



A review of the research on Deproteinizing agents as a first step toward improved bonding

Nidhi Gupta,¹ Neeti Mittal,² Sumita Giri,^{3*} Chetna Arora,⁴ Puja Malhotra,⁵ Rajiv Ahluwalia⁶

¹Professor & HOD, Department of Pediatric and Preventive Dentistry, Santosh Dental College & Hospital, Santosh Deemed to be University, Ghaziabad, Delhi NCR

²Professor, Department of Pediatric and Preventive Dentistry, Santosh Dental College & Hospital, Santosh Deemed to be University, Ghaziabad, Delhi NCR

³Professor & HOD, Department of Conservative Dentistry and Endodontics, Santosh Dental College & Hospital, Santosh Deemed to be University, Ghaziabad, Delhi NCR

⁴Professor, Department of Conservative Dentistry and Endodontics, Santosh Dental College & Hospital, Santosh Deemed to be University, Ghaziabad, Delhi NCR

⁵Professor & HOD, Department of Prosthodontics and Crown & Bridge, Santosh Dental College & Hospital, Santosh Deemed to be University, Ghaziabad, Delhi NCR

⁶Professor & HOD, Department of Orthodontics and Dentofacial Orthopedics, Santosh Dental College & Hospital, Santosh Deemed to be University, Ghaziabad, Delhi NCR

*Corresponding author- Sumita Giri

ABSTRACT

Deproteinization is the removal of proteins from a compound, typically as a step in chemical purification. In many processes for the chemical analysis of body fluids, it is a crucial step. Deproteinization can be used in dentistry to remove organic materials from the enamel surface prior to etching because it strengthens the orthodontic bond by primarily producing Type 1 and Type 2 etch patterns. Several writers have used 5.25% sodium hypochlorite (NaOCl) to investigate with the effects of enamel deproteinization on the shear bond strength of various adhesive systems as well as the etching pattern. The various investigations on the efficiency of NaOCl as a deproteinizing agent, including its alternatives, have been examined and summarised in this article.

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

INTRODUCTION

Producing materials that can improve the bond strength between the tooth enamel surface and orthodontic brackets at a reasonable level has always been crucial in the field of modern dentistry. The direct bonding of orthodontic brackets over the surface of tooth enamel has been encouraged since the 1960s. [1] To achieve a good and stable bond, the enamel surface must be adequately prepared regardless of the bonding processes used. Enamel conditioning is the process of preparing the

enamel surface for bonding by causing surface imperfections and the evacuation of the enamel pellicle. [2]

Either the acid-etching method or sandblasting are used. In order to develop surface microporosities that can be used to form a micromechanical link, Buonocore invented the acid-etching technique. [3] Routine etching with 37% phosphoric acid (H₃PO₄) for 15 s results in the creation of rough surface porosities up to 10 to 200 μm deep by removing 10 to 50 μm of enamel from the surface. Recent research, however,

has revealed that topographically, more than 69% of the H₃PO₄-treated enamel surface was left untreated, 7% had tenuous etching, and just 2% was optimally etched. [5-6] Clinically, it manifests as the failure of orthodontic brackets, sealants, and adhesive restorations. Numerous invasive and noninvasive approaches have been developed to overcome these restrictions. The impact of enamel deproteinization with 5.25% sodium hypochlorite (NaOCl) before H₃PO₄ etching on the etching pattern and shear bond strength (SBS) of various adhesive systems has been investigated by a number of writers. [7-12] The effectiveness of 10% papain gel as an enamel deproteinizing agent prior to the bonding technique has been studied by Pithon et al. Using regard to the SBS of orthodontic brackets attached with resin-modified glass ionomer cement, both NaOCl and papain gel demonstrated positive outcomes (RMGI).

Sodium Hypochlorite in Dentistry and its Mechanism of Action

Because of its effectiveness in pulpal dissolving and antibacterial activity, NaOCl is used as a root canal irrigating solution all over the world. NaOCl maintains a dynamic equilibrium, as evidenced by the following reaction: $\text{NaOCl} + \text{H}_2\text{O} \rightleftharpoons \text{NaOH} + \text{HOCl}$ $\text{Na}^{++}\text{OH}^{-} + \text{H}^{++}\text{OCl}^{-}$. There are three different ways that NaOCl and organic tissue react chemically [14-17].

By breaking down fatty acids into fatty acid salts (soaps) and glycerols, which lowers the surface tension of the residual solution, NaOCl works as an organic and lipid solvent. This is made clear by the chemical reactions that resulted in these conclusions (saponification reaction). By neutralisation reaction, NaOCl converts amino acid into salt and water, and the pH level decreases as hydroxyl ions leave the system.

When hypochlorous acid in NaOCl solutions comes into touch with organic tissue, it works as a solvent. When it interacts with the amino group of a protein, it releases chlorine, which turns into chloramines (chloramination reaction). These chloramines disrupt the metabolic process in bacterial cells. Since chlorine is a potent oxidizer, it inhibits bacterial enzymes, which causes the irreversible oxidation of SH groups (sulphydryl groups) in crucial bacterial enzymes.

The aforementioned responses undoubtedly imply that using 5.25% NaOCl as a deproteinizing agent can improve adhesion by loosening organic components of both the enamel structure and the acquired pellicle.

Sodium Hypochlorite as Deproteinizing Agent

In order to categorise enamel etching into three patterns, Gwinnett[18] and Silverstone et al. [19] used a scanning electron microscope to examine the enamel micromorphology (SEM). H₃PO₄ dissolves the prism's head in Type 1 etching patterns, but the interprismatic material is left untouched. In Type 2, the head of the prism remains intact while the periphery dissolves. In Type 3, the modifications are vague and only produce a minimal amount of disintegration. At any given location on the enamel surface, these three etching patterns can emerge together at random. [20]

Due to the larger size and depth of the porous surface, Type 1 and Type 2 etching patterns both exhibit the most retentive properties, according to Silverstone et al. [19], however Type 3 patterns lacked micromechanical bonding in comparison to the first two.

Prior to acid etching, Espinosa et al. [7] found that soaking and/or treating the

enamel surface with 5.25% NaOCl for 1 min improved the quality of the etching pattern by removing organic debris from the enamel surface (deproteinization). After applying 37% H₃PO₄, the authors showed that the outer organic layer limits successful etching of the enamel surface, leading to erratic etch patterns and an unreliable enamel surface for orthodontic bonding. When NaOCl was utilised, Type 1 and Type 2 etching patterns were produced, while Type 3 etching patterns predominated when enamel pretreatment wasn't done with NaOCl.

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

1. Espinosa et al. [7] studied 10 extracted lower first and second permanent molars in the year 2008. After polishing with pumice and water, teeth were split into four equal buccal regions with comparable physical and chemical characteristics. Each group received a unique formulation of care. Group A: For 15 seconds, 37% H₃PO₄ was used for acid etching. Group AH1: NaOCl 5.25% was used for 30 s, then 37% H₃PO₄ was utilised for 15 s of acid etching. Group AH2: 15 seconds of acid etching with 37% H₃PO₄ was followed by 60 seconds of NaOCl 5.25%.

The results showed that Group AH2's type 1 and type 2 etching patterns successfully etched an area of 76.6 mm² of the total surface, while Group AH1 only managed to etch an area of 55.9 mm² out of a total surface area of 75.12 mm² (74.1%) and Group A only managed to etch an area of 36.8 mm² (48.83%) out of a total surface area of 72.7 mm². The enamel deproteinization with 5.25% NaOCl

for 1 min prior to H₃PO₄ etching increased the enamel conditioning surface and the quality of the etching pattern, according to statistical difference (P 0.05) that was significant enough to draw this conclusion.

2. In 2010, Justus et al. [8] examined whether deproteinizing the surface of tooth enamel with 5.25% NaOCl before acid etching increased the SBS of two orthodontic bracket adhesive systems: composite resin and RMGI. The adhesive systems used were Transbond XT (3M Unitek Orthodontic Products, Monrovia, CA, USA) and Fuji Ortho LC during the experimentation on 76 removed human premolars (GC America, Inc., Alsip, IL, USA). Before etching and bonding orthodontic brackets with either primer and composite resin or RMGI, pretreatment was carried out using 5.25% NaOCl. Teeth were installed on acrylic rings and debonded using a universal testing machine after a rigorous trial process. To determine how much adhesive was still present on the tooth, the enamel surfaces were magnified by a factor of 10. A Chi-square test was utilised to evaluate the adhesive remnant index scores; an analysis of variance was used to see if there was a statistically significant difference in SBSs between the test groups, as well as a post hoc test to find any potential significant variations among the pair of means. In comparison to 9.4 MPa in the Transbond XT group with NaOCl, SBS was reported to have increased significantly from 5.7 to 9.6 MPa using NaOCl in the Fuji Ortho LC group. The author came to the conclusion that pretreatment with 5.25% NaOCl can

significantly increase bracket bond strength with RMGI, which is very similar to the composite adhesive system. Thus, after treating the enamel with NaOCl as a deproteinizing agent to limit the frequency of white spot lesions, fluoride-releasing RMGIs may be employed to bond brackets.

3. Ahuja B et al. [10] conducted a study to evaluate the topographical characteristics of enamel surfaces that had been etched with H₃PO₄ and deproteinized with NaOCl to those that had just been etched with H₃PO₄. There was no statistically significant difference between the two groups. They came to the conclusion that the optimal way for pretreating enamel is still using 37% H₃PO₄ for 15 seconds.
4. The SBS of Adper™ Single Bond 2 adhesive and Filtek™ Z-350 XT composite resin was examined in a different investigation by Harleen et al. [11] to determine the impact of enamel deproteinization with 5.25% NaOCl before H₃PO₄ etching. This investigation found that, prior acid etching, Adper™ Single Bond 2 adhesive and Filtek™ Z-350 XT composite resin's SBS was not significantly affected by NaOCl enamel deproteinization.
5. Ramakrishna et al. [21] carried out a study to determine the effects of enamel deproteinization after acid etching on the SBS of Adper™ Single Bond 2 adhesive and Filtek™ Z-350 XT composite resin as well as the topographical features of enamel surface deproteinized with 5.25% NaOCl after H₃PO₄ etching. However, no discernible difference between the SBS of adhesive resin and composite resin complex to the enamel

surface and types 1 and 2 etching patterns was discovered.

6. In order to determine the impact of deproteinizing human tooth enamel surfaces with 5.25% NaOCl prior to etching on orthodontic bracket SBS of the RMGI adhesive system, Ayman E et al. undertook a study in 2016[12]. While the experiment was very similar to that conducted by Justus et al. in 2010, the debonding force (SBS) was calculated using an Instron machine, and the amount of adhesive that remained on the tooth surface was marked in addition to profilometry measurements of enamel roughness and residual adhesive. In comparison to the untreated group, the study found that enamel treatment with NaOCl increases the bonding strength of brackets bonded with RMGIC.

Effect of 10% Papain gel on Enamel Deproteinization

The latex of the *Carica papaya* is used to make the alkaloid enzyme known as papain. It is an endoprotein having bacterial and anti-inflammatory effects. Without having any negative effects on critical tissue, it eliminates the fibrin coating created by the inflammatory process and cleaves partially degraded collagen fibrils. [22],[23],[24],[25] Justus et al.[26] recommended using 5.25% NaOCl for 60 s as a deproteinizing agent before applying 37% H₃PO₄ to the enamel surface in order to remove the impact of the organic matrix on the adhesion of composite to the enamel surface.

In 2012[13], Pithon et al. conducted an experiment to rule out the possibility that using 10% papain gel to deproteinize enamel might increase the SBS of orthodontic brackets bonded with RMGIC. The purpose of the study was to test the

claim that brackets bonded with RMGIC had higher SBS when 10% papain gel, a deproteinizing agent, is employed for 60 seconds. The theory was disproved by the results, and they came to the conclusion that 10% papain gel works well to deproteinize enamel.

Bromelain as Deproteinizing Agent

Raad Niama Dayeme conducted a study in which the deproteinizing power of the bromelain enzyme was evaluated and its effect was contrasted with that of the Nd: YAG laser and 10% NaOCl using SEM and polarised microscopy. Endopeptidases make up bromelain, which also has fibrinolytic and anti-inflammatory properties. Additionally, it eliminates the collagen network from the dentinal surface, reducing adhesive restoration leakage. [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. The teeth in the first group were deproteinized using a Nd: YAG laser, whereas the teeth in the second group were deproteinized using bromelain enzyme and the teeth in the third group were deproteinized using 10% NaOCl.

RESULTS

It was discovered that the bromelain enzyme considerably lowers the adhesive system's global leakage scores and is effective at eliminating the collagen network [27]. Another study by Chauhan, Basavanna, and Shivanna evaluated the deproteinizing effects of 5% NaOCl and bromelain enzyme [28]. Application of the bromelain enzyme had a substantial impact on the results for bond strength. There were no changes between the control group and the NaOCl-treated group that were statistically significant. The group treated

with bromelain enzyme demonstrated the highest bond strength because it was more successful than NaOCl at removing unsupported collagen fibrils [28].

DISCUSSION

White spot lesions and marginal gingivitis develop next to fixed orthodontic appliances as a result of the presence of bacterial biofilm. Decalcification is a significant consequence of orthodontic therapy on dental enamel, according to Bishara and Ostby[30]. There has been awareness of the usage of new fluoride-releasing products to reduce and prevent white spot lesions. [29]

Wilson and Kent's glass ionomer cements [31], in addition to releasing fluoride, permit chemical attachment to enamel, dentin, and other surfaces. Contrary to orthodontic composites, these cements have a weaker connection to the enamel surface. RMGICs, which combine key features of the aforementioned two materials (such SBS and fluoride release) and release fluoride without weakening the adhesive strength to the tooth surface, were later created. [32-34]

RMGIs, however, have a much lower initial bond strength than composite adhesives, which have a significantly higher initial bond strength, according to Bishara et al. [35]. Due to the low initial bond strength of RMGI, a second appointment is required to place the archwire, which raises the overall number of appointments required for orthodontic treatment and makes time management for the orthodontist more challenging. [36]

Espinosa et al. reported that increasing the enamel conditioning surface and the quality of the etching pattern by deproteinizing the enamel with 5.25% NaOCl for 1 min before H3PO4 etching. Roberto et al. [8] came to

the conclusion that pretreatment with 5.25% NaOCl can greatly boost bracket bond strength with RMGI, which is relatively similar to the composite adhesive method, in 2010. According to a study conducted in 2016[12] by Ayman E, Amara A, and Khursheed AM, applying NaOCl to the enamel increases the bonding strength of brackets adhered with RMGIC and is statistically significant when compared to the control group.

While Pithon et al. [13] utilised 10% papain gel as an enamel deproteinizing agent prior to the bonding technique, other studies[10],[11],[21] showed no significant influence of NaOCl generated enamel deproteinization on etching pattern or SBS between tooth surface bracket interface. Future research is required to determine whether papain gel, NaOCl, or bromelain is more efficient with regard to the SBS of orthodontic brackets bonded with RMGI. Both NaOCl and papain gel obtained positive findings in this regard.

CONCLUSION

According to the aforementioned studies, deproteinizing with 5.25% NaOCl for one minute prior to acid etching strengthens the bond, enabling the orthodontist to use fluoride-releasing RMGIs as bonding adhesives that may be able to prevent the enamel from developing white spot lesions, a significant iatrogenic effect of orthodontic treatment. To assess the true clinical advantages of NaOCl as a deproteinizing agent and to assess the deproteinizing effects of bromelain and 10% papain gel, additional study is urgently required.

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, Amara 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. [PUBMED] [Full text]
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 enzyme for deproteinizing dentin before application of adhesive system. *Contemp Clin Dent* 2013;4:421-6. [PUBMED] [Full text]
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. [PUBMED] [Full text]
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. [PUBMED] [Full text].