]

The enzyme bromelain is made up of various endopeptidase, obtained commercially from the pineapple fruit or stem. It also has fibrinolytic and antiinflammatory properties. Additionally, it eliminates the collagen network reducing the adhesive restoration leakage. [9]

According to our knowledge, no studies have been published comparing the efficacy of NaOCl (5.25%), 10% Papain enzyme and 6% Bromelain enzyme on pit and fissure sealant microleakage and SBS pre and post acid etching. The study's null hypothesis was that the shear bond strength and microleakage of pit and fissure sealant in permanent teeth would not be affected by the deproteinization of enamel surfaces with 5.25% NaOCl, 10% papain enzyme, and 6% bromelain enzyme before or after acid etching.

Therefore, this study was aimed to assess the efficacy of three enamel deproteinizing agents used pre and post acid etching on SBS and microleakage of sealants.

2. Materials and Method:

STUDY DESIGN, SAMPLE SIZE AND RANDOMIZATION:

After receiving prior approval and consent from the Karnavati University's Ethical and Research Committee in Gandhinagar, Gujarat, the intended study was carried out as an in vitro study. The sample included 154 extracted human maxillary and mandibular molars. Prior to use in the study, they were thoroughly rinsed under running water and cleaned with a fluoride-free polishing paste.

The exclusion criteria were:

- Molars having obvious fracture and/or crack;
- Caries;
- Hypoplastic lesion and/or developmental defects;
- Dental erosion

Sample size calculation was done using one-way ANOVA for mean and proportion, which was based on previous studies. [10,11] An absolute minimum of 84 teeth for the microleakage and 70 teeth for SBS were

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required when power was set at 80% and significance level at 5%.

Using the block randomization technique, teeth were randomly divided into three groups:

Group A (Deproteinization after acid etching), which were further divide into three groups: A1: NaOCl A2: Papain enzyme A3: bromelain enzyme

Group B (Deproteinization before acid etching), which were further divide into three groups: B1: NaOCl B2: Papain enzyme B3: bromelain enzyme

Group C (Control group) where, no deproteinization was done.

Ingredients used for papain and bromelain gel preparation: [12]

10% Papain Gel (5 g):

- Papain powder 250 mg
- Carbopol with purified water 200 mg + 2 mL
- Amylopectin with purified water 50 mg + 1 mL
- Propyl-p-hydroxy benzoate 100 mg
- Distilled water 1 mL

6% Bromelain gel (5 g):

- Bromelain powder 250 mg
- Carbopol with purified water 200 mg + 2 mL
- Amylopectin with purified water 50 mg + 1 mL
- Propyl-p-hydroxy benzoate 100 mg
- Distilled water 1 Ml

PROCEDURE:

Enamel treatment:

Samples were treated as follows:

Group A (Deproteinization after acid etching)

Group A1: Enamel surfaces were etched for 15 seconds with a 37% phosphoric acid gel (Prevest Denpro Actino Gel), then rinsed with sterile water and dried for 15 seconds with oil-free compressed air. Following that, 5.25% NaOCl (Vishal Dentocare Private Limited) was applied for 60 seconds with a micro applicator tip, rinsed and dried for 10 seconds. After that, sealant (3M ESPE Clinpro) was applied, and the light-curing process took 20 seconds. The curing light's tip was positioned 2 mm perpendicular to the sealant surface (guilins woodpecker -1000 Mw/cm2).

Group A2: Surface etching of enamel was done similar to Group A1. It was followed by the application of Papain gel (10%) for 60 seconds, followed by rinsing and drying for 10 seconds. After that, sealant (3M ESPE Clinpro) was used, and it was light-cured for 20 seconds.

Group A3: As in group A1, the enamel surface was etched. Then, for 60 seconds bromelain gel (6%) application was done. Further it was rinsed and dried for 10 seconds. After that, sealant (3M ESPE Clinpro) was applied, and light curing was carried out for 20 seconds.

Group B (Deproteinization before acid etching)

The same procedure was followed for different subgroups except that here, deproteinization was done before acid etching.

Group C: Etching of enamel was performed using phosphoric acid gel (37%) (Prevest Denpro Actino Gel) for 15s followed by rinsing and drying for 10s. Then sealant (3M ESPE Clinpro) application was done and light cured for 20s.

At room temperature, all specimens were incubated in the sterile saline solution for a day. Samples were subjected to 250 cycles of thermocycling between 5 and 55 degrees Celsius, with an immersion time of 30 seconds in each bath and a transfer time of 10 seconds.

PARAMETERS EVALUATED:

Microleakage evaluation: Two coats of nail polish were applied to the specimens, with the exception of the 2mm margin surrounding the sealant. Root apices were covered in waxy seals. The next step involved keeping samples in 2% methylene blue for 24 hours. Teeth were divided buccolingually into mesial and distal halves by a low-speed, water-cooled diamond disc after being thoroughly rinsed with tap water. Using a stereomicroscope (SZX7 Olympus) set to 40x magnification, an investigator who was unaware of the treatment the specimen had undergone assessed the dye penetration in each section. Based on Ovrebo and Raadal's [13] criteria, “Dye penetration was scored as

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follow: 0 indicates no dye penetration; 1 indicates that dye penetration is restricted to the outer half of the sealant; 2 indicates that dye penetration extends to the inner half of the sealant; and 3 indicates that dye penetration extends to the underlying fissure."

SBS assessment: From the root, 2 mm below CEJ crowns were sectioned using low speed diamond disc. Samples were then mounted on acrylic resin block. The prepared enamel surface was sealed with sealant using a cylindrical plastic tube (3*3 mm), which was then given 20 seconds to cure. Specimens were stored in sterile saline for a day at room temperature. On universal testing machine (Instron, 3369) they were sheared at 1.0 mm/min speed. The peak load at failure divided by the specimen surface area yielded a shear bond strength measurement in MPa (Fig. 2).

STATISTICAL ANALYSIS:

Microsoft Excel 2010 was used to create a spreadsheet from the data gathered. Data on continuous variables were presented as Mean & Standard Deviation (SD). The assumption of normality was validated using the Shapiro-Wilk test.

- Two continuous groups were compared using Unpaired t-test
- Four groups were compared with One Way Analysis of Variance and Post Hoc comparison between groups were done by Tukey HSD
- Comparison of Categorical groups was done with Chi-square (χ^2) test.

All of the data were statistically analysed using the Statistical Package for Social Sciences (SPSS version 20.0, IBM Corporation, USA) for MS Windows. Probabilities lower than 0.05 and less than 0.01(0.001) were used to define statistically significant and highly significant, respectively.

3. Results:

Table 1 depicts the microleakage score distribution according to the group using Chi-Square test, where scores were lowest for Bromelain group, followed by Papain group and NaOCl group and highest for control group. Table 2 illustrates the comparison of microleakage scores between groups using chi-square test. Statistically significant difference was found between Group C and Group A3 (P=0.011), Group C and Group B3 (P=0.000), Group B1 and Group B3(P=0.001) and also among Group B2 and B3 (P=0.033). Images obtained from stereomicroscope are shown in Figure 1.

Table 3 shows the Inter and Intra group comparison using One Way ANOVA and Unpaired t-test for shear bond strength. Shear bond strength was highest for Group B3 (18.65±3.56), followed by Group B2 (12.00±2.46), Group B1 (9.77±2.31) and control group (7.71±1.58). It 3 4 7 12 14 17 20 22 32 39 41 49 51 67 78 showed statistically significant difference between Group A2 and Group B2(P=0.047) and Group A3 and Group B3(P=0.047). Table 4 shows the comparison of mean SBS values between the groups. Statistically significant difference was found between Group C and A3 (P=0.000), Group C and B3 (P=0.000), Group A1 and A3 (P=0.000), Group C and B2 (0.004), Group A2 and A3 (P=0.000) and also between Group B2 and B3 (P=0.000).

4. Discussion:

The results of the present in vitro study rejected the null hypothesis, which held that the shear bond strength and microleakage of pit and fissure sealants in permanent teeth would not be affected by the deproteinization of enamel surfaces with 5.25% NaOCl, 10% papain enzyme, and 6% bromelain enzyme pre or post acid etching.

Etching with phosphoric acid alone does not always give uniformly etched surface. It depends on factors like concentration, time, and agent used for etching.[14] Various techniques have been proposed and researched to improve the quality of etching. Among these, enamel deproteinization appears to be a potential strategy for enhancing the material adherence [5]

According to the studies, deproteinization done using 5.25% sodium hypochlorite (NaOCl) will eliminate the organic material from the surface of enamel and also significantly increases the quality of etching. [15] When hypochlorous acid in NaOCl, comes into contact with organic material, it works as a solvent. When it interacts with the amino group of a protein, it releases chlorine, which turns into chloramines (chloramination reaction), which interferes with the metabolism of bacterial cell. Due to this mechanism of action NaOCl can act as a promising deproteinizing agent. [16] But, NaOCl has certain disadvantages like its strong

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oxidizing nature, cytotoxicity, unpleasant odor and flavor, which lead to the search of more natural deproteinizing agents. [7] Results of the present study suggests that there was no statistically significant difference between the control group and NaOCl group before or after acid etching for SBS and microleakage both. Similar results were also found in study conducted by, Ahuja B [17] and Ramakrishna [18] in which they found no significant difference between the control group and NaOCl group. Whereas, according to Espinosa et al. [14] better results were found when prior acid etching was done,

Papain was first used as a potent chemomechanical caries removal agent in dentistry in 2003. It is currently being researched as a natural deproteinizing agent because of its ability to remove partially degraded collagen without causing any harm to vital tissue and also due to its antibacterial and antiinflammatory characteristics. [19] Results of this in vitro study implies that Papain group showed higher SBS values and lower microleakage score than the Control group and NaOCl group before and after acid etching. According to Pithon et al. [20], 10% of papain gel can increase the SBS of braces bonded with RMGIC. Papacarie® has been proposed by Bayrak GD et al. [2] as an alternative to NaOCl as a surface pre-treatment agent.

Another naturally occurring deproteinizing agent with anti-inflammatory and fibrinolytic properties is bromelain. Additionally, it eliminates the collagen network, which in turn reduces the leakage of restorations or sealants. [9] Results of the present study showed the difference which was statistically significant between the Bromelain group and the control group for SBS and microleakage before and after acid etching. Compared to NaOCl group and Papain group, Bromelain group possessed higher SBS values and lower microleakage score. These were in line with the findings of the study by Chauhan et al. [21] in which they compared the deproteinizing effects of 5% NaOCl and bromelain enzyme. They discovered that the bromelain enzyme significantly affected the bond strength findings. Application of the bromelain enzyme, according to Dayem RN et al. [9], resulted in the removal of the collagen network and significantly decreased the adhesive system's leakage scores.

Present study also showed that, superior results were obtained when deproteinization was done before acid etching. This can be due to the reason that, deproteinization before acid etching will remove the organic residue from the enamel surface which are abundant in interprismatic spaces. The shear bond strength of pit and fissure sealants is increased by the process of etching because it can impact both the interprismatic spaces and the core of the enamel prisms. The research conducted by Mowiena et al. [7] was used to support this finding. On the other hand, deproteinization following acid etching was found to significantly increase the SBS values in comparison to conventional etching by Ekambaram et al. [22], Hasija et al. [23], and Aras et al. [24].

LIMITATIONS OF THE STUDY:

- As this study was carried out in an in vitro setting, the oral environment cannot be simulated.
- The role of saliva and pellicle was not possible to assess.
- The amount of organic material and the pattern of etching were not assessed.

5. Conclusions:

The following conclusions were drawn within the parameters of the current study:

- Deproteinization prior to the application of pit and fissure sealant significantly increased the shear bond strength values and showed reduction in sealant microleakage score than the conventional acid etching.
- 10% Papain enzyme and 6% Bromelain enzyme can be successfully used as an alternative deproteinizing agents to 5.25% Sodium hypochlorite which has a number of drawbacks, particularly when working with young patients.

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TABLES:

Table 1: Microleakage scores distribution according to the group

N=12	Group A microleakage score				N=12	Group B microleakage score				P value$
Groups	0	1	2	3	Groups	0	1	2	3	
C	0	1	5	6	C	0	1	5	6	---
A1	1	2	8	1	B1	0	4	6	2	0.515
A2	1	3	6	2	B2	2	5	3	2	0.607
A3	4	3	5	0	B3	8	4	0	0	0.039
P value$	0.042				P value$	0.000				

$: Chi-Square test

Table 2: Comparison of microleakage scores between the groups

Group A microleakage score		Group B microleakage score	
Groups	P value$	Groups	P value$
C-A1	0.133	C-B1	0.143
C-A2	0.251	C-B2	0.066
C-A3	0.011	C-B3	0.000
A1-A2	0.845	B1-B2	0.374
A1-A3	0.296	B1-B3	0.001
A2-A3	0.273	B2-B3	0.033

$: Chi-Square test

Table 3: Inter and Intra group comparison for shear bond strength

Group A SBS		Group B SBS		P value*
Groups	Mean±SD	Groups	Mean±SD	
C (n=10)	7.71±1.58	C (n=10)	7.71±1.58	---
A1 (n=10)	8.25±1.70	B1 (n=10)	9.77±2.31	0.111
A2 (n=10)	9.84±2.07	B2 (n=10)	12.00±2.46	0.047
A3 (n=10)	15.60±2.80	B3 (n=10)	18.65±3.56	0.047

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P value**	0.000	P value**	0.000	

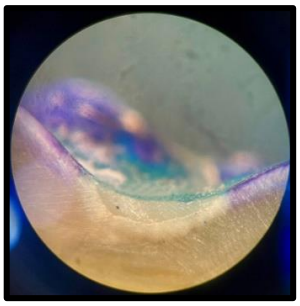
*: Unpaired t-test, **: One Way ANOVA

Table 4: Comparison of mean shear bond strength values between the groups

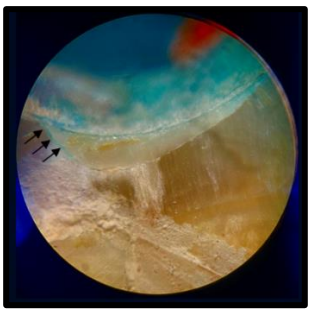
Group A SBS		Group B SBS	
Groups	P value#	Groups	P value#
C-A1	0.939	C-B1	0.295
C-A2	0.124	C-B2	0.004
C-A3	0.000	C-B3	0.000
A1-A2	0.341	B1-B2	0.232
A1-A3	0.000	B1-B3	0.000
A2-A3	0.000	B2-B3	0.000

#: Tukey HSD Post Hoc Test

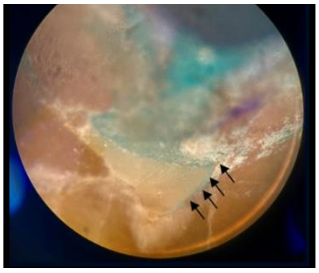
Figures:



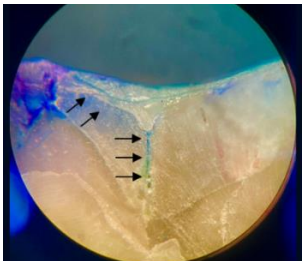
0- no dye penetration



1- Dye penetration limited to the outer half of the



2- dye penetration extending to the inner half of the sealant



3- dye penetration extending to the underlying fissure

Figure 1 Stereomicroscopic images of microleakage scoring

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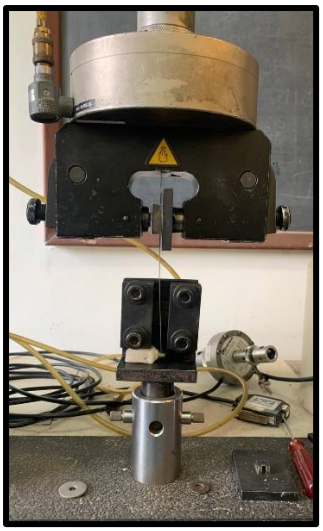

Figure 2 Evaluation of shear bond strength by Universal Testing machine