Evaluation of Deproteinization on Clinical Success and Longevity of Pit and Fissure Sealants on Erupting Permanent First Molars – An *In vivo* Study

K. B. Roopa, Ashwin Bahanan Abraham, P. Poornima, K. Mallikarjuna, N. B. Nagaveni, I. E. Neena

Department of Pedodontics and Preventive Dentistry, College of Dental Sciences, Davangere, Karnataka, India

Abstract

Objective: Higher protein content in the maturing enamel could hamper adequate etching for sealant application. Removing the organic content could improve its adhesion due surface alterations in the enamel. To enhance retention of sealants, higher protein content in immature permanent molar enamel could be deproteinized with sodium hypochlorite. Hence, the purpose of the study was to evaluate the effect of deproteinization with 5% sodium hypochlorite before and after acid etching on the longevity of pit and fissure sealants. **Methodology:** One hundred and five immature first permanent molar in 35 children aged 6–9 years were included. In Group A, a protocol of etching, bonding and sealant application was followed. In Group B and Group C, deproteinization was done after and before etching, respectively, followed by bonding and sealant application. Groups were examined at 3, 6, and 9 months for sealant retention using Simonsen's criteria. **Results:** Intergroup comparison for retained sealants across all three groups over 9 months revealed that retention is more in Group A, followed by Group B and least in Group C. **Conclusion:** Deproteinization does not have an added advantage in the retention of pit and fissure sealant over routine acid etching method. Deproteinization after etching is better compared to deproteinization before etching.

Keywords: Deproteinization, longevity, pit and fissure, sealants retention, sodium hypochlorite

Introduction

The tooth enamel is a highly mineralized structure and one of the hardest calcified substances in the human body. Composed of 96% mineral, rest 4% water and organic material.[1] The immature enamel of the first permanent molar in the eruption period is the most caries-susceptible, as it is immature during this stage. The ideal site for retention of bacteria and food debris is the pits and fissures over the crown surface. These faults or imperfections in cuspal odontogenesis have a complex morphology, making hygiene maintenance practices ineffective. Preventive measures such as control of bacterial plaque and topical application of fluoride solutions have little effect on such surfaces. Preventing the formation of such a caries susceptible environment by sealing the entry into the susceptible pits and fissure with sealants or other effective measures is of utmost importance.[2] Organic and cellular debris is present in the fissures of recently erupted teeth. Such teeth have a porous enamel lining. Theoretically, this porous zone of enamel bordering the fissures offers a three-dimensional honeycombed structure into which fissure sealants could be locked. Any procedure must be carried out at the earliest possible time after the eruption to make effective preventive use of fissure sealants owing to the higher protein content in the maturing enamel.[3] In erupting teeth with high organic content, removing the organic content could improve its adhesion due surface alterations in the enamel.[4,5] Studies have showed that removing the organic content from the enamel surface with 5.2% sodium hypochlorite (NaOCl) as a deproteinizing agent doubles significantly enamel retentive surface and increased the Type 1 and 2 etched enamel.[6]

Access this article online

Quick Response Code:

Website: www.ijcommdent.com

DOI: 10.4103/ijcd.ijcd_1_20

Address for correspondence: Dr. Ashwin Bahanan Abraham, Department of Pedodontics and Preventive Dentistry, College of Dental Sciences, Davangere, Karnataka, India.
E-mail: drashwinabraham@gmail.com

How to cite this article: Roopa KB, Abraham AB, Poornima P, Mallikarjuna K, Nagaveni NB, Neena IE. Evaluation of deproteinization on clinical success and longevity of pit and fissure sealants on erupting permanent first molars – An *In vivo* study. Int J Community Dent 2019;7:42-8.

Received: 11-02-20; **Accepted:** 25-06-20; **Web Published:** 30-10-20.

The retentive microporosities on the enamel surface post etching were vital in providing good adaptation and retention. An ideal quality of enamel etching with phosphoric acid is not achieved over the entire adhesion surface. Only a minimal 2% of the surface showed ideal etching and 7% slight etching, unlike the rest of the 69% of the treated surface without significant etching.[5]

By the use of sodium hypochlorite, noninvasive disinfection of root canals with effective pulp extirpation and dissolution is possible. It has a good antibacterial action without affecting the tooth structure adversely. It lowers surface tension by the saponification of fatty acids. Although many aspects and possibilities are explored, a not adequately explored aspect of sodium hypochlorite is its use as a deproteinization agent.[5]

The use of sodium hypochlorite as a deproteinizing agent may be a possible strategy to optimize adhesion by removing organic elements of both the enamel structure and the acquired pellicle in combination with acid etching during pit and fissure sealant application. Hence, this study was carried out with an objective to evaluate the effect of sodium hypochlorite deproteinization on the retentiveness of pit and fissure sealants.

Methodology

The present randomized, experimental, split-mouth *in vivo* study was done on a total of 35 children aged 6–9 years, irrespective of sex, race, and economic status. Children of both sexes were selected from the outpatient clinic in the Department of Pedodontics and Preventive Dentistry, College of Dental Sciences, Davangere, Karnataka, India. Written informed consent was obtained before the study after parents were informed about the objective of this study and the methodology to be employed. Treatment was performed after written consent had been obtained. Healthy newly erupted immature first permanent molars[6] with deep retentive, caries susceptible pits and fissures[7] were included in the study. Teeth that had enamel cracks or fractures, malformations, carious lesions, restorations, erosions, and dental pathology[6] were excluded from the study.

The teeth were randomly divided into three groups, and sealant application was made with and without deproteinization. In the control Group A, occlusal surface was etched for 15 s with 37% phosphoric acid, rinsed with water for 5 s, and gently air-dried for 1–2 s. Two layers of bonding agent were applied using an applicator tip and photopolymerized for 10 s. Pit and fissure sealant application was made, and photopolymerized for 20 s.[8] In the experimental Group C, a solution of sodium hypochlorite was applied with an applicator tip [Figure 1] for one minute on the occlusal surface and then washed with water and air spray. Then, it was etched for 15 s with 37% phosphoric acid, washed with water for 5 s, and gently air-dried for 1–2 s. Two layers of bonding agent (Adper Single bond 2, 3M ESPE) were applied using an applicator tip and photopolymerized for 10 s. Pit and fissure sealant (Clinpro Sealant, 3M-ESPE) application was made and photopolymerized for 20 s.[8] In the next experimental Group B, the occlusal surface was etched for 15 s with 37% phosphoric acid, washed with water for 5 s, and gently air-dried for 1–2 s. A solution of sodium hypochlorite was applied for 1 min and then rinsed with water spray. Two layers of bonding agent were applied using an applicator tip and photopolymerized for 10 s. Pit and fissure sealant application was made and photopolymerized for 20 s from each side.[8]

Figure 1: Deproteinization

The procedure was carried out by a single examiner and was assisted by an alert recorder to follow the instruction. All patients in the study were followed for 3, 6, 9, and 12 months to check for clinical success based on Simonsen's criteria.[2] Score 1 was given for complete retention of sealant [Figure 2], score 2 was given for partially retained sealant [Figure 3], and score 3 was given for completely missing sealant.

Statistical analysis

The result data collected were entered into Microsoft excel spreadsheet, and the statistical analysis was performed using IBM Statistical Package for the Social Sciences (SPSS) version 24 (IBM Corp., Armonk, NY, USA) software. Intergroup comparison of retention at each interval has been depicted by the Chi-square test. Intergroup comparison of retention status at each interval has been depicted by Kruskal–Wallis test, while Mann–Whitney U test has been used to compare every group with each other. Intragroup comparison of retention at different intervals has been depicted by the Friedman's test, whereas the Wilcoxon's signed-rank test has been used to compare every interval with each other.

Results

This study was done to evaluate the effectiveness of deproteinization on the retentiveness of pit and fissure sealants, compare it with etching, bonding, and sealing technique and to evaluate if deproteinization before or after etching is better. More retention was noted with Group A without deproteinization, followed by Group B with deproteinization after etching and Group C on deproteinization before etching [Table 1]. The fractured restoration was seen most with Group

Figure 2: Completely retained sealant

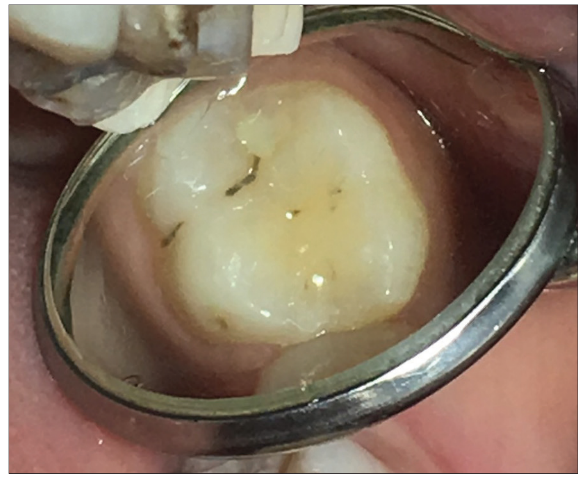

Figure 3: Fractured sealant

C [Table 2], lesser with Group B [Table 3] and least with Group A [Table 4] throughout the 12 months follow-up. At 3months and 6 months, Group A showed more retention followed by Group B, then Group C [Table 5 and Table 6]. At 9 months and 12 months, Group B showed most retention, followed by Group A, then Group C [Table 7 and Table 8].

Discussion

In 90% of cases, the pits and fissures of the permanent tooth are involved. Although with a community approach, a huge number of people have been exposed to oral hygiene education, water fluoridation, and sugar control, all of this aid better to reduce smooth surface caries and not pit and fissure caries. The plaque retentive design of the pit and fissures pose a threat against effective cleansing.[9,10] Thus, preventive measures like pit and fissure sealants have a vital role to play in this arena.

Because erupting permanent molars are easily susceptible to carious attacks than other permanent teeth, they were included in the study.[11,12] Such teeth show higher caries prevalence in the first 4 years of eruption, and until the occlusion is achieved, plaque retains over the surface due to inadequate forces for removal. Erupting immature tooth enamel has less mineralization, hence is more susceptible to acid attack and demineralization unlike a matured enamel in a fully erupted tooth. Thus, 6 –9-year-old children were included in the study.[13]

Rubber dam isolation was chosen for excellent isolation as well as to increase the incidence of retention.[13]

Thrity-seven percent phosphoric acid etching was done for 15 s to indirectly enhance sealant retention.[11] A fifth-generation bonding agent was used with the sealant to enhance the bond strength of sealant to the enamel.[14-16]

A color-changing, opaque, fluoride-releasing sealant[11] was used in this study basically to keep the teeth caries resistant during the study period and to evaluate the flow, extent, and retention.

Enamel etching is done to prepare the enamel surface for the resin to flow into the porosities created for better retention of the sealant. The enamel surface treated with phosphoric acid should give an equally and adequately etched surface. Studies show that >69% and 50% area, respectively, which is not effectively etched. Other Studies also show inefficiency in achieving this evenly etched enamel surface. Traditional phosphoric acid is only able to demineralize the inorganic enamel and not able to completely remove the protein content in enamel. Some proteins are also embedded in the enamel crystals. The inefficiency of resin to penetrate into enamel could also be because of a Type 3 etching pattern seen after etching it conventionally. Studies showed that more of Type 1 and 2 etching pattern after using sodium hypochlorite to remove protein in enamel.[5] Literature shows that the enamel deproteinization technique with sodium hypochlorite is an effective way to remove organic material on the occlusal enamel surfaces of teeth.[17] Thus, this could be a boon to the retention rates of the sealant applied to the treated enamel.

Other authors highlighted that <50% of the enamel surface was conditioned with deproteinization with sodium hypochlorite before traditional etching. A study with a resin replica model showed that deproteinizating enamel before phosphoric acid etching multiplies the retentive surface.[8]

An enamel surface of immature permanent enamel contains more protein, are low in minerals and is more porous than mature permanent teeth.[6] Deproteinization has been good enough to increase resin tag penetration in Type 1 and Type 2 surfaces. Furthermore, bracket bond strength showed an increase when the enamel surface was deproteinized before etching.[18]

Better surface area retention was noticed with 60 s of deproteinization rather than 15 or 30 s. Compared to conventional etching, results were better with 30 s of deproteinization.[5]

It was in the early 1900s that sodium hypochlorite was used as an irrigant for wounds and introduced into endodontics as an irrigant. It is an economical, antibacterial, antiviral, low viscous,

Table 1: Comparison of retention status between the study groups at different time intervals

Time interval	Score	Group			Total (%)	Chi-square test	
		A (%)	B (%)	C (%)		χ^2	*P*
3 months	1	32 (91.4)	30 (85.7)	25 (71.4)	87 (82.9)	5.23	0.07 (NS)
	2	3 (8.6)	5 (14.3)	10 (28.6)	18 (17.1)		
6 months	1	30 (85.7)	26 (74.3)	17 (48.6)	73 (69.5)	11.96	0.003*
	2	5 (14.3)	9 (25.7)	18 (51.4)	32 (30.5)		
9 months	1	21 (67.7)	21 (67.7)	10 (32.3)	52 (55.9)		0.002*,#
	2	8 (25.8)	10 (32.3)	21 (67.7)	39 (41.9)		
	3	2 (6.5)	0 (0.0)	0 (0.0)	2 (2.2)		
12 months	1	21 (67.7)	21 (67.7)	10 (32.3)	52 (55.9)		0.002*,#
	2	8 (25.8)	10 (32.3)	21 (67.7)	39 (41.9)		
	3	2 (6.5)	0 (0.0)	0 (0.0)	2 (2.2)		

**P*<0.05 statistically significant, *P*>0.05 NS. NS: Nonsignificant

Table 2: Comparison of retention status at different time intervals in group C

Group C	*n*	Mean (SD)	Range	Median (Q1-Q3)	Friedman test		Wilcoxon Sign Rank Test			
					χ^2	*P*	3-6	3-9, 12	6-9, 12	9-12
3 months	31	1.32 (0.48)	1-2	1 (1-2)	26.19	<0.001*	0.005*	0.001*	0.04*	1.00 (NS)
6 months	31	1.55 (0.51)	1-2	2 (1-2)						
9 months	31	1.68 (0.48)	1-2	2 (1-2)						
12 months	31	1.68 (0.48)	1-2	2 (1-2)						

**P*<0.05 statistically significant, *P*>0.05 NS. NS: Nonsignificant, SD: Standard deviation

Table 3: Comparison of retention status at different time intervals in group B

Group B	*n*	Mean (SD)	Range	Median (Q1-Q3)	Friedman test		Wilcoxon Sign Rank Test			
					χ^2	*P*	3-6	3-9, 12	6-9, 12	9-12
3 months	31	1.16 (0.37)	1-2	1 (1-1)	11.82	0.008*	0.04*	0.03*	0.16 (NS)	1.00 (NS)
6 months	31	1.26 (0.45)	1-2	1 (1-2)						
9 months	31	1.32 (0.48)	1-2	1 (1-2)						
12 months	31	1.32 (0.48)	1-2	1 (1-2)						

**P*<0.05 statistically significant, *P*>0.05 NS. NS: Nonsignificant, SD: Standard deviation

Table 4: Comparison of retention status at different time intervals in group A

Group A	*n*	Mean (SD)	Range	Median (Q1-Q3)	Friedman test		Wilcoxon Sign Rank Test			
					χ^2	*P*	3-6	3-9, 12	6-9, 12	9-12
3 months	31	1.10 (0.30)	1-2	1 (1-1)	20.48	<0.001*	0.16 (NS)	0.01*	0.008*	1.00 (NS)
6 months	31	1.16 (0.37)	1-2	1 (1-1)						
9 months	31	1.39 (0.62)	1-3	1 (1-2)						
12 months	31	1.39 (0.62)	1-3	1 (1-2)						

**P*<0.05 statistically significant, *P*>0.05 NS. NS: Nonsignificant, SD: Standard deviation

and nonspecific proteolytic agent. Concentrations ranging from 1% to 5% are used generally for irrigating root canals to disinfect, remove debris and organic matter. It is being used effectively to remove the organic pulp during root canal treatment. The chloride content in chlorine has a vital role to play in the proteolytic action of sodium hypochlorite. When exposed to organic material, a saponification reaction takes place as the fatty acids react with sodium hydroxide. A neutralization reaction too takes place when the amino acids react with sodium hydroxide creating salt and water. Organic tissue reacting with hypochlorous or chloride content forms makes water-soluble chloramines. Alterations also take place in the dentin as its collagen fibrils while other organic components are lysed. All of this causes liquefaction necrosis in the organic tissue. Hence, sodium hypochlorite was used as a deproteinizing agent in this study against the protein content in the immature permanent molars.[19]

Studies on deproteinization of amelogenesis imperfecta affected enamel before etching to enhance bracket bonding strength concluded that it gave fruitful results.[17] Furthermore, research

Table 5: Comparison of retention status between different study groups at 3 months

3 months	*n*	Mean (SD)	Range	Median (Q1-Q3)	Kruskal-Wallis test	
					χ^2	*P*
A	31	1.10 (0.30)	1-2	1 (1-1)	5.18	0.08 (NS)
B	31	1.16 (0.37)	1-2	1 (1-1)		
C	31	1.32 (0.48)	1-2	1 (1-2)		

**P*<0.05 statistically significant, *P*>0.05 NS. NS: Nonsignificant, SD: Standard deviation

Table 6: Comparison of retention status between different study groups at 6 months

6 months	*n*	Mean (SD)	Range	Median (Q1-Q3)	Kruskal-Wallis test		Mann-Whitney U-test		
					χ^2	*P*	A versus B	A versus C	B versus C
A	31	1.16 (0.37)	1-2	1 (1-1)	11.84	0.003*	0.24 (NS)	0.001*	0.03*
B	31	1.26 (0.45)	1-2	1 (1-2)					
C	31	1.55 (0.51)	1-2	2 (1-2)					

**P*<0.05 statistically significant, *P*>0.05 NS. NS: Nonsignificant, SD: Standard deviation

Table 7: Comparison of retention status between different study groups at 9 months

9 months	*n*	Mean (SD)	Range	Median (Q1-Q3)	Kruskal-Wallis test		Mann-Whitney U-Test		
					χ^2	*P*	A versus B	A versus C	B versus C
A	31	1.39 (0.62)	1-3	1 (1-2)	9.10	0.01*	0.86 (NS)	0.02*	0.006*
B	31	1.32 (0.48)	1-2	1 (1-2)					
C	31	1.68 (0.48)	1-2	2 (1-2)					

**P*<0.05 statistically significant, *P*>0.05 NS. NS: Nonsignificant, SD: Standard deviation

Table 8: Comparison of retention status between different study groups at 12 months

12 months	*n*	Mean (SD)	Range	Median (Q1-Q3)	Kruskal-Wallis test		Mann-Whitney U Test		
					χ^2	*P*	A versus B	A versus C	B versus C
A	31	1.39 (0.62)	1-3	1 (1-2)	9.10	0.01*	0.86 (NS)	0.02*	0.006*
B	31	1.32 (0.48)	1-2	1 (1-2)					
C	31	1.68 (0.48)	1-2	2 (1-2)					

**P*<0.05 statistically significant, *P*>0.05 NS. NS: Nonsignificant, SD: Standard deviation

has shown clogged organic material in the etched enamel specimens.[19]

The external organic layer prevents the 37% phosphoric acid from effectively acting on the enamel surface, thus providing inferior quality of etched surface for bonding. Type 1 and 2 etching pattern is observed when etched after 5.25% sodium hypochlorite deproteinization, which could give the better inward flow of resin than the inferior Type 3 pattern seen without deproteinization.[5]

These studies also found that enamel deproteinization did not grossly alter the surface topography features of enamel before acid etching. Other studies showcased that shear bond strength did not increase after enamel deproteinization with 5.25% sodium hypochlorite before etching.[20] Thus, deproteinization was done clinically before etching to evaluate if it enhances the retention of sealant by increasing the etched surface area of enamel.

Deproteinization was also tried after etching to enhance enamel bond strength in hypocalcified amelogenesis imperfecta teeth. An increase in the shear bond strength was noted after deproteinization.[21] Resin veneering was done on amelogenesis imperfecta associated tooth by treating with deproteinization after etching, which showed successful results on long-term basis.[22] Reports also show that deproteinization after etching improved bond strength values in primary and immature teeth enamel, unlike before etching.[6] Hence, deproteinization was done clinically after etching to evaluate if it enhances retention of sealant by increasing the etched surface area of enamel.

To the best of our knowledge, no *in vivo* studies have been done to evaluate sealant retention after deproteinization. Moreover, intraorally saliva and pellicle have a role to play, unlike a laboratory setting, which may not simulate the effect of deproteinization and acid etching *in vivo*. It is also difficult to maintain standardization during the processing of the samples and application of different surface treatments in the laboratory.[23] Thus, we did an *in vivo* study to evaluate the effect of deproteinization.

The modified Simonsen's criteria evaluate the occurrence of caries along with sealant retention status.[24] Similarly, the

USPHS criteria for sealants help determine factors such as marginal integrity and caries occurrence apart from sealant retention.[25] The modified USPHS criteria and Freigals criteria determine the marginal integrity, caries presence, color match, surface evaluation, and anatomical form.[21,26,27] The color, coverage, and caries sealant evaluation criterion[28] only indicates the level of surface coverage. It encompasses scoring criteria for sealant retention on the surface of the teeth. However we intended to evaluate the effectiveness of deproteinization on retention, we chose the Simonsen's criteria. The Simonsen's criteria for sealant retention have been proved to have high validity, good reliability, and also known to be simple and convenient.[11] Thus, every follow-up at 3, 6, 9, and 12 months was evaluated by these criteria. Since the failure probability of sealants is highest soon after application, they should be evaluated clinically for a partial or total loss within 1 year of placement.[28]

Partially retained sealants do not provide ideal caries protection; hence, it needs to be monitored and reapplied if needed.[29] Hence, we reapplied the sealants in case of partially retained cases.

Our results show significant retention loss when deproteinized before acid etching, unlike after etching or without deproteinization. Results from *in vitro* studies by Harleen *et al*.[20] and Gandhi *et al*.[30] find a resemblance to our *in vivo* study, which concluded that enamel deproteinization does not have added advantage in the retention of pit and fissure sealant over routine acid etching method. Gandhi *et al*.[30] found that deproteinizing before etching did not enhance the tag quality for penetration.

Deproteinization before etching was found to be effective by a number of authors. Sharma *et al*.[31] found it to cause a rougher surface on fluorosed teeth, Espinosa *et al*.[5,8] found it to provide a Type 1 and 2 etching pattern with an increase in the total etched area., Roberto *et al*.[3] adds that etching of enamel with 37% phosphoric acid after deproteinizing the enamel surface results into longer adhesive tags that penetrate the enamel adding to better retention. Thus, highly increasing the mechanical retention of adhesives to the enamel, Ayman *et al*.[32] stated that though NaOCl has a lower etching ability, it deproteinizes and increases the surface and give a chance to the etching material to penetrate more deeply creating Type 2 etching pattern and also increasing bond strength. Unlike our study, Venezie *et al*.[17] found it effective in bonding brackets to hypocalcified amelogenesis imperfect affected teeth. While Trindade *et al*.[33] found the reduction of shear bond strength of brackets.

Similar to this study, Ekambaram *et al*.,[34] Hasija *et al*.,[18] Aras *et al*.[6] found that deproteinization after acid etching significantly enhanced bond strength values than conventional etching. Aras *et al*.[6] also add that it was better than deproteinization before etching. In immature permanent tooth, it increased shear bond strength to be like mature teeth.[6] Studies by Ahuja *et al*.,[19] Ramakrishna *et al*.[35] showed no significant enhancive effect of enamel on deproteinization after etching. Ahuja *et al*.[19] did not find any significant topographical changes to enhance retention.

According to Abdelmegid *et al*., deproteinization before and after acid etching increased surface roughness of immature human enamel of permanent teeth like conventional etching. However, deproteinizing before etching with phosphoric acid gave higher surface roughness than deproteinizing after etching or without deproteinization.

To the best of our knowledge, there is no *in vivo* study done to evaluate the effectiveness of 5% sodium hypochlorite deproteinization on the retention of pit and fissure sealants.

Conclusion

The present study was done to evaluate the effectiveness of deproteinization on the retentiveness of pit and fissure sealants. The following conclusion was concluded from the study.

1. Deproteinization does not have added advantage in retention of pit and fissure sealant over routine acid etching method
2. Deproteinization after etching is better compared to deproteinization before etching
3. More *in vivo* studies are required in this arena to determine the effectiveness of deproteinization on the retention of pit and fissure sealants.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Kumar GS. Enamel. In: Orbans Oral Histology and Embryology. 12th ed.. Delhi: Elsevier India; 2009. p. 45-6.
2. Reddy VR, Chowdhary N, Mukunda KS, Kiran NK, Kavyarani BS, Pradeep MC. Retention of resin-based filled and unfilled pit and fissure sealants: A comparative clinical study. Contemp Clin Dent 2015;6:S18-23.
3. Grewal N, Chopra R. The effect of fissure morphology and eruption time on penetration and adaptation of pit and fissure sealants: An SEM study. J Indian Soc Pedod Prev Dent 2008;26:59-63.
4. Ijaz S, Choudary S, Awaiz F, Javed M, Asad R, Malik S, *et al*. The importance of enamel deproteinization in clinical dentistry: A review. PODJ 2018;33:374-7.
5. 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.
6. Aras S, Küçükeşmen C, Küçükeşmen HC, Sönmez IS. Deproteinization treatment on bond strengths of primary, mature and immature permanent tooth enamel. J Clin Pediatr Dent 2013;37:275-9.
7. Sanders BJ, Feigal RJ, Avery DR. Pit and fissure sealants and preventive resin restorations. In: Mcdonald RE, Avery DR, Dean JA, editors. Dentistry for the Child and Adolescent. 8th ed.. Missouri: Mosby; 2004. p. 357.
8. 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.
9. Pardi V, Sinhoreti MA, Pereira AC, Ambrosano GM, Meneghim Mde C. *In vitro* evaluation of microleakage of different materials used as pit-and-fissure sealants. Braz Dent J 2006;17:49-52.

10. Naaman R, El-Housseiny AA, Alamoudi N. The use of pit and fissure sealants-A literature review. Dent J (Basel) 2017;5:1-19.
11. Bhushan U, Goswami M. Evaluation of retention of pit and fissure sealants placed with and without air abrasion pretreatment in 6-8 year old children An *In vivo* study. J Clin Exp Dent 2017;9:e211-7.
12. Macek MD, Beltrán-Aguilar ED, Lockwood SA, Malvitz DM. Updated comparison of the caries susceptibility of various morphological types of permanent teeth. J Public Health Dent 2003;63:174-82.
13. Kishor A, Goswami M, Chaudhary S, Manuja N, Arora R, Rallan M. Comparative evaluation of retention ability of amorphous calcium phosphate containing and illuminating pit & amp; fissure sealants in 6-9 year old age group. J Indian Soc Pedod Prev Dent 2013;31:159-64.
14. Velpula L, Nirmala SV, Mallineni SK, Nuvvula S. The effectiveness and ease of a one-step conditioning agent with conventional acid etch and priming in the placement of sealants: A 6-month follow-up. Int J Pedod Rehabil 2018;3:23-7.
15. Das UM, Suma G. Bonding agents in pit and fissure sealants: A review. Int J Clin Pediatr Dent 2009;2:1-6.
16. Torres CP, Balbo P, Gomes-Silva JM, Ramos RP, Palma-Dibb RG, Borsatto MC. Effect of individual or simultaneous curing on sealant bond strength. J Dent Child (Chic) 2005;72:31-5.
17. Venezie RD, Vadiakas G, Christensen JR, Wright JT. Enamel pretreatment with sodium hypochlorite to enhance bonding in hypocalcified amelogenesis imperfecta: Case report and SEM analysis. Pediatr Dent 1994;16:433-6.
18. Hasija P, Sachdev V, Mathur S, Rath R. Deproteinizing agents as an effective enamel bond enhancer-an *in vitro* study. J Clin Pediatr Dent 2017;41:280-3.
19. Ahuja B, Yeluri R, Baliga S, Munshi AK. Enamel deproteinization before acid etching-a scanning electron microscopic observation. J Clin Pediatr Dent 2010;35:169-72.
20. Harleen N, Ramakrishna Y, Munshi AK. Enamel deproteinization before acid etching and its effect on the shear bond strength-an *in vitro* study. J Clin Pediatr Dent 2011;36:19-23.
21. Saroğlu I, Aras S, Oztaş D. Effect of deproteinization on composite bond strength in hypocalcified amelogenesis imperfecta. Oral Dis 2006;12:305-8.
22. Sönmez IS, Aras S, Tunç ES, Küçükeşmen C. Clinical success of deproteinization in hypocalcified amelogenesis imperfecta. Quintessence Int 2009;40:113-8.
23. Abdelmegid FY. Effect of deproteinization before and after acid etching on the surface roughness of immature permanent enamel. Niger J Clin Pract 2018;21:591-6.
24. Bhat PK, Konde S, Raj SN, Kumar NC. Moisture-tolerant resin-based sealant: A boon. Contemp Clin Dent 2013;4:343-8.
25. Guler C, Yilmaz Y. A two-year clinical evaluation of glass ionomer and ormocer based fissure sealants. J Clin Pediatr Dent 2013;37:263-7.
26. Askarizadeh N, Heshmat H, Zangeneh N. One-year clinical success of embrace hydrophilic and helioseal-f hydrophobic sealants in permanent first molars: A clinical trial. J Dent (Tehran) 2017;14:92-9.
27. Eskandarian T, Baghi S, Alipoor A. Comparison of clinical success of applying a kind of fissure sealant on the lower permanent molar teeth in dry and wet conditions. J Dent (Shiraz) 2015;16:162-8.
28. Subramaniam P, Girish Babu KL, Jayasurya S. Evaluation of solubility and microleakage of glass carbomer sealant. J Clin Pediatr Dent 2015;39:429-34.
29. Wendt LK, Koch G, Birkhed D. On the retention and effectiveness of fissure sealant in permanent molars after 15-20 years: A cohort study. Community Dent Oral Epidemiol 2001;29:302-7.
30. Gandhi S, Crawford P, Shellis P. The use of a 'bleach-etch-seal' deproteinization technique on MIH affected enamel. Int J Paediatr Dent 2012;22:427-34.
31. Sharma R, Kumar D, Verma M. Deproteinization of fluorosed enamel with sodium hypochlorite enhances the shear bond strength of orthodontic brackets: An *In vitro* study. Contemp Clin Dent 2017;8:20-5.
32. Ayman E, Amera A, Khursheed AM. Sodium hypochlorite as a deproteinizing agent optimize orthodontic brackets adhesion using resin modified glass ionomer cement. Austin J Dent 2016;3:1-7.
33. AM Trindade, TB Pereira, SN Perrin, MC Horta, MM Pithon, E Akaki, *et al*. Consequences of enamel preparation with sodium hypochlorite, polyacrylic and phosphoric acids for the bonding of brackets with resin-modified glass ionomer cements. Materials Res 2013;16:1423-7.
34. Ekambaram M, Anthonappa RP, Govindool SR, Yiu CKY. Comparison of deproteinization agents on bonding to developmentally hypomineralized enamel. J Dent 2017;67:94-101.
35. Ramakrishna Y, Bhoomika A, Harleen N, Munshi AK. Enamel Deproteinization after Acid Etching-Is it Worth the Effort? Dentistry 2014;200:1-5.