Original Article

Access this article online

Quick Response Code:

Website: www.jorthodsci.org

DOI: 10.4103/jos.JOS_26_19

Effects of different deproteinizing agents on topographic features of enamel and shear bond strength - An *in vitro* study

Santy Panchal, Akram Ansari[1], Abhay Kumar Jain[2] and Yogesh Garg[3]

Abstract:

OBJECTIVES: To evaluate and compare the effect of different enamel deproteinizing agents on topographic features of enamel and shear bond strength before acid etching.

MATERIALS AND METHOD: In total, 120 sound human maxillary premolars were taken and divided into three groups: Group 1 control (37% phosphoric acid (H_3PO_4), Group 2, (5.25% Sodium Hypochlorite (NaOCl)+ 37% H_3PO_4, and Group 3, (10% Papain gel + 37% H_3PO_4). These groups were further divided into A and B subgroups. In subgroups 1A, 2A, and 3A (n30) topographic features were evaluated using Scanning electron microscope (SEM) at different magnifications. Insub-groups 1B, 2B, and 3B (n90) metal brackets were bonded with Transbond™ XT, and all the samples were subjected for Shear Bond Strength (SBS) evaluation using universal testing machineat a cross speed of 0.5 mm^2/min. The failure mode was analyzed using adhesive remnant index (ARI).Statistical analysis was done using one-way ANOVA for the shear bond strength, and Kruskal-Wallis test followed by Mann-Whitneywas performed for ARI scores.

RESULTS: SEM showed predominance of type 3 etching pattern in control Group (1A) and type 1 and type 2 in deproteinizedGroups (2A and 3A).Mean values of shear bond strength showed statistically significant differences between evaluated groups ($P < 0.005$).The lowest and highest shear bond strength was attributed to Group 1B (Control) and 3B (10% papain gel), respectively. Statisticallysignificant differences were noted for the mean ARI scores between control and deproteinized group ($P < 0.05$).

CONCLUSION: 10% papain geland 5.25% NaOCl can be used as deproteinizing agents on enamel surface before acid etchingto enhance the shear bond strength of orthodontic brackets.

Keywords:

Adhesive remnant index, deproteinization, phosphoric acid, scanning electron microscope, shear bond strength

Dentalign Mutlispeciality Dental Clinic and Orthodontic Care Centre, Private Practitioner, Panipat, [1]Department of Orthodontics and Dentofacial Orthopedics, Yamuna Institute of Dental Sciences, Yamuna Nagar, Haryana, [2]Department of Orthodontics and Dentofacial Orthopedics, Hazaribag College of Dental Sciences and Hospital, Jharkhand, [3]Department of Public Health Dentistry, Surrender Dental College, Ganganagar, Rajasthan, India

Address for correspondence:
Dr. Abhay Kumar Jain, Department of Orthodontics and Dentofacial Orthopedics, Hazaribag College of Dental Sciences and Hospital, Jharkhand, India.
E-mail: docabhayjain@gmail.com

Introduction

Orthodontic bonding involves attaching brackets or other attachments directly to enamel surface, by means of orthodontic adhesives. Bonding involves the following steps: cleaning, enamel conditioning (etching), sealing, and bonding.[1]

Retention of dental resin materials is enhanced greatly by pretreatment of the enamel surface with certain inorganic acids or chelators. The acid solutions act by partially decalcifying the enamel and creating micro irregularities on the surface of the teeth. The procedure, referred to as acid etching, has received much attention from investigators because the quality of the acid etch is a crucial factor in the retention of materials.[2]

How to cite this article: Panchal S, Ansari A, Jain AK, Garg Y. Effects of different deproteinizing agents on topographic features of enamel and shear bond strength - An *in vitro* study. J Orthodont Sci 2019;8:17.

The purpose of acid etching is to clean the dental surface, remove the smear layer, microscopically enhance the roughness or fuzziness by erasing the prismatic and inter prismatic crystals, and to enhance the free surface energy to yield acceptable monomer penetration, block the surface with adhesive, and promote retention of the composite restorations. Phosphoric acid (H_3PO_4) ranging from 30% to 40% has been utilized for decades to bond resin-based dental materials to enamel. The micro mechanical retention of resin materials into the enamel porosities, resulting from acid etching, thus creates a strong and durable bond.[3]

The superficial changes created in the surface of enamel after acid etching was first reported by Gwinett (1971)[4] using a scanning electron microscope (SEM). Silverstone[5] later categorized the enamel micro-morphology and divided the enamel etching into 3 patterns of acid etching. In the type 1 etching pattern, acid dissolves the head of the prism, with inter prismatic substance (peripheral material) remaining intact, bringing about a honeycomb appearance. In type 2, the peripheral zones of the prisms are diluted by the acid, leaving the prism head relatively intact. In type 3, as such the surface shows no changes but displays some superficial dissolution that does not alter the deeper strata where the enamel prisms are present.In type 1 and type 2, the etching patterns are considered to be ideal for good bonding strength.[5]

Bracket bond failure is one of the common problems in orthodontic practice. The consequences include an increase in treatment time, additional costs in material, personnel, and additional patient visits. Along with the adhesive material, conditioning of the tooth surface is one of the most important factors affecting the bond strength between bracket and enamel surface.[6]

The bond strength of adhesive and attachments should be sufficient to withstand all the forces and stresses exerted by mastication and arch wires. There is no formally accepted minimal clinical bond strength. In-vitro studies have shown that orthodontic brackets must be able to sustain loads from 5.9–7.8 Megapascals (MPa) of shear bond strength to be considered clinically successful for orthodontic purposes.[7,8]

It has been firmly established that the essence of adhesion lies in achieving the best acid etching, with a generalized retentive morphological condition over the enamel surface.[9] However, recent studies have shown that the topographic quality of enamel etching with phosphoric acid is not achieved over the entire adhesive surface; more than 69% of this surface was no etched, whereas 7% presented tenuous etching and only 2% was ideally etched.[10]

To counteract these limitations, the use of deproteinizing agent may be considered as a possible alternative to optimize adhesion by removing organic elements of both the enamel structure and the acquired pellicle before acid etching to achieve better bond strength.

Papain was introduced into dentistry in 2003 and used as a chemo-mechanical caries removal agent in pediatric patients. Papain is derived from the *Carica papaya*latex. This is a proteolytic cysteine enzyme having antibacterial and anti-inflammatory properties and helps in removing debris with no harmful effect on tissues owing to the enzyme specificity.[11]

Currently, Pithon *etal*.[12] explained that by using 10% papain gel as a protein removal agent prior to enamel etching and documented that this kind of elimination of chemically organic substances aggravated the bond strength. Enamel deproteinization using sodium hypochlorite (NaOCl) was done by Venezie *et al*. to improve the bonding efficacy to hypocalcifiedamelogenesisimperfecta enamel.[13]

Because NaOCl and 10% papain gel have been proved to be an effective enamel deproteinizing agents, which helps to enhance the shear bond strength. Few studies in orthodontic fields have been published in the literature on this subject, but none of which have compared the various deproteinizing agents with each other.

Hence, the aim of this study was to evaluate and correlate the topographic features and shear bond strength of the enamel surface by using different enamel deproteinizing agents.

Materials and Method

The present study was approved by the Ethics Committee of Teerthanker Mahaveer Dental College and Research Centre, affiliated to Teerthanker Mahaveer University, Moradabad, India In total, 120 human maxillary premolars extracted for the orthodontic purpose was taken and stored in normal saline solution at room temperature. Selection criteria included no surface defect or carious lesion. Samples were randomly divided into 3 groups; Group 1 (Control *n*=40): acid etching with 37% H_3PO_4, Group 2 (Experimental *n*=40):5.25% NaOCl + acid etching with 37% H_3PO_4, and Group 3 (Experimental n40): 10% papain + acid etching with 37% H_3PO_4. All the groups were further divided into subgroups A (n10) and B (n30). Subgroups 1A, 2A, and 3A were subjected for SEM. Subgroups 1B, 2B, and 3B were subjected shear bond strength testing after bonding the brackets.

Preparation of the samples for scanning electron microscopy

To obtain enamel samples for the SEM evaluation, the buccal surface of each crown was divided into 9 parts by marking two horizontal and two vertical lines equidistant to each other, and then, the crown portion of the tooth was cut in transverse direction with the thickness of less than 5 mm (middle portion of the enamel) with a high-speed double-sided diamond disk. The middle portion of the enamel surface of each tooth was obtained and trimmed to 1 mm^2. All the samples were stored in normal saline at room temperature for 24 h until the surface preparation.

Group 1A (n10): Enamel surface of premolars was etched with 37% H_3PO_4 for 15 s followed by washing and drying with sterile water and oil-free compressed air, respectively and then stored in artificial saliva for 24 h at room temperature.

Group 2A (n10): In this group, on the enamel surface of premolars 5.25% NaOCl was applied for 60 s, washed with sterile water, and air sprayed for 10 s, and then dried with oil-free compressed air, followed by etching with 37% H_3PO_4 for 15 s, as with Group 1A and stored in artificial saliva for 24 h at room temperature.

Group 3A (n = 10): In this group, on the enamel surface of premolars 10% papain gel was applied for 60 s washed with distilled water and dried, followed by etching with 37% H_3PO_4 for 15 s and stored in artificial saliva for 24 h at room temperature as with Group 1A.

Scanning electron microscopeanalysis

All specimens were mounted on aluminum stubs for gold sputtering and were coated with gold electrodepositing, using a sputtering effacoater (QUORUM, Model EMS 7620). Randomly, two different sites were selected for microscopic observation for each sample. Microphotographs of each site were obtained at 5000× magnification. All the photographs were subjected to evaluate the etching patterns and topographic features of enamel surface of all the samples from the SEM images by the observer and comparing with the patterns given by Silverstone *etal*.[5]

Preparation of the samples for Shear Bond Strength

All the samples (1B, 2B, and 3B, n = 90), with different color coding, were mounted with self-cure acrylic resin in plastic molds diameter of 2 × 4″, keeping the crown exposed.

Group 1 B (n30): The enamel surface was etched with 37% H_3PO_4 (3M ESPE Scotch bond etching gel, St Paul, MN) for 15 s, followed by washing and drying with sterile water and air. The orthodontic brackets (3M Gemini, MBT 0.022 slot) were fixed using Transbond XT adhesive (3M/Unitek, Monrovia, Calif) after primer application, followed by photopolymerization (LED, Woodpecker) for 40 s (10 s on each particular side).

Group 2 B (n30): The enamel surface was deproteinized with 5.25% NaOCl for 60 s, followed by rinsing, drying and, followed by the same procedure of etching, bracket bonding, and photopolymerizationas in Group 1B.

Group 3 B (n30): The enamel surface was deproteinized with 10% papain gel for 60 s, followed by rinsing, drying and, followed by the same procedure of etching, bracket bonding, and photopolymerization as in Group 1B.

All the prepared samples were preserved in artificial saliva (Wet mouth) at room temperature for 24 h and then subjected for shear bond strength testing.

Samples testing

Each sample was subjected with shear load in a Universal Testing Machine, (WDW-5, SERIAL NO. 20070802 Instron Machine, Taiwan), applied by a knife-edged blade at a cross-head speed of 0.5 mm/min. The applied force was directly parallel to the external surface of the tooth on top of base of each bracket, and the load of shear bond strength was recorded at the point of debonding. This force (kilonewton) was converted into Mpa by the following formula.

MPa Force (in N)/Surface area (In mm^2).

Bracket base was 10.61 mm^2 according to the company specification.

Adhesive remnant index

The enamel surfaces of all the test samples were examined after shear bond strength estimation under a stereomicroscope at 16× magnification to determine the amount of the adhesive resin remaining on the surface and then classified according to the adhesive remnant index (ARI). The ARI scores arranged according to the criteria given by Artun and Bergland[14] 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.

Statistical analysis

All statistical analyses were performed on SPSS 21.0 software for Windows (SPSS, Chicago, III). Descriptive statistics such as mean, median, standard deviation, and minimum and maximum values were calculated. Chi-square test for SEM, ANOVA, and post hoc Bonferroni test for SBS, and Kruskal-Wallis and Mann-Whitney test

were used for assessing ARI scores. Significance for all statistical tests was pre-determined at $P \leq 0.05$.

Results

Scanning electron microscopeanalysis

In control/1A Group, type 3 etching pattern [Figure 1] was observed in 40% of the samples, in experimental group 2A, type 2 etching pattern [Figure 2] was observed in 50% of the samples, and in group 3A, type 1 etching patterns [Figure 3] were seen in 50% of the samples. The Chi-square test shows statistically significant differences (p 0.046) among the three groups [Table 1].

Shear bond strength

Descriptive statistics (Mean and SD) of shear bond strength measured for all the 3 groups is shown in Table 2. Minimum SBS of 5.35 Mpa in control group, and maximum SBS of 40.56 Mpa in group 3B (10% papain gel) was recorded.

Group 3B (10% papain Gel)>Group 2B (5.25% NaOCl) >Group 1B (H_3PO_4)

An high statistical significant difference ($P < 0.001$) existed between the groups when the analysis of variance test was applied [Table 2]. Pairwise comparison of SBS using post-hoc Bonferroni method, between 1B and 2B and 2B and 3B showed statistical significant difference (p 0.046) and (p 0.049) respectively, which is statistically significant. When shear bond strength of Group 1B and 3B was compared; statistically highly significant difference ($P < 0.001$) was found [Table 3].

Adhesive remnant index scores

Table 4 shows the descriptive statistics (Mean and SD) and comparison of the ARI scores for all the groups using Non parametric Kruskal-Wallis test. The test showed statistically significant differences in the ARI scores ($P < .05$) among all the groups.

Mann-Whitney Utest was performed to find out significant differences among the groups. It is evident that in intergroup comparison using Mann-Whitney Utest; statistically significant difference were found between 1B and 2B and 1B and 3B groups ($P < .05$), but no statistical difference was reported between 2B and 3B ($P > 0.05$) [Table 5].

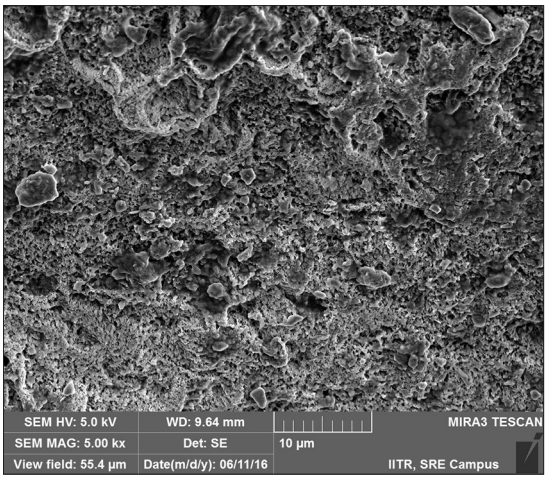

Figure 1: SEM × 5000Micrographs of Group 1A (37% H_3PO_4) showing type 3 etching pattern of enamel

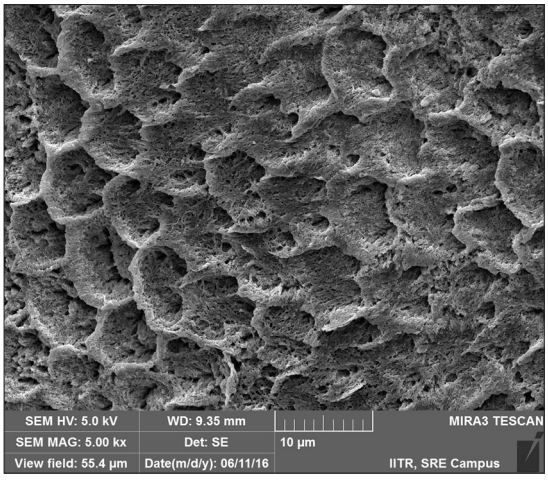

Figure 2: SEM × 5000Micrographs of Group 2A (5.25% NaOCl) showing type 2 etching pattern of enamel

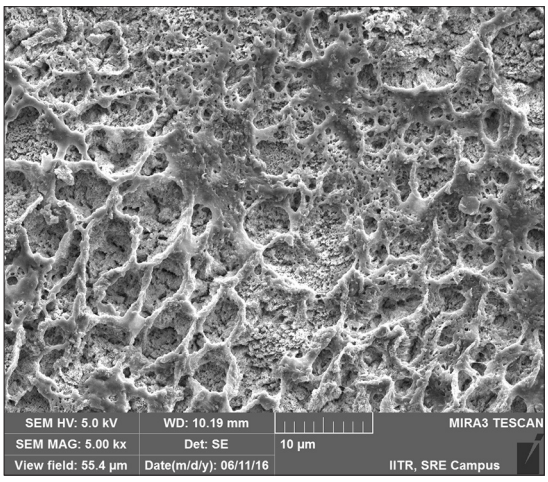

Figure 3: SEM × 5000Micrographs of Group 3A (10% papain gel) showing type 1 etching pattern of enamel

Discussion

Two key factors responsible for adhesive failure are the quantity of the etched surface and the quality of the etching pattern. The presence of the acquired pellicle, comprised with organic elements lead to a poorly defined acid etching, results into decrease shear bond strength. To achieve good bond strength, proper enamel conditioning

Table 1: Percentage distribution of etching patterns observed in control and experimental groups using Chi-square test

Group	*n*	Type 1 Etching Pattern	Type 2 Etching Pattern	Type 3 Etching Pattern
Control	10	3	3	4
H_3PO_4	%	30%	30%	40%
5.25%	10	2	5	3
NaOCl	%	20%	50%	30%
10%	10	5	4	1
Papain Gel	%	50%	40%	10%

Chi-square value=3.650, *P*=0.046*, Chi-square test, **P*<0.05

Table 2: Descriptive Statistics and Comparative Mean Shear Bond Strength of all the 3 groupsusing One-Way ANOVA test

Group	*n*	Min.	Max.	Mean	SD	*F*	*P*
Control 37% H_3PO_4	30	5.35	27.54	17.47	5.98	9.662	<0.001*
5.25% NaOCl	30	7.19	30.75	21.98	6.68		
10% Papain Gel	30	8.9	40.5	25.73	8.87		

**P*<0.05

Table 3: Intergroup Comparison of the Mean Shear Bond Strength of all the 3 groups using *Post-hoc* Bonferroni test

Group	Group	Mean difference	*P*
Control 37%H_3PO_4	5.25% NaOCl	-4.51	0.046*
Control 37%H_3PO_4	10% Papain gel	-8.25	<0.001*
5.25% NaOCl	10% Papain gel	-3.74	0.049*

**P*<0.05

Table 4: Mean and Kruskal-Wallis test result for the ARI scores of different groups

	n	Min	Max	Mean	SD	Mean rank	Critical value	*P*
Control H_3PO_4	30	0	3	2.17	0.79	34.87	11.236	0.004*
5.25% NaOCl	30	0	3	2.53	0.73	47.37		
10% Papain gel	30	1	3	2.73	0.58	54.27		

**P*<0.05

Table 5: Intergroup Comparison of the Adhesive Remnant Index of all the 3 groups using Mann-Whitney U test

Groups	Groups	Mean difference	*Z*	*P*
Control H_3PO_4	5.25% NaOCl	−0.37	−2.076	0.038*
Control H_3PO_4	10% Papain Gel	−0.57	−3.227	0.001*
5.25% NaOCl	10% Papain Gel	−0.20	−1.331	0.183

**P*<0.05

is a must. Justus *et al*.[15] suggested the use of 5.25% NaOCl a non-invasive method to eliminate the organic pellicle. Other materials with considerable deproteinization characteristics include sodium hydroxide and papain. It has been seen that NaOCl exhibits a dynamic balance as is shown by the reaction:

$$NaOCl + H_2O \leftrightarrow NaOH + HOCl \leftrightarrow Na^+ + OH^- + H^+ + OCl^-$$

When NaOClcomes in contact with organic material, several chemical reactions take place, i.e., fatty acids react with sodium hydroxide creating soap and glycerol (saponification reaction), amino acids react with sodium hydroxide creating salt and water (neutralization reaction), and also reacts with hypochlorous acid creating chloramines and water. These reactions occur simultaneously and synergistically leading to liquefaction of organic tissues.[16]

The action of phosphoric acid on the enamel surface occurs mostly on its mineralized part i.e., its inorganic matter. Unfortunately, this acid does not eliminate the organic matter on the enamel surface, which comprises of less than 1% but can be effective in enhancing the etching pattern.

Papain is an alkaloid enzyme extracted from the papaya. It has a proteolytic action and presents antibacterial and anti-inflammatory properties. Its use as a deproteinizing agent is preferable because of the specificity of itsaction; it only acts on the organic part with no harmful effects on the sound inorganic tissue.[11]

Hence, the present study is a pioneering effort to investigate the effect of the application of deproteinizing agents on topographic features of enamel and shear bond strength of the orthodontic brackets before acid etching.

In the present study, the SEM evaluation of Control group samples etched with 37% H_3PO_4, mostly showed type 3 etching pattern. In contrast to this experimental groups, samples which were deproteinized with 5.25% NaOCl and 10% papain gel prior to acid etching, predominantly showed type 2 and type 1 etching pattern, respectively, which is considered best for orthodontic bonding. 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.

According to Silverstone,[6] the most retentive etching patterns were types 1 and 2 because the porous surface offered retentive areas of greater size and depth. The type 3 etching pattern did not present a defined and deep morphology and lacked the micro mechanical retention. The finding of the present SEM study is also supported by Espinosa,[17] Justus *et al*.[15] and Agarwal *et al*.[18] who also found type 1 and type 2 etching pattern when enamel surface was treated with 5.25% NaOCl and 10% papain gel, as a deproteinizing agent before phosphoric acid etching.

SBS result of the current study indicates that the control group with deproteinizing agents showed best and statistically significant shear bond strength as compared to the control group ($P < 0.05$). NaOCl and papain are an efficient alternative for deprotenization of the enamel

surface before bonding. The group deproteinized with 10% papain gel obtained the highest SBS than 5.25% NaOCl. This proves that papain gel is better deproteinizing agent than NaOCl.

The findings of the present study are in agreement with Pithon*et al*.[12] who also used 10% papain gel. Justus *et al*.,[15] Pereira *et al*.,[19] and Ayman *et al*.[20] also reported a significant increase in shear bond strength when the enamel surface was pretreated withNaOCl.

Thus it can be concluded from the above findings that enamel deproteinization is an important step in the overall bracket bonding procedure. The improved marginal seal of the bracket base to the enamel is obtained because of type 1 and type 2 acid etching patterns. The facts further emphasize that a better bond strength can be obtained by deproteinization of enamel surface prior to acid etching.

The ARI scores were more in the deproteinized group as compared to the control group, which can be considered a favorable finding as lower ARI scores means that the mode of failure is closer to the enamel or adhesive interface and the risk of enamel fracture increases as shown by Liu *et al*.[21] Greater ARI scores can be attributed to better adhesion of composite resin to the deproteinized enamel surface. More the adhesive left on the tooth surface, safer is the debonding as the fracture interface shifts to the bracket adhesive interface thus preventing enamel damage. These findings are also supported by Pithon *et al*.[12] and Justus *et al*.[15]

The bracket failure at each of the 2 interfaces has its own advantages and disadvantages;[21] brackets failure at the bracket-adhesive interface is advantageous as it indicates good adhesion to the enamel. However, considerable chair 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 treatment, disrupting chair time and prolonging the duration of orthodontic treatment.

Conclusion

The conclusions drawn from the present study are:

1. Conventional enamel etching using phosphoric acid has significant limitations in most cases owing to the presence of acquired pellicle. Enamel deproteinization with papain gel and NaOCl, improve the quality of acid etching by removal of a tenacious acquired pellicle layer, which inturn changes the topographical features significantly from type 3 to type 1 and type 2 etching patterns
2. Deproteinization with 5.25% NaOCl and 10% papain gel provides a significant increase in the shear bond strength
3. Increase in the ARI scores in the deproteinized groups demonstrates better adhesion to the enamel surface, leading to a safer debonding after the treatment as the fracture interface shifts from enamel adhesive to the adhesive bracket interface thus preventing enamel microfractures
4. Thus, by incorporating deproteinization as a routine procedure in enamel pretreatment, loss of clinical time caused by debonded brackets can be averted.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Graber WL, Vanarsdall LR, Vig WL. Orthodontics: Current Principles and Techniques. 5th ed. St. Louis: Mosby; 2102.
2. Galil KA, Wright GZ. Acid etching patterns on buccal surfaces of permanent teeth. Pediatr Dent 1979;1:230-34.
3. Harleen N, Yeluri R, Munshi AK.Enamel deproteinization before acid etching and its effect on the shear bond strength – An *in vitro* study. J Clin Pediatr Dent 2011;36:19-24.
4. GwinettAJ. Histological changes in human enamel following treatment with acidic adhesive conditioning agents. Arch Oral Biol 1971;16:731-8.
5. Silverstone LM, Saxton CA, Dogon IL, Fejerskov O. Variation in the pattern of acid etching of human dental enamel examined by scanning electron microscopy. Caries Res 1975;9:373-87.
6. Aksu B, Caniklioglu MC, Yildiz O. Comparison of bond strength of lingual brackets under different composite base forming method. Hellenic Orthod Rev 2005;8:81-91.
7. Lopes MC, Mascarini RC, da Silva BMCG, Flório FM, Basting RT. Effect of a papain-based gel for chemomechanical caries removal on dentin shear bond strength. J Dent Child 2007;74:93-7.
8. Reynolds IR. A review of direct orthodontic bonding. Br J Orthod 2016;2:171-8.
9. Espinosa R, Valencia R, Uribe M, Ceja I, Cruz J, Saadia M. Resin replica in enamel deproteinization and its effect on acid etching. J Clin Pediatr Dent 2010;35:47-51.
10. Hobson RS, Rugg-Gunn AJ. Acid etch patterns on the buccal surface of human permanent teeth. Arch Oral Biol 2002;47:407-12.
11. BotelhoAmaral FL, Martao Florio F, BoviAmbrosano GM, Basting RT. Morphology and microtensile bond strength of adhesive systems to in situ-formedcaries-affected dentin after the use of a papain based chemomechanical gel method. Am J Dent 2011;24:13-9.
12. Pithon MM, FerrazCde S, de Oliveira GC, Pereira TB, Oliveira DD, de Souza RA, *et al*. Effect of 10% papain gel on enamel deproteinization before bonding procedure. Angle Orthod 2012;82:541-5.
13. Venezie RD, Vadiakas G, Christensen JR, Wright TJ. Enamel pre-treatment with sodium hypochlorite to enhance bonding in hypocalcifiedamelogenesis imperfect: Case report and SEM analysis, J ClinPediatr Dent 1994;16:433-6.
14. ArtunJ, Bergland S. Clinical trials with crystal growth conditioning as an alternative to acid-etch enamel pretreatment. Am J Orthod 1984;85:333-40.

15. Justus R, Cubero T, Ondarza R, and Morales F. A new technique with sodium hypochlorite to increase bracket shear bond strength of fluoride-releasing resin-modified glass ionomer cements: Comparing shear bond strength of two adhesive systems with enamel surface deproteinization before etching. Semin Orthod 2010;16:66-75.
16. Scott DB, Wyckoff RWG. Carbon surface replicas for electron microscopy and electron diffraction. JR Microsc Soc 1955;75:217-22.
17. Espinosa R, Valencia R, Uribe M, Ceja I, Saadia M. Enamel deproteinization and its effect on acid etching: An *in vitro* study. J Clin Pediatr Dent 2008;33:13-9.
18. AgarwalRM, Yeluri R, Singh C, Munshi AK. Enamel deproteinization using papacarie and 10% papain gel on shear bond strength of orthodontic brackets before and afteracid etching. J ClinPediatr Dent 2015;39:348-57.
19. Pereira TB, Jansen WC, Pithon MM, Souki BQ, Tanaka OM, Oliveira DD. Effects of enamel deproteinizationon bracket bonding with conventional and resin-modified glass ionomercements. Eur J Orthod 2013;35:442-46.
20. Ayman E, Amera A, Khursheed AM. Sodium hypochlorite as a deproteinizing agent optimize orthodontic brackets adhesion using resin modified glass ionomercement. Austin J Dent 2016;3:1037.
21. Hegarty DJ, Macfarlane TV. *In vivo* bracket retention comparison of resin-modified glass ionomer cement and a resin-based bracket adhesive system after a year. Am J Orthod Dentofacial Orthop 2002;121:496-501.