

**Evaluation of the Effect of Different
Deproteinizing Agents on the Clinical
Success of Fissure Sealants in
Primary Teeth: Splint Mouth Clinical
Study**

18/12/2023

RESEARCH PROTOCOL

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1. PURPOSE and HYPOTHESIS OF THE RESEARCH

Dental caries is a chronic infectious, preventable disease in which prolonged exposure to fermentable carbohydrate causes an ecological shift in the composition and activity of the bacterial biofilm, resulting in the physical and chemical destruction of dental hard tissues and disrupting the balance between demineralization and remineralization of tooth surfaces.¹ The teeth with the highest caries risk are primary molars, respectively.² The retentive areas formed by pits and fissures on the occlusal surfaces of molars are more favorable areas for caries initiation from the eruption of teeth.³ Pit and fissure sealants are the most effective method used to prevent caries formation in these areas. Pit and fissure sealants are a non-invasive method that has been used for many years to prevent caries formation or to stop the progression of initial caries lesions limited to enamel on tooth surfaces considered to be at high risk of caries.⁴ There are many clinical studies in the literature reporting that fissure sealants applied with non-invasive techniques are highly effective in preventing pit and fissure caries.^{5,6} Various methods such as pumice prophylaxis and deproteinization have been recommended as surface preparation methods to increase the success of fissure sealants.^{7,8} During the application of pit and fissure sealants, acid roughening of the enamel as a pre-application is an inevitable step.⁹ Removal of organic materials from the enamel surface before acid roughening provides better acid roughening on the enamel, increases bond strength, improves adhesion and retention of the adhesive material. There are many studies in the literature showing that the use of 5.25% NaOCl as an enamel deproteinization agent is successful in positively affecting adhesion by removing the organic elements of the pellicle both in the enamel structure and on the enamel surface before acid application.¹⁰⁻¹³ Hypochlorous acid is produced as a result of oxidative reactions by cells of the body immune system, neutrophilic polymorphonuclear leukocytes, to overcome pathogenic microorganisms and fight infection.^{14,15} Recently, slightly acidic HOCl solutions developed by electrolyzing NaOCl have been widely used as disinfectants.¹⁶ Hypochlorous acid has been shown to significantly reduce the lipopolysaccharide concentration of *Porphyromonas gingivalis* compared to NaOCl and chlorhexidine, and is a well-tolerated agent by oral tissues.¹⁷ Furthermore, HOCl used as a mouthwash has been reported to produce no systemic side

effects.¹⁸ In clinical dentistry, HOCl has been shown to be highly effective as a result of its antimicrobial and deproteinizing properties. With its low acidity, HOCl is an antiseptic irrigant with exceptional properties, exhibiting not only biocompatibility and low cytotoxicity, but also rapid and highly effective antimicrobial activity in physiological concentration ranges.¹⁹ The aim of this study was to compare the effects of sodium hypochlorite and hypochlorous acid, deproteinizing agents used to prepare the tooth surface for fissure sealant application in primary teeth, on the clinical success of fissure sealants.

The null hypotheses of our study;

- I. There is no significant difference in terms of retention loss and new caries formation between the fissure sealants in the group treated with sodium hypochlorite as deproteinizing agent and the fissure sealants in the group treated with hypochlorous acid.
- II. There is no significant difference in edge integrity between the fissure sealants in the group treated with sodium hypochlorite as deproteinizing agent and the fissure sealants in the group treated with hypochlorous acid.
- III. There is no significant difference in edge coloration between the fissure sealants in the group treated with sodium hypochlorite as deproteinizing agent and the fissure sealants in the group treated with hypochlorous acid.
- IV. There is no significant difference in anatomical form between the fissure sealants in the group treated with sodium hypochlorite as deproteinizing agent and the fissure sealants in the group treated with hypochlorous acid.

Keywords: Deproteinization, Fissure sealant, Hypochlorous acid, Sodium hypochlorite

REFERENCES

1. Young DA, Novy BB, Zeller GG, Hale R, Hart TC, Truelove EL, et al. The American Dental Association Caries Classification System for clinical practice: a report of the American Dental Association Council on Scientific Affairs. *J Am Dent Assoc.* 2015;146(2):79-86.
2. Batchelor PA, Sheiham A. Grouping of tooth surfaces by susceptibility to caries: a study in 5-16 year-old children. *BMC Oral Health.* 2004;4(1):2.
3. Horst JA, Tanzer JM, Milgrom PM. Fluorides and Other Preventive Strategies for Tooth Decay. *Dental Clinics of North America.* 2018;62(2):207-34.

4. Salama, F. S., and Al-Hammad, N. S. (2002). Marginal seal of sealant and compomer materials with and without enameloplasty. *International Journal of Paediatric Dentistry*, 12(1), 39-46.
5. Mertz-Fairhurst EJ, Adair SM, Sams DR, Curtis JW, Jr, Ergle JW, Hawkins KI, et al. Cariostatic and ultraconservative sealed restorations: nine-year results among children and adults. *ASDC J Dent Child* 62: 97-107, 1995.
6. Morphis TL, Toumba KJ, Lygidakis NA. Fluoride pit and fissure sealants: a review. *Int J Paediatr Dent* 10: 90-98, 2000.
7. Garrocho-Rangel A, Lozano-Vázquez C, Butrón-Tellez-Girón C, Escobar-García D, Ruíz-Rodríguez S, Pozos-Guillén AJEjopd. In vitro assessment of retention and microleakage in pit and fissure sealants following enamel pre-etching with sodium hypochlorite deproteinisation. 2015;16(3):212-6.
8. Hegde RJ, Coutinho RCJJJoISoP, Dentistry P. Comparison of different methods of cleaning and preparing occlusal fissure surface before placement of pit and fissure sealants: An in vivo study. 2016;34(2):111.
9. Buonocore, M. G. (1955). A simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. *Journal of Dental Research*, 34(6), 849-853.
10. Espinosa, R., Valencia, R., Uribe, M., Ceja, I., and Saadia, M. (2008). Enamel deproteinization and its effect on acid etching: an in vitro study. *Journal of Clinical Pediatric Dentistry*, 33(1), 13-19.
11. Valencia, R., Espinosa, R., Borovoy, N., Perez, S., Ceja, I., and Saadia, M. (2018). Deproteinization Effectiveness on Occlusal Enamel Surfaces and Resultant Acid Etching Patterns: An in vitro Study. *Journal of Clinical Pediatric Dentistry*, 42(6), 434-441.
12. Justus, R., Cubero, T., Ondarza, R., & Morales, F. (2010, March). 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. *Seminars in Orthodontics*, 16(1), 66-75.
13. Harleen N, Ramakrishna Y, Munshi AJJoCPD. Enamel deproteinization before acid etching and its effect on the shear bond strength-An in vitro study. 2011;36(1):19-24.
14. Mainnemare, A., Megarbane, B., Soueidan, A., Daniel, A., and Chapple, I. L. (2004). Hypochlorous acid and taurine-N-monochloramine in periodontal diseases. *Journal of Dental Research*, 83(11), 823-831.
15. Fukuzaki, S. (2006). Mechanisms of actions of sodium hypochlorite in cleaning and disinfection processes. *Biocontrol Science*, 11(4), 147-157
16. Wang, L., Bassiri, M., Najafi, R., Najafi, K., Yang, J., Khosrovi, B., Hwong, W., Barati, E., Belisle, B., Celeri, C., and Robson, M. C. (2007). Hypochlorous acid as a potential wound care agent: part I. Stabilized hypochlorous acid: a component of the inorganic armamentarium of innate immunity. *Journal of Burns and Wounds*, 6(e5).
17. Block, M. S., and Rowan, B. G. (2020). Hypochlorous Acid: A Review. *Journal of Oral Maxillofacial Surgery*, 78(9), 1461-1466.

18. Morita, C., Nishida, T., and Ito, K. (2011). Biological toxicity of acid electrolyzed functional water: effect of oral administration on mouse digestive tract and changes in body weight. *Archives of Oral Biology*, 56(4), 359-366.
19. Pashley, D. H., Tao, L., Boyd, L., King, G. E., and Horner, J. A. (1988). Scanning electron microscopy of the substructure of smear layers in human dentine. *Archives of Oral Biology*, 33(4), 265-270.

2. MATERIALS AND METHOD

Case Selection

This study will include patients between the ages of 4 and 6 years old who are admitted to Tokat Gaziosmanpaşa University, Faculty of Dentistry, Department of Pedodontics for routine check-ups, have no systemic problems, have a score of 3 and 4 according to the Frankl Behavior Scale 20 (Table 1), have no parafunctional habits such as clenching and/or grinding, have erupted caries-free primary lower second molars, and have an indication for fissure sealant application. In our single-center, split mouth clinical study, sodium hypochlorite (Wizard, Turkey) will be applied to one of the lower second molars and hypochlorous acid (Superox, Anolit Hijyen ve Kimya Sanayi Anonim Şirketi, Ankara, Turkey) will be applied to the other as deproteinizing agent before non-invasive (without any abrasion on the tooth) fissure sealant application under rubber-dam isolation. Then 37% phosphoric acid (K-ETCHANT Syringe, Kuraray Noritake Dental Inc., Japan) will be applied. Clinpro™ Sealant (3M ESPE, St. Paul, MN, United States of America), a resin-based sealant that polymerizes with light, changes color during polymerization, contains fluorine and has a low filler content, will be used as fissure sealant in both groups.

Table 1. Frankl Behavior Scale

1	-	Definitely negative	Refusal of treatment, crying forcefully, fearful or any other overt evidence of extreme negativism.
2	--	Negative	Reluctant to accept treatment; uncooperative, some evidence of negative attitude but not

			pronounced, i.e. /sullen, withdrawn.
3	+	Positive	Acceptance of treatment; at times cautious, willingness to comply with the dentist, at times with reservation but patient follows the dentist's directions cooperatively.
4	++	Definitely positive	Good rapport with the dentist, interested in the dental procedures, laughing and enjoying the situation.

Power Analysis of the Study

As a result of the power analysis of the study with 95% confidence ($1-\alpha$), 95% test power ($1-\beta$) and Chi-square test with an effect size of $w=0.576$, the number of samples to be included in the study was found to be 47. When the dropout rate was taken as 20%, the study was planned to be conducted with 60 samples.²¹

Clinical Assessments

The patients included in the study will be followed up by two observers at 3,6, 12 and 24 months. At the control sessions, loss of retention and new caries formation (Table 2) according to the Modified Simonsen Criteria (1991) (22), edge integrity (Table 3) and edge coloration (Table 4) according to the criteria of Feigal et al. (2000) and anatomical form (Table 5) will be evaluated.

Table 2. Modified Simonsen Criteria

Score 0 Fissure sealant completely in the mouth and no new caries formation.

Score 1 Partial loss of fissure sealant, no new caries formation.

Score 2 Partial loss of fissure sealant and new caries formation.

Score 3 Complete loss of fissure sealant, no new caries formation.

Score 4 Complete loss of fissure sealant and new caries formation.

Table 3. Edge Integrity Evaluation Criteria

0 Fissure sealant and tooth surface are intact and indistinguishable with the probe.

1 The edge of the fissure sealant can be distinguished with a probe.

2 There is spacing along the edge of the fissure seal and deep cracks reaching the central fossa.

Table 4. Edge Coloration Evaluation Criteria

0 No discoloration between fissure sealant and tooth

1 Discoloration in only one area

2 Discoloration in many areas

3 Severe discoloration indicating the presence of leakage

Table 5. Anatomical Form Evaluation Criteria

0 Consistent and continuous and structure in harmony with occlusal form

1 Change in anatomical form but all pits and fissures covered

2a Partial loss of one or two pits or fissures, but no need to repair or replace fissure sealant

2b Partial loss of pits and fissures, replacement or repair of fissure sealant required

3 Loss in all pits and fissures

7 Partial loss due to occlusion

9 Bubble not connected to margins

Statistical Analysis

All statistical analyses will be analyzed using IBM SPSS V23. Chi-square test will be used to compare categorical data by groups.

REFERENCES

20. Frankl S, Shiere F, Fogels H. Should the parent remain with the child in the dental operator. J Dent Child 1962; 29(1): 150-163.
21. Rishika, Garg, N., Mayall, S. S., Pathivada, L., & Yeluri, R. (2018). Combined Effect of Enamel Deproteinization and Intermediate Bonding in the Retention of Pit and Fissure Sealants in Children: A Randomized Clinical Trial. The Journal of clinical pediatric dentistry, 42(6), 427-433.
22. Simonsen RJ. Retention and effectiveness of dental sealant after 15 years. J Am Dent Assoc 1991; 122(10): 34-42.
23. Feigal RJ, Musherure P, Gillespie B, Levy-Polack M, Quelhas I, Hebling J. Improved sealant retention with bonding agents: A clinical study of two-bottle and single-bottle systems. J Dent Res 2000; 79(11): 1850-1856.

PLACE(S) OF IMPLEMENTATION OF THE RESEARCH☒ Single Center☐ Multicenter

	Name of the center	Name of the principal investigator	phone
Center 1	Tokat Gaziosmanpasa University, Faculty of Dentistry		

INFORMATION ABOUT RESEARCHERS

Name-Surname	Title	Mission	phone
	Research assistant	Obtaining and evaluating data, writing the research	
	Assistant professor	Planning the research, Evaluation of the data	

INFORMATION ABOUT VOLUNTEERS

Qualification of volunteers	Patients aged 4-6 years with no systemic problems, a score of 3 or 4 on the Frankl Behavior Scale, no habit of clenching and/or grinding, erupted caries-free primary lower second molars, and indication for fissure sealant application
Working group	60
Number of control groups	60
<i>Inclusion criteria</i>	<p>He has no systemic problems and scores 3 and 4 on the Frankl Behavior Scale,</p> <p>No habit of clenching and/or grinding teeth,</p> <p>He has erupted primary lower second molars without decay,</p> <p>Patients aged 4-6 years with an indication for fissure sealant application.</p>
Exclusion criteria	<p>Teeth with fissure sealant applied,</p> <p>Teeth with developmental defects such as hypoplasia,</p> <p>Teeth with approximal or initial carious lesions on one or both lower primary second molars,</p> <p>Children who are not systemically healthy,</p> <p>Non-cooperative children with scores of 1 and 2 on the Frankl Behavior Scale,</p> <p>Children and parents who refuse to attend follow-up appointments,</p> <p>Children with parafunctional habits such as teeth clenching.</p>

I will comply with the World Medical Association (WMA) HELSINKI Declaration (and/or World Psychiatric Association HAWAI Declaration), Good Clinical Practices and Good Laboratory Practices Rules during this research, and I will immediately notify the Clinical Research Ethics Committee in case of any unexpected adverse effect or event, I promise to notify the committee in writing in case of any changes in the study protocol during the research,

to submit a report on the progress of the research to the Clinical Research Ethics Committee every 6 months according to the duration of the research, and to notify the Clinical Research Ethics Committee immediately if the research is stopped.

RESEARCH RESPONSIBLE COORDINATOR

Asst. Prof. 