Enamel Deproteinization using Papacarie and 10% Papain Gel on Shear Bond Strength of Orthodontic Brackets Before and After Acid Etching

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Objective: *To suggest Papacarie® as a new deproteinizing agent in comparison with indigenously prepared 10% papain gel before and after acid etching that may enhance the quality of the bond between enamel surface and composite resin complex.* ***Study design:*** *One hundred and twenty five extracted human premolars were utilized and divided into five groups: In the group 1, enamel surface was etched and primer was applied. In group 2, treatment with papacarie® for 60 seconds followed by etching and primer application. In group 3, etching followed by treatment with papacarie® for 60 seconds and primer application. In group 4, treatment with 10% papain gel for 60 seconds followed by etching and primer application. In group 5, etching followed by treatment with 10% papain gel for 60 seconds and primer application . After bonding the brackets, the mechanical testing was performed using a Universal testing machine. The failure mode was analyzed using an adhesive remnant index. The etching patterns before and after application of papacarie® and 10% papain gel was also evaluated using SEM. The values obtained for shear bond strength were submitted to analysis of variance and Tukey test ($p < 0.05$).* ***Results:*** *It was observed that group 2 and group 4 had the highest shear bond strength and was statistically significant from other groups ($p=0.001$). Regarding Adhesive remnant index no statistical difference was seen between the groups ($p=0.538$).* ***Conclusion:*** *Papacarie® or 10% papain gel can be used to deproteinize the enamel surface before acid etching to enhance the bond strength of orthodontic brackets.*

Key words: *Enamel deproteinization, Etching, Papacarie®, Papain gel, Shear bond strength*

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INTRODUCTION

Since Buonocore[1] described the concept of acid etching in 1955, this procedure has become a standard technique in the placement of composite resin, fissure sealants, orthodontic attachments etc. Previous studies[2,3] have shown that the topographic quality of the enamel etching with phosphoric acid (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 tenacious etching and only 2% was ideally etched. Therefore it is not necessary that the area required for bonding is ideally etched. Thus to counteract the limitations of acid etching alone, various invasive and noninvasive techniques such as enamel abrasion[4] or grinding, air abrasion[5], lasers[6] etc. were carried out, but good results were not obtained. Silverstone[7] identified the enamel micromorphology and classified enamel etching into 4 patterns. Type 1 and type 2 etching patterns were considered to be ideal for a good bond.

Human enamel contains 96% of inorganic substance and less than 1% of organic substance. Less than one half of the organic component contains protein.[8] The action of H_3PO_4 etching on the enamel surface occurs mostly on the mineralized tissues (inorganic matter). Moreover, H_3PO_4 does not eliminate the organic matter. It is this outer organic layer that prevents the conventional 37% H_3PO_4

from effectively etching the surface, resulting in inconsistent pattern and an unreliable enamel surface for bonding.[9] Thus it is necessary to remove the organic matter from the enamel surface to enhance the quality of etching pattern, which gave rise to the concept of deproteinization. Deproteinization of the enamel surface before bonding the orthodontic bracket was first proposed by Justus[10] et al using 5.25% sodium hypochlorite (NaOCl). Recently, Pithon[11] et al proved 10% papain gel to be effective as an enamel deproteinizing agent which increases the shear bond strength of the orthodontic brackets. Papain is extracted from the latex of the Carica papaya, belonging to the Caricaceae family. It is a proteolytic cysteine enzyme with antibacterial and anti-inflammatory properties, and it acts as an agent for removing debris without any harmful effect on normal tissues. In 2003, Papain was introduced into dentistry. Bussadori[12] et al conducted a study on Papacarie® and concluded it as an effective chemo-mechanical caries removal agent. One of the active ingredient of Papacarie® is papain gel which helps in degradation and elimination of fibrin "mantle" formed by carious process and cause breakdown of collagen molecules.

Since 10% papain gel has been proved to be an effective deproteinizing agent[11] and papacarie contains papain gel as one of the active ingredient, the objective of the study was to assess the effect of enamel deproteinization using Papacarie® and 10% papain gel on the shear bond strength of the orthodontic brackets bonded to the enamel surface before and after the acid etching. The null hypothesis tested was "Enamel deproteinization with Papacarie® does not increase the bond strength of orthodontic brackets bonded to the enamel when compared with 10% Papain gel

MATERIALS AND METHOD

This study was partially carried out in the Department of Pedodontics and Preventive Dentistry, K. D. Dental College and Hospital, Mathura, India and Department of Mechanical Engineering, Sardar Vallabhbhai National Institute of Technology, Surat, India. Ethical clearance was obtained from the institutional ethics committee before beginning of the study.

Two hundred and thirteen human premolar teeth extracted for orthodontic reasons were collected from the patients attending the outpatient department of Oral and Maxillofacial Surgery. All the extracted teeth were thoroughly washed under running water immediately after extraction to remove any blood and were subjected to scaling to remove any debris and adherent tissues. The teeth with enamel defects, enamel cracks, restorations, erosions etc. were excluded[13] from the study and 133 such teeth were selected. Each tooth was then polished with fluoride-free pumice slurry, rinsed, and dried. All the premolars were stored in saline with thymol crystals (Qualikems Fine Chemicals, New Delhi, India) added to it to inhibit the bacterial growth. All the collected teeth were utilized for this study within a span of five months.

Out of 133 premolars, 125 were used for shear bond strength evaluation and 8 teeth were used for Scanning electron microscopic evaluation. The premolars for shear bond strength test were randomly chosen and divided into five groups of 25 teeth each.

Preparation of 10% papain gel

The following ingredients were used in preparing papain gel: Papain, an anti-oxidant (α-tocopherol acetate), emulsifier (amylopectin), thickener (Carbomer), pH adjuster (Triethanolamine), preservative (methyl paraben & propyl paraben), metal complexing agent (EDTA) and distilled water as a vehicle.

Shear Bond Strength Evaluation

A rectangular adhesive tape (5x4 mm) was placed on the middle portion of the buccal surface of each tooth so as to delineate an area for surface treatment as per the protocol followed in the study by Pithon *et al* [11] for standardization and comparison if any. The remaining portion of the tooth was covered with nail varnish of different colors for different groups. Each tooth was mounted in a stainless steel mold of the height 1 inch and diameter of 2.5 cm with the help of self cure acrylic resin (DPI, India). The teeth were inserted within the mold such that the buccal surface of the crowns was exposed and brackets could be bonded 1 mm above the mounting surface. The mounted specimen was placed immediately in water to reduce the heat generated by polymerization reaction.

Group 1- Control group

The delineated enamel surface was etched with 37% H_3PO_4 for 15 seconds, washed with distilled water and then air dried with oil free compressed air for 20 seconds. A single coat of primer (Transbond XT, 3M/ Unitek, Monrovia, Calif) was applied onto the etched surface and on the base of the curved orthodontic brackets according to the manufacturer's instructions and photopolymerized for 20 seconds. Transbond XT adhesive resin (3M/ Unitek, Monrovia, Calif) was applied on the base of the bracket and was placed on the delineated area of the tooth to be bonded.

Group 2

The delineated enamel surface was treated with Papacarie® gel and left for 60 seconds. The gel was washed with distilled water and then air dried with oil free compressed air for 10 seconds. The treated surface was etched with 37% H_3PO_4 for 15 seconds, washed with distilled water and then air dried with oil free compressed air for 20 seconds. A single coat of primer (Transbond XT, 3M/ Unitek, Monrovia, Calif) was applied onto the etched surface and on the base of the curved orthodontic Begg bracket according to the manufacturer's instructions. Transbond XT adhesive resin (3M/ Unitek, Monrovia, Calif) was applied on the base of the bracket and was placed on the delineated area of the tooth to be bonded

Group 3

The delineated enamel surface was etched with 37% H_3PO_4 for 15 seconds, washed with distilled water and then air dried with oil free compressed air for 20 seconds. Papacarie® gel was applied onto the tooth for 60 sec, washed with distilled water and then air dried with oil free compressed air. A single coat of primer (Transbond XT, 3M/ Unitek, Monrovia, Calif) was applied onto the treated surface and on the base of the curved orthodontic Begg bracket according to the manufacturer's instructions. Transbond XT adhesive resin (3M/ Unitek, Monrovia, Calif) was applied on the base of the bracket and was placed on the delineated area of the tooth to be bonded.

Group 4

The delineated enamel surface was treated with 10% Papain gel applied for 60 seconds. The gel was washed with distilled water and then air dried with oil free compressed air for 10 seconds. The surface was etched with 37% H_3PO_4 for 15 seconds, washed with distilled water and then air dried with oil free compressed air for 20 seconds. A single coat of primer (Transbond XT, 3M/ Unitek, Monrovia, California) was applied onto the etched surface and on the base of the curved orthodontic Begg bracket according to the manufacturer's instructions. Transbond XT adhesive resin was applied on the base of the bracket and was placed on the delineated area of the tooth to be bonded

Group 5

The delineated enamel surface was etched with 37% H_3PO_4 for 15 seconds, washed with distilled water and then air dried with oil free compressed air for 20 seconds. 10% Papain gel was applied onto the tooth for 60 sec, washed with distilled water and then air dried with oil free compressed air. A single coat of primer (was applied onto the treated surface and on the base of the curved orthodontic Begg bracket according to the manufacturer's instructions. Transbond XT adhesive resin was applied on the base of the bracket and was placed on the delineated area of the tooth to be bonded.

All the brackets were positioned with a seating force of approximately 0.250 kgf[14] with a Dontrix gauge (3M Unitek Dental Products) and photopolymerized for 60 seconds. All the test samples were stored in distilled water at room temperature (37°C) for 24 hours (ISO Test type-1[15]) following which they were subjected to thermocycling of 500 cycles at 5°c and 55°c with 20 seconds exposure in the water bath and the dwelling time between the baths was 5 seconds (ISO Test type-2[15]). The shear bond strength of all the samples was measured on Universal Testing Machine (LR- 100 K, Lloyd Instruments, UK) using a stainless steel shear probe with a crosshead speed of 1 mm/minute.

The enamel surfaces of all the test samples were examined after shear bond strength estimation under a stereomicroscope at 16X magnification to determine the amount of adhesive resin remaining on the surface and then classified according to the Adhesive Remnant Index (ARI). The ARI scores ranged according to the criteria given by Arthur and Bergland[16] from 0 to 3, with 0 indicating no composite left on the enamel; 1, less than half of the composite left; 2, more than half of the composite left; and 3, all of the composite remained on the tooth surface.

The remaining 8 premolars were subjected to scanning electron microscope analysis to evaluate the effect of Papacarie® and 10% Papain gel on the quality of Type I and Type II etching patterns[8,9] of the enamel surface.

To obtain enamel samples for the SEM evaluation, the buccal surface of each crown was divided into 9 parts by marking two horizontal lines and two vertical lines equidistant to each other and the middle portion of the enamel was cut with a high speed double sided diamond disk (Shofu, Japan). The middle portion of the enamel block was obtained from each tooth and trimmed to $1mm^2$. Thus 8 enamel blocks were obtained from 8 premolars. All the enamel blocks were stored in distilled water until the surface preparation.

Sample 1: No treatment was carried out on the enamel surface

Sample 2: Etching with 37% H_3PO_4 for 15 seconds, washed with distilled water and then air dried with oil free compressed air for 20 seconds.

Sample 3: Treatment with Papacarie® gel for 60 seconds, washed with distilled water and then air dried with oil free compressed air for 10 seconds

Sample 4: Treatment with Papacarie® gel for 60 seconds, washed with distilled water and then air dried with oil free compressed air for 10 seconds. The treated enamel surface was then etched with 37% H_3PO_4 for 15 seconds, washed with distilled water and then air dried with oil free compressed air for 20 seconds.

Sample 5: Etching with 37% H_3PO_4 for 15 seconds, washed with distilled water and then air dried with oil free compressed air for 20 seconds. Then Papacarie® gel was applied on the etched surface for 60 seconds, washed with distilled water and then air dried with oil free compressed air for 20 seconds.

Sample 6: Treatment with 10% Papain gel with a micro brush for 60 seconds, washed with distilled water and then air dried with oil free compressed air for 20 seconds.

Sample 7: Treatment with 10% Papain gel for 60 seconds, washed with distilled water and then air dried with oil free compressed air for 20 seconds. The treated enamel surface was then etched with 37% H_3PO_4 for 15 seconds, washed with distilled water and then air dried with oil free compressed air for 20 seconds.

Sample 8: Etching with 37% H_3PO_4 for 15 seconds, washed with distilled water and then air dried with oil free compressed air for 20 seconds. 10% Papain gel was then applied to the etched surface with a micro brush for 60 seconds, washed with distilled water and then air dried with oil free compressed air for 20 seconds.

All the samples were placed on stubs for gold sputtering and were coated with gold electrodepositing, using a Sputtering Effa-coater and prepared for surface SEM analysis and 5 microphotographs of each sample were obtained at 500X magnification randomly, covering the entire treated surface making a total of 40 photographs. All the photographs were subjected to evaluation for the quality of Type I and Type II etching patterns[7] of the enamel surface in percentage and the evaluation was done using Gimp 2.8 software.

The data thus obtained was subjected to statistical analysis which was performed using SPSS (Statistical package for social sciences) version 14 for Windows with one way ANOVA along with post hoc tests to compare the shear bond strength values of all the groups and Kruskal- Wallis test was used for assessing the ARI scores. All the tests were performed at 95% confidence level with the level of significance set at 0.05 (5%).

RESULTS

The mean shear bond strength of group 4 (40.27 Mpa) is highest followed by group 2 (36.6 Mpa). Both the groups are statistically significant with group 1, 3 and 5 ($p<0.05$) (Table-1). Regarding ARI score, no statistical significant difference is seen between all the 5 groups($p>0.05$) (Table-2). Table-3 shows the percentage distribution of the Type I and Type II etching patterns after the values were obtained from the image manipulation software GIMP. The percentage of etching pattern ranged from 17.52% to a maximum of 44.98% on an average.

Figure 1: Normal enamel surface characteristics and remnants of organic pellicle can be seen.

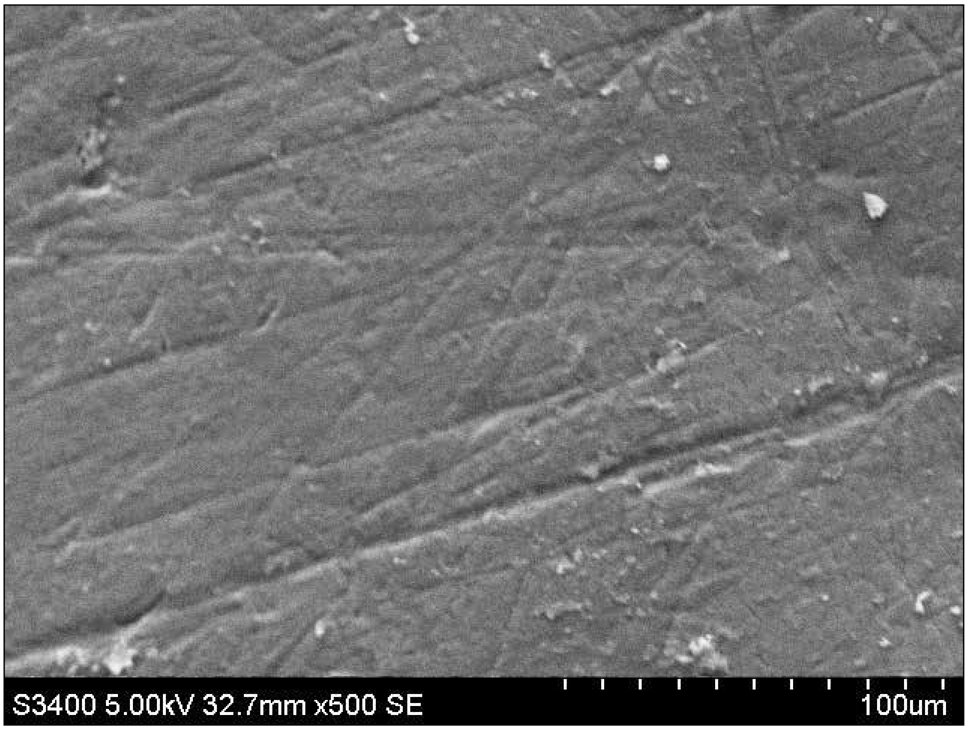


Figure 2: No uniform pattern of etching is observed on the enamel surface treated with phosphoric acid alone.

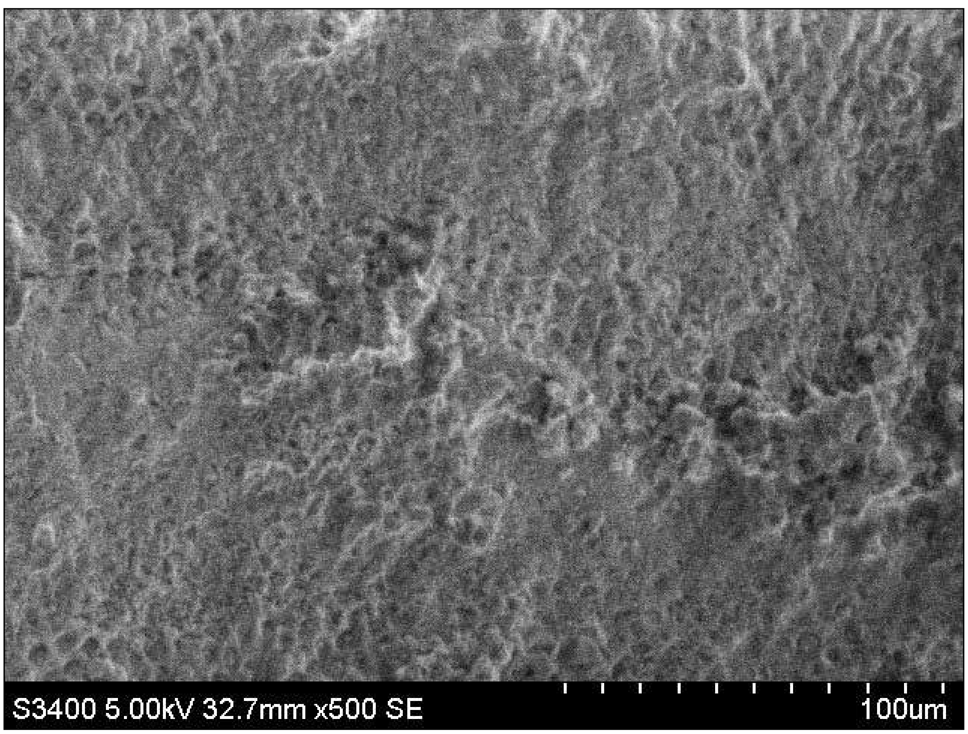


Figure 3: No remnants of organic pellicle is seen on the enamel surface treated with Papacarie® for 60 seconds

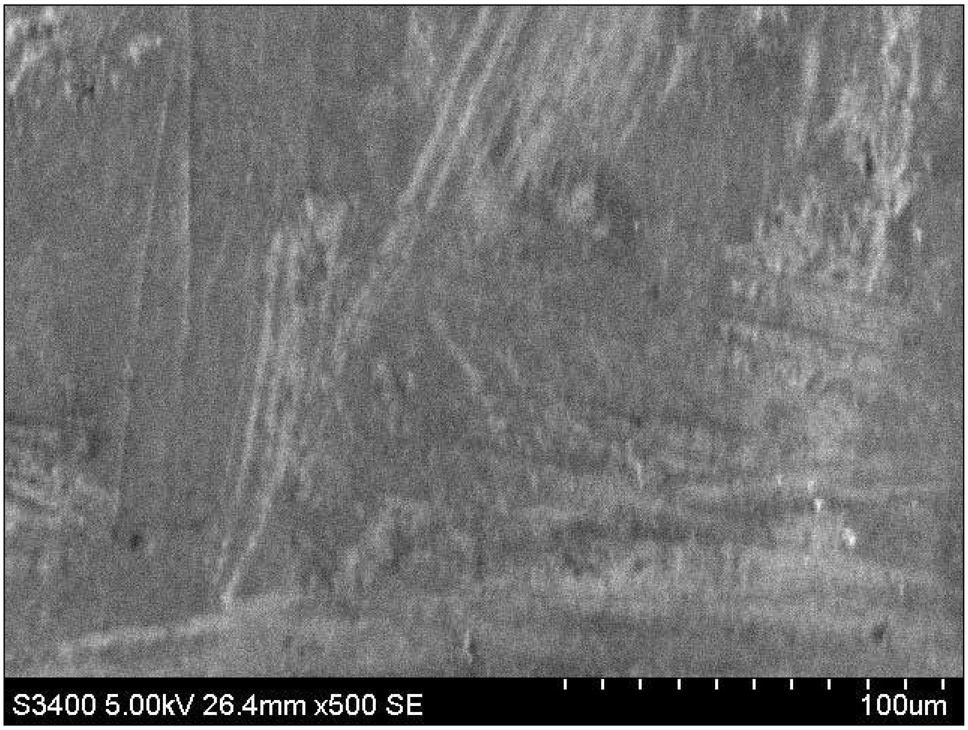


Figure 4: Type I and II etching patterns seen in most of the areas of the enamel surface treated with Papacarie® for 60 seconds before phosphoric acid etching.

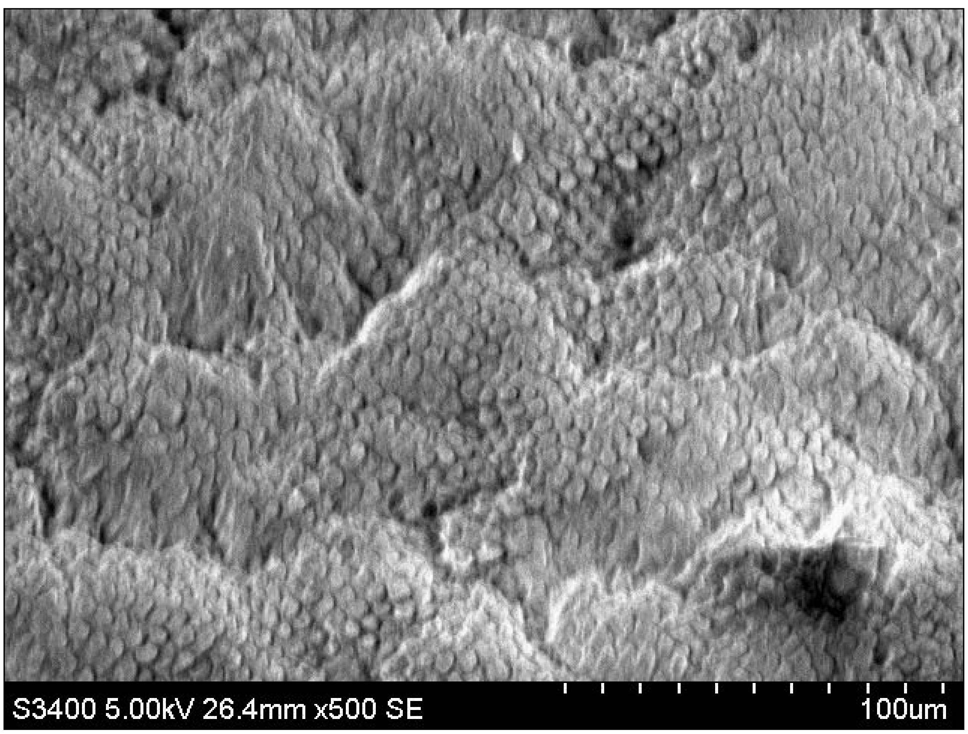


Figure 5: Etching pattern is not uniform and is filled with organic debris on the enamel surface treated with Papacarie® for 60 seconds after phosphoric acid etching.

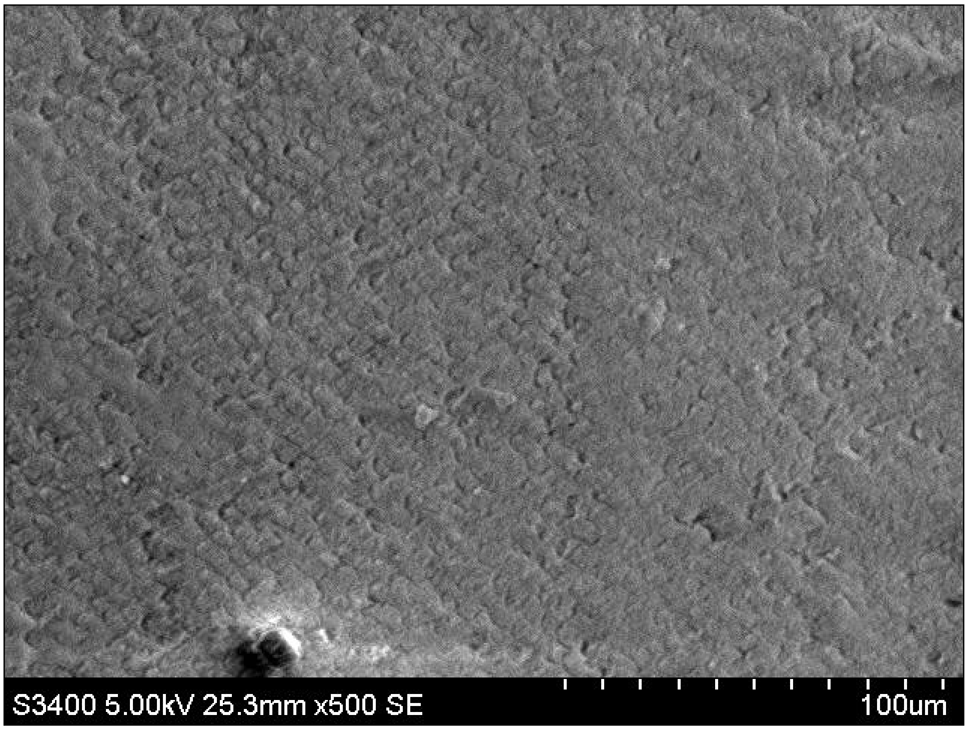


Figure 6: No remnants of organic pellicle is seen on the enamel surface treated with Papacarie® for 60 seconds

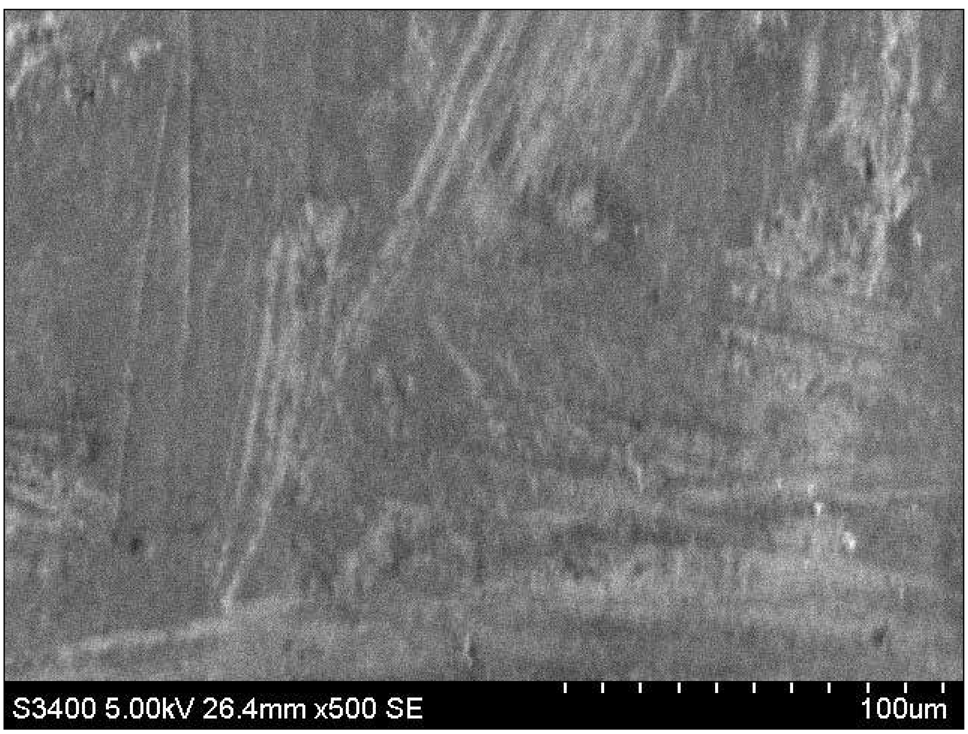


Figure 7: Type II etching pattern is seen in which prism core is intact and prism peripheries are not seen in most of the area of the enamel surface treated with 10% papain gel before acid etching.

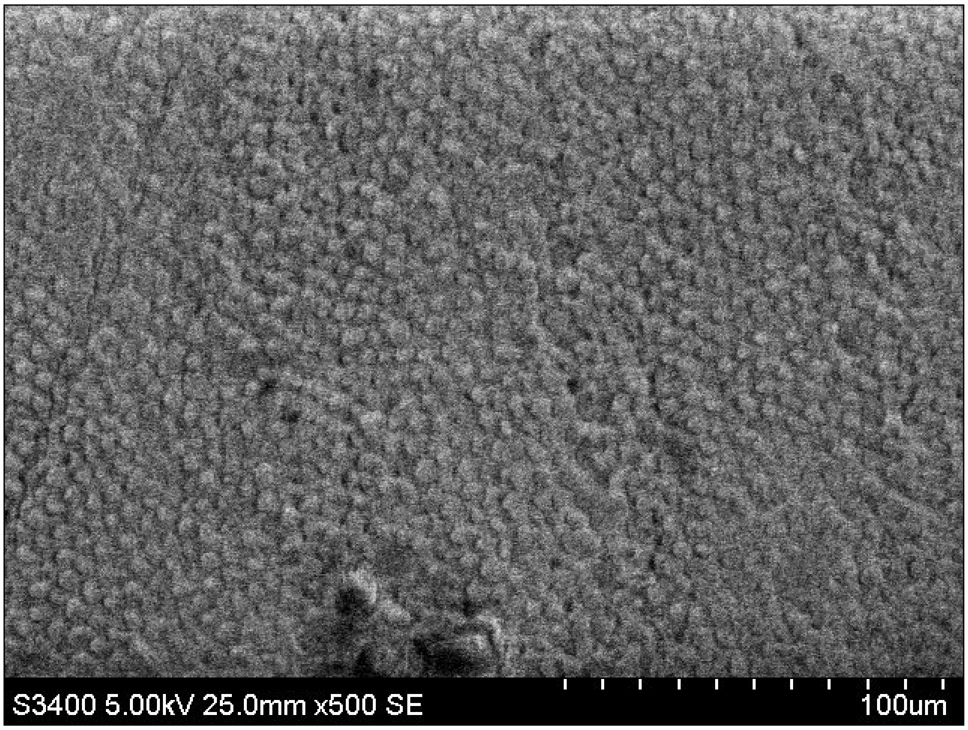
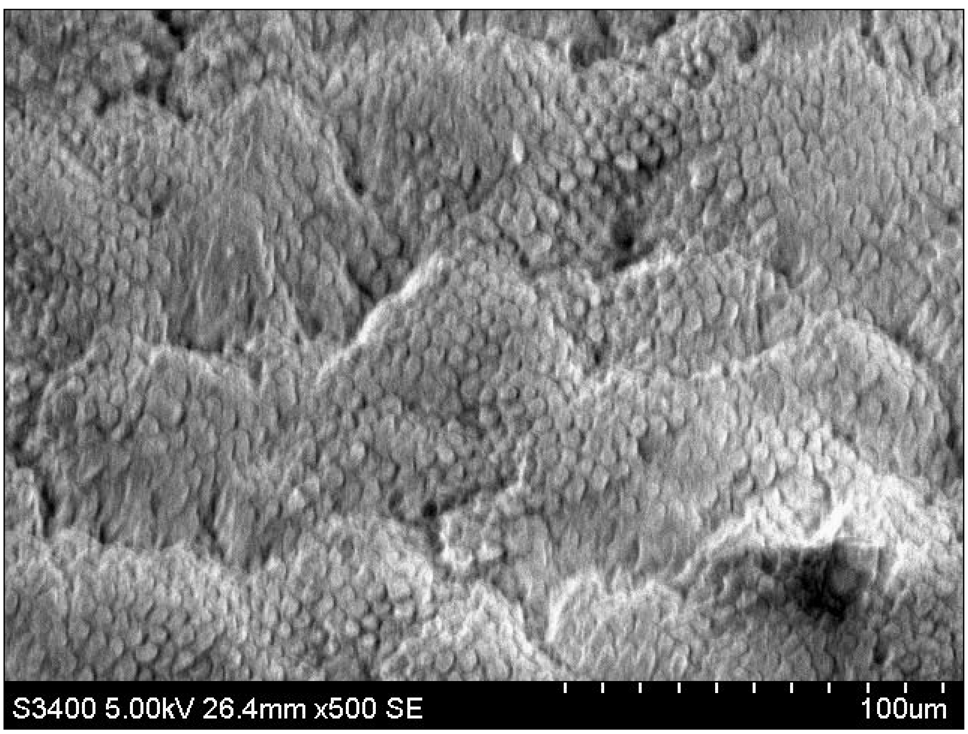


Figure 8: Type II etching pattern in which prism core is intact and prism peripheries are not seen in most of the areas of the enamel surface treated with 10% papain gel after acid etching



DISCUSSION

Direct bonding of orthodontic brackets has been advocated since the 1960s[17]. The bond strength between orthodontic accessories and the enamel may also be compromised by the presence of acquired pellicle, which covers the soft and hard tissues in the oral region, particularly the surface of tooth enamel. This membrane is a biofilm, free of bacterial colonization, and its most abundant components are proteins, glycoproteins, enzymes, and mucins or their derivatives. These organic elements make it difficult for the composite resin to adhere to the tooth enamel surface, diminishing its shear bond strength.[18] Hence, the present study is a pioneering effort to investigate the influence of the application of papacarie® and indigenously prepared 10% papain gel as a deproteinizing agent on the shear bond strength of the orthodontic brackets before and after acid etching by 37% phosphoric acid.

In the present study, the mean shear bond strength of all the groups were 29.77± 6.51 MPa, 35.13 ± 7.23 MPa, 30.96 ± 6.39 MPa, 36.88 ± 7.96 MPa, 31.90 ± 5.85 MPa respectively which was statistically significant (p= 0.001). The groups in which papacarie and 10% papain gel was used as a deproteinizing agent before acid etching showed highest shear bond strength and was statistically significant from the control group in which only acid etching was used (p<0.05).

Table 1: Groups, Minimum, Maximum, Mean and standard Deviation of the shear bond strength values and statistical analysis of the groups evaluated.

Group	n	Minimum MPa	Maximum MPa	Mean(SD)	Significance
1	25	7.32	40.41	29.77(6.51)	2- p=0.049* 3- p=0.972 4- p=0.003** 5- p=0.803
2	25	12.70	47.35	35.13(7.23)	3- p=0.203 4- p=0.894 5- p=0.455
3	25	10.74	40.10	30.96(6.39)	4- p=0.022* 5- p=0.989
4	25	13.78	50.38	36.88(7.96)	5- p=0.081
5	25	14.12	42.13	31.90(5.85)	

F=4.742
* Significant
** Highly significant
SD- Standard Deviation

Table 2: Mean ARI scores of different groups

Group	n	Minimum	Maximum	Mean(SD)	Mean Rank
1	25	0	3	2.16(.800)	61.36
2	25	1	3	2.40(.707)	71.68
3	25	0	3	2.12(.726)	58.58
4	25	1	3	2.28(.737)	65.98
5	25	0	3	2.04(.773)	57.40

	ARI
Chi-Square	3.118
Df	4
value	.538*

* Not significant

Table 3: Mean percentage distribution of type I-II etching patterns obtained from 5 different sites for each sample (Values obtained from the GIMP software)

Sample No	Images	Type I-II (%)	Mean (SD) %
1	1	0	
	2	0	
	3	0	0 (0)
	4	0	
	5	0	
2	1	16.63	
	2	31.86	
	3	7.69	17.52 (10.50)
	4	7.59	
	5	23.81	
3	1	0	
	2	0	
	3	0	0 (0)
	4	0	
	5	0	
4	1	35.94	
	2	34.18	
	3	25.33	36.90 (8.06)
	4	45.46	
	5	43.60	
5	1	10.29	
	2	15.11	
	3	43.16	36.90 (8.06)
	4	4.23	
	5	21.99	
6	1	0	
	2	0	
	3	0	0 (0)
	4	0	
	5	0	
7	1	34.70	
	2	45.97	
	3	63.43	44.98 (11.61)
	4	45.44	
	5	35.35	
8	1	36.94	
	2	43.85	
	3	29.21	33.08 (11.00)
	4	39.57	
	5	15.85	

In most of the studies, the effect of deproteinizing agent has been observed before acid etching. But Saroglu[19] has observed the deproteinizing effect of NaOCl after acid etching on hypocalcified amelogenesis imperfecta teeth. They predicted that treating the acid-etched enamel surface with NaOCl could make the enamel crystals more accessible to the bonding agent resulting in clinically more favorable bond strength. Recently, Aras S[20] et al concluded that deproteinization with NaOCl after acid etching significantly enhances the shear bond strength in primary and immature permanent teeth. But, in the present study, papacarie® and 10% papain gel, when used as a deproteinizing agent after acid etching showed no statistical significant result (p>0.05) from that of the group in which acid etching alone was used.

Regarding Adhesive Remnant index, no statistical difference was observed between the groups (p= .538). The mean ARI score of all the groups 1-5 were 2.16 ± .800, 2.40 ± .707, 2.12 ± .726, 2.28 ± .737, 2.04 ± .889 respectively. In all the groups the average score was more than 2 which indicates that more than half of the composite has remained on the enamel surface. Bracket failure at each of the 2 interfaces has its own advantages and disadvantages. Bracket failure at the bracket adhesive interface is advantageous as it indicates good adhesion to the enamel. However, considerable chair side time is needed to remove the residual adhesive, with the added possibility of damaging the enamel surface during the cleaning process. In contrast when brackets fail at the enamel adhesive interface, less residual adhesive remains on the enamel, but then bracket failure probably occurs more often during the treatment, disrupting chair time and prolonging the duration of orthodontic treatment. A study by O'Brien[21] suggested that the ARI score depends on many factors like bracket base design, the type of adhesive and not only the bond strength at the interface.

The results of the present study were in contrast with our previous study by Harleen[22] where enamel deproteinization using 5.25% NaOCl has no effect on the shear bond strength of Adper™ Single bond 2 adhesive and Filtek™ Z-350 XT composite resin.

The SEM results showed that the enamel surface conditioned first with 10% papain gel and followed with H_3PO_4 was qualitatively rougher than when only phosphoric acid was used. Moreover, the enamel surface conditioned first with Papacarie ®gel and followed with H_3PO_4 also showed rough surface compared to when only phosphoric acid was used. The enamel surface conditioned with Papain gel and papacarie® after phosphoric acid treatment showed no difference in roughness compared to H_3PO_4 alone. In the present study, etching pattern of Type I and II were observed on the enamel surface conditioned with papain gel and papacarie followed by acid etching. Type III etching patterns were observed in most of the areas on the enamel surface treated with H_3PO_4 alone. Etching of the enamel with 37% H_3PO_4 after eliminating the organic elements from the enamel surface probably produces longer adhesive tags that penetrate the enamel. The longer tags greatly increases the mechanical retention of adhesives to the enamel, thus increases the bond strength. In the present study, to evaluate the type of etching patterns, GIMP 2.8 image manipulation software was used. The likely possible areas resembling Type I and Type II etching patterns were marked manually and the percentage of the areas which it covered was determined by using GIMP software. The group in which only phosphoric acid etching was used showed 17.52 ± 10.50 % of type I and type II etching patterns. The group in which enamel surface was deproteinized using papacarie before acid etching showed 36.9 ± 8.06 % type I and type II etching patterns. The group in which enamel surface was deproteinized using 10% papain gel before acid etching showed 44.98 ± 11.61% type I and type II etching patterns. The group in which enamel surface was deproteinized using papacarie and 10% papain gel after acid etching showed 18.96 ± 15.01% and 33.08 ± 11.00% respectively.

The results of this study were in accordance in comparison with Espinosa[13] where they found that by deproteinizing the enamel surface using 5.25% NaOCl, the enamel's retentive surface were enhanced and increased the type 1 and type 2 etching pattern. The results also supported their later study[23] where they 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 phosphoric acid etching almost doubled the enamel's retentive surface from 46% to 73% and the topographical features of the resin replica penetration surface increased significantly with Type 1 and Type 2 etching pattern. But, the present study does not support the results of previous study by Bhoomika[24] et al where they found that the effect of 5.25% NaOCl deproteinization has no effect on type I and II etching patterns and concluded that etching with 37% H_3PO_4 is still the best method for pre-treatment of the enamel surface. In the present study, it is not possible to draw conclusions regarding the etching patterns based on only one sample and compare with other studies.

Since papain based gel has been proved as an effective enamel deproteinizing agent, it will help the Pediatric dentists to achieve effective bonding before any adhesive procedure but more extensive research is further needed to derive a firm conclusion

CONCLUSION

Within the limitations, the following conclusions were drawn from the present study:

Enamel deproteinization using papacarie® and 10% papain gel enhances the shear bond strength of the orthodontic brackets bonded to the enamel.

Both Papacarie® and 10% papain gel were found to be effective in deproteinizing the enamel surface before acid etching and enhancing the shear bond strength of the orthodontic brackets.

There is no role of either papacarie® or 10% papain gel as an enamel deproteinizing agent after acid etching on the shear bond strength of the orthodontic brackets.

Enamel deproteinization using papacarie® and 10% papain gel before acid etching increases the type I-II etching patterns which is essential for good bonding.

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