Review Article

Deproteinizing Agent, a Fore Step to Better Bonding: A literature Review

Shubhabrata Pal, Sauvik Galui[1], Subir Sarkar[1]

West Bengal University of Health Sciences, [1]Department of Pediatric and Preventive Dentistry, Dr. R Ahmed Dental College and Hospital, Kolkata, West Bengal, India

Abstract

Deproteinization can be defined as a process of removing the protein from a substance, usually as a stage in chemical purification. It is a necessary step in many procedures for the chemical analysis of body fluids. In dentistry, deproteinization can be used for elimination of the organic substances from the enamel surface before etching, as it increases the orthodontic bond strength by creating predominantly Type 1 and 2 etch patterns. Various authors have experimented the effect of enamel deproteinization with 5.25% sodium hypochlorite (NaOCl) prior to phosphoric acid etching and its effect on the etching pattern and shear bond strength of different adhesive systems. This article has reviewed and summarized the different studies regarding the effectiveness of NaOCl as a deproteinizing agent including its alternatives.

Keywords: Bond strength, bromelain, deproteinizing agent, papain gel, sodium hypochlorite

Introduction

In the field of modern dentistry, it has always been extremely essential to produce such materials that can enhance the bond strength between the tooth enamel surface and orthodontic brackets at an acceptable range. Since the 1960s, direct bonding of orthodontic brackets over tooth enamel surface has been promoted.[1] Irrespective of the bonding techniques, it is necessary to prepare the enamel surface properly to obtain a good and stable bond. This preparation includes the expulsion of enamel pellicle and creation of surface irregularities over the enamel surface prior to bonding which is called enamel conditioning.[2] It is done either by acid-etching technique or by sandblasting. Buonocore introduced acid-etching technique, which involves enamel dissolution to form surface microporosities that are utilized to create a micromechanical bond.[3] During routine etching with 37% phosphoric acid (H_3PO_4) for 15 s, 10 to 50 μm enamel is removed from the surface, wherein rough surface porosities up to 10 to 200 μm deep are created.[4] However, recent studies have shown that topographically, more than 69% of the H_3PO_4-treated enamel surface were left untreated, 7% showed tenuous etching, and only 2% was ideally etched.[5,6] Clinically, it can be found in adhesive restorations, sealants, and orthodontic brackets failure. To surpass these limitations, various invasive and noninvasive techniques have been introduced. Various authors have experimented the effect of enamel deproteinization with 5.25% sodium hypochlorite (NaOCl) prior to H_3PO_4 etching on the etching pattern and shear bond strength (SBS) of different adhesive systems.[7-12] Pithon *et al*.[13] have evaluated the effect of 10% papain gel as an enamel deproteinizing agent prior to the bonding procedure. Both NaOCl and papain gel showed good results with respect to the SBS of orthodontic brackets bonded with resin-modified glass ionomer cement (RMGI).

Sodium Hypochlorite in Dentistry and its Mechanism of Action

NaOCl is used as a root canal irrigating solution all over the world due to its efficacy for pulpal dissolution and antimicrobial activity. A dynamic balance is maintained by NaOCl and it is shown by the reaction:

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

Submitted: 26-Mar-2020 **Revised:** 16-Feb-2021
Accepted: 21-Feb-2021 **Published:** 11-Jan-2022

Access this article online

Quick Response Code:

Website: www.ijpedor.org

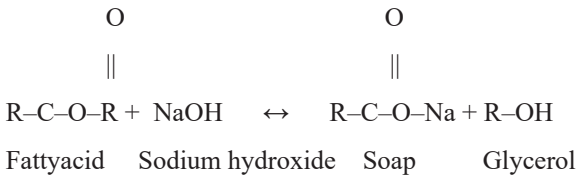
DOI: 10.4103/ijpr.ijpr_11_20

Address for correspondence: Dr. Shubhabrata Pal, WBUHS, Kolkata, West Bengal, India.
E-mail: spshubho25@gmail.com

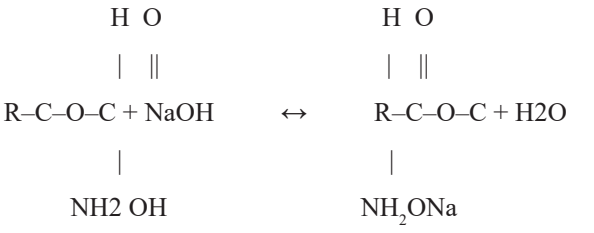
How to cite this article: Pal S, Galui S, Sarkar S. Deproteinizing agent, a fore step to better bonding: A literature review. Int J Pedod Rehabil 2021;6:1-5.

The chemical reaction between NaOCl and organic tissue takes place in three schemes.[14-17]

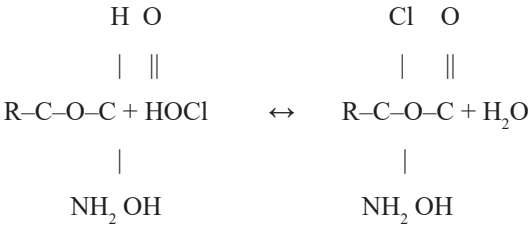
Scheme 1. Saponification reaction



Scheme 2. Amino acid neutralization reaction



Scheme 3. Chloramination reaction



After elucidating these chemical reactions, it can be seen that NaOCl acts as an organic and lipid solvent by degrading fatty acids into fatty acid salts (soaps) and glycerols that reduces the surface tension of the remaining solution (saponification reaction).

By neutralization reaction, NaOCl neutralizes amino acid into water and salt and the pH level drops with the exit of hydroxyl ions.

Hypochlorous acid present in NaOCl solutions acts as a solvent when it comes in contact with organic tissue. It releases chlorine that forms chloramines after combining with protein amino group (chloramination reaction).

These chloramines interfere in bacterial cell metabolism. Being a strong oxidant, chlorine shows its antimicrobial action by inhibiting bacterial enzymes leading to an irreversible oxidation of SH groups (sulphydryl group) of essential bacterial enzymes.

The above reactions clearly suggest that the use of 5.25% NaOCl as a deproteinizing agent can be a way to optimize adhesion by unfastening organic elements of both the enamel structure and the acquired pellicle.

Sodium Hypochlorite as Deproteinizing Agent

Gwinnett[18] and Silverstone *et al*.[19] classified enamel etching into three patterns after observing the enamel micromorphology using a scanning electron microscope (SEM). In Type 1 etching pattern, head of the prism is dissolved by H_3PO_4, but the interprismatic substance remains intact. In Type 2, the peripheral zone gets dissolved and the prism head remains intact. In Type 3, the changes are nonspecific creating only some superficial dissolution. These three etching patterns appear randomly together at any point on the enamel surface.[20] Silverstone *et al*. showed that both Type 1 and 2 etching patterns exhibit the most retentive features due to greater size and depth of porous surface,[19] whereas the Type 3 patterns lacked the micromechanical bonding as compared with the previous two.

Espinosa *et al*.[7] revealed that wetting and/or conditioning the enamel surface with 5.25% NaOCl for 1 min, prior to acid etching, enhanced the quality of the etching pattern because NaOCl eliminated the organic matter from the enamel surface (deproteinization). The authors demonstrated that the outer organic layer prevents successful etching of the enamel surface after application of 37% H_3PO_4, resulting in inconsistent etch patterns and an undependable enamel surface for orthodontic bonding. Type 1 and 2 etching patterns were created when NaOCl was used, whereas Type 3 etching patterns predominated when enamel pretreatment was not done using NaOCl.

Inference of different studies conducted to assess the effectiveness of sodium hypochlorite as a deproteinizing agent

1. In the year 2008, Espinosa *et al*.[7] conducted a study on ten extracted lower first and second permanent molars. Teeth were divided into four equal buccal sections having similar physical and chemical properties after polishing with pumice and water. Each group was treated with different formulations. Group A: Acid etching was done using 37% H_3PO_4 for 15 s. Group AH1: NaOCl 5.25% was used for 30 s followed by acid etching with 37% H_3PO_4 for 15 s. Group AH2: NaOCl 5.25% was used for 60 s followed by acid etching with 37% H_3PO_4 for 15 s.
   The results showed that Group AH2 etching technique achieved an area of 76.6 mm^2 of the total surface, with a 71.8 mm^2 (94.47%), type 1 and 2 etching pattern, Group AH1 with 55.9 mm^2 out of 75.12 mm^2 (74.1%), and Group A with only 36.8 mm^2 (48.83%) out of an area of 72.7 mm^2. Statistical difference ($P < 0.05$) was significant, giving rise to the conclusion that enamel deproteinization with 5.25% NaOCl for 1 min prior to H_3PO_4 etching increases the enamel conditioning surface as well as the quality of the etching pattern.
2. Justus *et al*.[8] in 2010 evaluated whether deproteinization of tooth enamel surface with 5.25% NaOCl prior to acid etching increases orthodontic bracket SBS of two adhesive systems: a composite resin and an RMGI. They experimented with 76 extracted human premolars and the adhesive systems were Transbond XT (3M Unitek Orthodontic Products, Monrovia, CA, USA)

and Fuji Ortho LC (GC America, Inc., Alsip, IL, USA). Pretreatment was done using 5.25% NaOCl before etching and orthodontic brackets were bonded, either with primer and composite resin or with RMGI. After a thorough experimental process, teeth were mounted on acrylic rings and debonded using a universal testing machine. The enamel surfaces were viewed at ×10 magnification to assess the amount of residual adhesive remaining on the tooth. An analysis of variance was done to determine whether there was a statistically significant difference in SBSs between the test groups, along with a *post hoc* test to determine possible significant differences among the pair of means; a Chi-square test was used to compare the adhesive remnant index scores. It was reported that SBS was significantly increased from 5.7 to 9.6 MPa using NaOCl in the Fuji Ortho LC group (compared with 9.4 MPa in the Transbond XT group with NaOCl) and the author concluded with the fact that pretreatment with 5.25% NaOCl can significantly increase the bracket bond strength with RMGI which is quite similar to the composite adhesive system. Thereby, fluoride-releasing RMGIs may possibly be used to bond brackets after conditioning the enamel with NaOCl as deproteinizing agent to reduce the incidence of white spot lesions

3. Ahuja B *et al*.[10] undertook a study to determine the topographical features of enamel surface deproteinized with NaOCl and etched with H_3PO_4 compared to H_3PO_4 alone using SEM analysis. Between the two groups, no statistically significant difference was observed. They concluded that the use of 37% H_3PO_4 for 15 s still remains the best method for pretreatment of the enamel
4. Another study was done by Harleen *et al*.[11] to analyze the effect of enamel deproteinization with 5.25% NaOCl preceding H_3PO_4 etching on the SBS of AdperTM Single Bond 2 adhesive and FiltekTM Z-350 XT composite resin. This study ended up with the fact that there is no significant effect of NaOCl enamel deproteinization on the SBS of AdperTM Single Bond 2 adhesive and FiltekTM Z-350 XT composite resin before acid etching
5. Ramakrishna *et al*.[21] conducted a study to observe the topographical features of enamel surface deproteinized with 5.25% NaOCl after H_3PO_4 etching by SEM analysis and also the effect of enamel deproteinization after acid etching on the SBS of Adper™ Single Bond 2 adhesive and Filtek™ Z-350 XT composite resin. However, there was no significant effect found regarding types 1 and 2 etching patterns and the SBS of adhesive resin and composite resin complex to the enamel surface
6. In 2016,[12] Ayman E *et al*. conducted a study to evaluate the effect of deproteinization of human dental enamel surfaces, with 5.25% NaOCl prior to etching on orthodontic bracket SBS of RMGI adhesive system. The experiment was quite similar to what Justus *et al*. did in 2010, but the debonding force (SBS) was estimated using the Instron machine and the residual adhesive remain on the tooth surface was marked as well as enamel roughness was measured using profilometry. The study concluded that enamel treatment with NaOCl raises the bonding strength of brackets bonded with RMGIC and was statistically significant when compared to the untreated group.

Effect of 10% Papain gel on Enamel Deproteinization

Papain is an alkaloid enzyme extracted from the latex of the *Carica papaya.* It is an endoprotein with anti-inflammatory and antibacterial properties. It cleaves partially degraded collagen fibrils and also removes fibrin coating formed by inflammatory process without causing any harmful effect on vital tissue.[22-25] To eliminate the influence of the organic matrix on the adhesion of composite to the enamel surface, it was suggested by Justus *et al.*[26] that 5.25% NaOCl should be used for 60 s as a deproteinizing agent before application of 37% H_3PO_4.

Pithon *et al*. in 2012[13] experimented to test the null hypothesis that 10% papain gel as an enamel deproteinizing agent does not increase the SBS of orthodontic brackets bonded with RMGIC. The aim of the study was to verify the hypothesis that 10% papain gel as a deproteinizing agent used for 60 s increases the SBS of brackets bonded with RMGIC. After obtaining the result, the hypothesis was rejected and they concluded with the fact that 10% papain gel is effective as an enamel deproteinizing agent.

Bromelain as Deproteinizing Agent

To assess the deproteinizing effect of the bromelain enzyme, a study was done by Raad Niama Dayeme and its effect was compared with Nd: YAG laser and 10% NaOCl by using SEM and polarized microscope.[27] Bromelain is a mixture of endopeptidases and it has fibrinolytic and anti-inflammatory activities. It also removes the collagen network from the dentinal surface and thereby decreases the leakage of adhesive restoration.[27] Sixty extracted human upper premolars were selected and standardized buccal and lingual class V cavities were prepared and the teeth were divided into three groups consisting of 20 in each. In the first group, teeth were deproteinized with Nd: YAG laser, whereas in the second group, teeth were deproteinized with bromelain enzyme and in the third group, teeth were deproteinized with 10% NaOCl.

Results

It was found that the bromelain enzyme was effective in removing the collagen network and significantly decreases the global leakage scores of the adhesive system.[27]

In an another study by Chauhan K, Basavanna RS, and Shivanna V, deproteinizing effect of bromelain enzyme and 5% NaOCl was compared.[28] The bond strength results were significantly influenced by the application of bromelain enzyme. Statistically significant differences were not demonstrated in

the control group and NaOCl-treated group. The highest bond strength was seen in bromelain enzyme-treated group as it was more effective in the removal of unsupported collagen fibrils than NaOCl.[28]

Discussion

Due to the presence of bacterial biofilm, the formation of white spot lesions and marginal gingivitis adjacent to fixed orthodontic appliances takes place.[29] Bishara and Ostby[30] stated that decalcification is an important effect of orthodontic therapy on dental enamel. To minimize and prevent white spot lesions, there has been cognizance about the use of new fluoride-releasing materials.[29] Glass ionomer cements developed by Wilson and Kent[31] allow chemical bonding to enamel, dentin, and other surfaces, in addition to releasing fluoride. However, these cements have lower bond strength to the enamel surface than orthodontic composites. Later on, RMGICs were developed to combine important characteristics of the above two materials (such as SBS and fluoride release) which release fluoride without compromising the bond strength to the tooth surface.[32-34]

However, Bishara *et al*.[35] concluded that RMGIs have significantly lower initial bond strength in comparison with composite adhesives which have significantly higher initial bond strength. Hence, the low initial bond strength of RMGI necessitates a second appointment for placing the archwire; which increases the total number of appointments during orthodontic treatment making time management more difficult for the orthodontist.[36]

It was Espinosa *et al*.[7] who reported that enamel deproteinization with 5.25% NaOCl for 1 min prior to H_3PO_4 etching increases the enamel conditioning surface as well as the quality of the etching pattern. Consequently, Roberto *et al*.[8] in 2010 concluded that pretreatment with 5.25% NaOCl can significantly increase the bracket bond strength with RMGI which is quite similar to the composite adhesive system. In 2016,[12] Ayman E, Amera A, and Khursheed AM conducted a study and reported that enamel treatment with NaOCl raises bonding strength of brackets bonded with RMGIC and was statistically significant when compared to the untreated group.

On the contrary, some other studies[10,11,21] reported no significant effect of NaOCl induced enamel deproteinization on etching pattern either or SBS between tooth surface bracket interface, whereas Pithon *et al*.[13] have applied 10% papain gel as an enamel deproteinizing agent prior to the bonding procedure. Both NaOCl and papain gel got good results with respect to the SBS of orthodontic brackets bonded with RMGI and future studies are needed to conclude whether papain gel or NaOCl or bromelain is more effective.

Conclusion

From the studies mentioned above, it can be concluded that the use of 5.25% NaOCl for 1 min, as a deproteinizing agent prior to acid etching, increases the bond strength which allows the orthodontist to use fluoride-releasing RMGIs as bonding adhesives that are able to possibly protect the enamel from developing white spot lesions, which is a major iatrogenic effect of orthodontic treatment. Further research is much needed to evaluate the real clinical benefits of NaOCl as a deproteinizing agent and to evaluate the deproteinizing effect of bromelain and 10% papain gel.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Newman GV. Epoxy adhesives for orthodontic attachments: Progress report. Am J Orthod 1965;51:901-12.
2. Canay Ş, Kocadereli İ, Akça E. The effect of enamel air abrasion on the retention of bonded metallic orthodontic brackets. Am J Orthod Dentofacial Orthop 2000;117:15-9.
3. Buonocore MG. A simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. J Dent Res 1955;34:849-53.
4. Graber TM, Eliades T, Athanasiou A. Risk management in orthodontics: Experts' guide to malpractice. Br Dent J 2004;198:114-5.
5. Hobson RS, Rugg-Gunn AJ, Booth TA. Acid-etch patterns on the buccal surface of human permanent teeth. Arch Oral Biol 2002;47:407-12.
6. Hobson RS, Crotty T, Thomason JM, Jepson NJ. A quantitative study of enamel acid etch patterns on surfaces used for retention of resin-bonded fixed prostheses. Eur J Prosthodont Restor Dent 2005;13:123-8.
7. 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.
8. Roberto J, Tatiana C, Ricardo O, Fernando M. 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.
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. 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.
11. 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;36:19-24.
12. 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;3:1037.
13. Pithon MM, Ferraz Cde S, de Oliveira Gdo C, 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.
14. Pecora JD, Sousa-Neto MD, Estrela C. Irrigating Solutions Used in the Biomechanical Preparation of Root Canals. In: Estrela C, Figueiredo JA, editors. Endodontia-Princípios Biológicos e Mecânicos. São Paulo: Artes Médicas; 1999. p. 552-69.
15. Barbin EL. *In vitro* study of the effect of the addition of lauryl diethylene glycol ether sulfate in sodium hypochlorite solutions on their physical and chemical properties before and after the dissolution of bovine pulp tissue.. [Master's thesis]. Ribeirão Preto: University of São Paulo; 1999. p. 108. Available from: http://www.forp.usp.br/restauradora/Teses/Barbin/mestrado_barbin.html. [Last accessed on 2020 Mar 11]
16. Santos TC. Estudo *"in vitro*" do efeito do aumento da temperatura nas soluções de hipoclorito de sódio sobre suas propriedades físico-químicas anteriores e posteriores à dissolução do tecido pulpar bovino. [Master's

thesis]. Ribeirão Preto: University of São Paulo; 1999. p. 108. Available from: http://www.forp.usp.br/restauradora/Teses/Tanit/tanit_mestrado.html. [Last accessed on 2020 Mar 08].
17. Spanó JC, Barbin EL, Santos TC, Guimarães LF, Pécora JD. Solvent action of sodium hypochlorite on bovine pulp and physico-chemical properties of resulting liquid. Braz Dent J 2001;12:154-7.
18. Gwinnett AJ. Histological changes in human enamel following treatment with acidic adhesive conditioning agents. Arch Oral Biol 1971;16:731-8.
19. 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.
20. Kodaka T, Kuroiwa M, Higashi S. Structural and distribution patterns of surface "prismless" enamel in human permanent teeth. Caries Res 1991;25:7-20.
21. Ramakrishna Y, Bhoomika A, Harleen N, Munshi AK. Enamel deproteinization after acid etching - Is it worth the effort? Dentistry 2014;4:200.
22. Bruno LH, Guedes CC, Motta LJ, Santos EM, Bussadori SK. Comparison between the use of low speed rotary elements and mechanical chemical treatment of dentinal caries in deciduous dentition. Acta Odontol Venez 2009;47:1-9.
23. Piva E, Ogliari FA, Moraes RR, Cora F, Henn S, Correr-Sobrinho L. Papain-based gel for biochemical caries removal: Influence on microtensile bond strength to dentin. Braz Oral Res 2008;22:364-70.
24. Motta LJ, Martins MD, Porta KP, Bussadori SK. Aesthetic restoration of deciduous anterior teeth after removal of carious tissue with Papacarie. Indian J Dent Res 2009;20:117-20.
25. Silva LR, Murillo JH, Santos EM, Guedes-Pinto AC, Bussadori SK. Use of papaya gel for caries removal. Acta Odontol Venez 2005;43:1-5.
26. Justus R, Cubero T, Ondarza R, 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.
27. Dayem RN, Tameesh MA. A new concept in hybridization: Bromelain enzymefor deproteinizing dentin before application of adhesive system. Contemp Clin Dent 2013;4:421-6.
28. Chauhan K, Basavanna RS, Shivanna V. Effect of bromelain enzyme for dentin deproteinization on bond strength of adhesive system. J Conserv Dent 2015;18:360-3.
29. Pithon MM, Dos Santos RL, de Oliveira MV, Ruellas AC, Romano FL. Metallic brackets bonded with resin-reinforced glass ionomer cements under different enamel conditions. Angle Orthod 2006;76:700-4.
30. Bishara SE, Ostby AW. White spot lesions: formation, prevention, and treatment. Semin Orthod 2008;14:174-82.
31. Wilson AD, Kent BE. A new translucent cement for dentistry. The glass ionomer cement. Br Dent J 1972;132:133-5.
32. Wheeler AW, Foley TF, Mamandras A. Comparison of fluoride release protocols for in-vitro testing of 3 orthodontic adhesives. Am J Orthod Dentofacial Orthop 2002;121:301-9.
33. Bishara SE, Ostby AW, Laffoon JF, Warren J. Shear bond strength comparison of two adhesive systems following thermocycling. A new self-etch primer and a resin-modified glass ionomer. Angle Orthod 2007;77:337-41.
34. Bishara SE, Ostby AW, Laffoon J, Warren JJ. A self-conditioner for resin-modified glass ionomers in bonding orthodontic brackets. Angle Orthod 2007;77:711-5.
35. Bishara SE, VonWald L, Olsen ME, Laffoon JF. Effect of time on the shear bond strength of glass ionomer and composite orthodontic adhesives. Am J Orthod Dentofacial Orthop 1999;116:616-20.
36. Sharma P, Valiathan A, Arora A, Agarwal S. A comparative evaluation of the retention of metallic brackets bonded with resin-modified glass ionomer cement under different enamel preparations: A pilot study. Contemp Clin Dent 2013;4:140-6.