CJDR Caspian Journal of Dental Research
Caspian Journal of Dental Research
e-ISSN: 2322-2395 p-ISSN: 2251-9890
Babol University Of Medical Sciences

Effect of chlorhexidine solution on microleakage of pit and fissure sealants in permanent teeth with and without the use of fifth- and sixth-generation adhesive systems

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Article type

Research Paper

ABSTRACT

Introduction: Due to concerns about histological caries and recurrent caries after the use of sealants, adhesive systems containing fluoride and antibacterial agents have been proposed. The aim of this study was to evaluate the microleakage of sealants after the use of antibacterial chlorhexidine solution on etched enamel with and without the use of fifth- and sixth-generation adhesive systems.

Materials & Methods: In this experimental study, sixty sound human premolars were divided into 4 groups as follows: Group A: Etching, fissure sealant; Group B: Etching, chlorhexidine solution (2 %), fissure sealant; Group C: Etching, chlorhexidine solution, single bond, fissure sealant; Group D: Etching, chlorhexidine solution, Clearfil SE Bond, fissure sealant. The samples were thermocycled for 500 cycles and immersed in basic fuchsine 0.5%. Then, the teeth were embedded in acrylic resin and cut buccolingually parallel to the long axis. Microleakage of the specimens was observed under ×40 magnification and graded from 0 to 3. Data were analysed using the Kruskal-Wallis and Mann-Whitney U tests. A value of $p<0.05$ was considered significant.

Results: Sealant micro-leakage was statistically lower in group A (etching, fissure sealant) than in groups B, C and D, the groups with the chlorhexidine solution ($P < 0.05$). There was no statistically significant difference between groups B, C and D.

Conclusion: The use of chlorhexidine solution on the etched enamel increases the sealant microleakage; with and without the application of the adhesive systems, Single Bond or Clearfil SE Bond. Therefore, it cannot be used as one of the steps in the application of the sealant to reduce the colonization of bacteria around the fissure sealant.

Received: 1 Dec 2022
Revised: 21 Mar 2023
Accepted: 8 May 2023
Pub. online: 14 Jun 2023

Keywords: Chlorhexidine, Dental leakage, Pit and Fissure Sealants

Cite this article: Paryab M. Effect of chlorhexidine solution on microleakage of pit and fissure sealants in permanent teeth with and without the use of fifth- and sixth-generation adhesive systems. Caspian J Dent Res 2023; 12: 50-6.

Publisher: Babol University of Medical Sciences

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[DOR: 20.1001.1.22519890.2023.12.2.1.6]
[DOI: 10.22088/cjdr.12.2.50]

Introduction

Some anatomical pits and fissures in the occlusal surfaces of posterior teeth are not self-cleansing and are prone to caries. [1] These areas with less enamel thickness at the base of a fissure or pit absorb little fluoride. [2] Pit and fissure sealants create an impermeable barrier that prevents colonization of the sealed fissure by other oral microorganisms and isolates the remaining viable organisms from the nutrient source. [1, 2]

Caries may be present histologically before it is detected clinically and radiographically. It is possible that sealants over the enamel cause histologic caries and cariogenic organisms in pits and fissures. This has caused great concern among dentists and has led to the restricted use of sealants. [2] The acid etching procedure itself eliminates 75% of viable microorganisms from pits and fissures. But etching gel and sealant materials may penetrate less deeply into the I-shaped pits and fissures that are the main indication for sealant use. [3] On the other hand, sealant materials, like restorative materials, have a higher surface energy than enamel, which increases microorganism colonization, especially during the first week after sealant placement, [1, 4] and clinical follow-up demonstrates sealant loss and wear over time. [5]

Fluorides and antibacterial agents have been added to the composition of adhesive systems or sealants to inhibit caries formation, especially at enamel margins. [6-8] Chlorhexidine solution and varnish were used in the prepared cavity [9-15] or during bracket placement. [16-21] There is evidence that chlorhexidine varnish is effective in suppressing oral mutans for at least 3 months and up to 7 months after application when used prior to the placement of fixed orthodontic appliances. [22] Little or no information is available on the use of chlorhexidine after enamel etching and before the placement of sealants. Therefore, the aim of the current study was to investigate whether the application of chlorhexidine on the etched enamel as an antibacterial agent can be one of the procedural steps in fissure sealing.

Materials & Methods

This experimental study was a research project (No: 2212) supported and funded by Zahedan University of Medical Sciences. According to the study of Darabi et al., [13] and considering the statistical formula

$$n = \frac{(Z_{1-\alpha/2}+Z_{1-\beta})^2\,(p_1 q_1 + p_2 q_2)}{(P_1-P_2)^2}$$

with coefficients of α=0.05 and β=0.05, the sample size of each group was set to 10 teeth. For more confidence, 15 teeth were considered. Sixty sound human premolars extracted for orthodontic purposes within a 6-month period were selected and disinfected with thymol 0.2%. The teeth were cleaned with water/pumice slurry and randomly divided into 4 equal groups. The occlusal pits and fissures of the teeth were etched with 37% phosphoric acid (Ultra Etch, Ultra dent product INC, South Jordan, Utah, USA) for 15 seconds. They were then rinsed thoroughly for 5 seconds and dried with oil-free compressed air to obtain a uniform whitish, dull, chalk-like appearance. Bonding procedures were performed in each group as follows:

[DOR: 20.1001.1.22519890.2023.12.2.1.6]

[DOI: 10.22088/cjdr.12.2.50]

Group A: Following the above procedures, fissure sealant (Concise, 3M ESPE, St. Paul, USA) was applied to the etched pits and fissures including 2 to 3 mm of cusp inclination and cured for 20 seconds with a visible light-curing unit (Coltolux 75, Colten, California, USA) at 450 mw/mm² output power.

Group B: A thin layer of chlorhexidine mouth rinse (2%) (Consepsis; Ultradent Product Inc, South Jordan, UT, USA) was brushed onto the enamel so that the entire surface of the enamel was covered. This was left undisturbed for 10 seconds and then the pits and fissures were dried and sealed.

Group C: Disinfection with chlorhexidine solution was performed as in group B. Two consecutive coats of Single Bond (3M EPSE, St. Paul, USA) were applied to the etched enamel for 15 seconds with gentle agitation using a fully saturated applicator. The surface was then gently dried for 15 seconds to evaporate the solvents. The adhesive was light-cured for 10 seconds, and finally the pits and fissures were sealed.

Group D: Disinfection with chlorhexidine solution was performed as in group B. Prior to sealing, Clearfil SE Bond System (Kuraray Medical Inc, Tokyo, Japan) was applied, according to the manufacturer's instructions, then the teeth were dried for 15 seconds and finally light-cured for 10 seconds.

According to the standard ISO/TR 11405 [23], samples were stored in distilled water at 37° C for at least 24 hours before testing and thermocycled for 500 cycles between 5 and 55 with a retention time of 50 hours. After thermo-cycling, the teeth were sealed with glass ionomer cement at the root tips and covered with two layers of nail polish, except for the restorations and a 1 mm rim of tooth structure around the restoration. The specimens were immersed in basic fuchsine (0.5 wt %) for 24 hours and then rinsed thoroughly with tap water. Next, the teeth were embedded in self-curing acrylic resin to prevent chipping of the material. Using a diamond disk (Leitz1600, Leica Instruments GmbH, Wetzlar, Germany), the resin blocks were buccolingually cut parallel to the long axis into mesial and distal fragments under water. After the above procedures, the length of dye penetration at the interface between the sealant and tooth was examined under (SMZ800N, Nikon, Tokyo, Japan) at ×40 magnification. The criterion for the amount of dye microleakage was the level of maximum dye penetration. Grading was based on the following criteria:

Grade 0: no penetration; **Grade 1:** dye penetration of more than zero to one-third of the sealant-tooth interface; **Grade 2:** dye penetration of one-third to two-thirds of the sealant- tooth interface length; **Grade 3:** dye penetration of more than two-thirds of the sealant- tooth interface length. Data were analyzed using the Kruskal-Wallis and Mann-Whitney U tests. All statistical tests were performed at a significance level of $p < 0.05$.

Results

A total of 120 sections were examined for microleakage. The percentage of each result for all groups is shown in table 1. The results of the statistical analysis indicated a significant difference in the total microleakage between ($p<0.05$). The results of the pairwise statistical tests represented that the microleakage was lower in group A compared to the other groups ($p< 0.05$). There were no differences between the dentin adhesives studied ($p>0.05$) (table 2).

Table 1. Distribution of microleakage grades in experimental groups

	Grade 0	Grade 1	Grade 2	Grade 3
Group A	27(90.00%)	2(6.66%)	1(3.33%)	0(0.00%)
Group B	16(53.33%)	9(30.00%)	2(6.66%)	3(10.00%)
Group C	12(40.00%)	14(46.66%)	2(6.66%)	2(6.66%)
Group D	16(53.33%)	10(33.33%)	4(13.33%)	0(0.00%)

Group A: etching, sealant
Group B: etching, chlorhexidine solution, sealant
Group C: etching, chlorhexidine solution, Single Bond, sealant
Group D: etching, chlorhexidine solution, Clearfil SE Bond

Table 2. Comparing the experimental groups using the Mann-Whitney U

Groups	P- value (OR: 1.96)	Groups	P- value (OR: 1.96)
Groups A &B	0.002	**Groups B & C**	0.58
Groups A & C	0.00	**Groups B & D**	0.74
Groups A &D	0.002	**Groups C & D**	0.36

Group A: etching, sealant
Group B: etching, chlorhexidine solution, sealant
Group C: etching, chlorhexidine solution, Single Bond, sealant
Group D: etching, chlorhexidine solution, Clearfil SE Bond

Discussion

Recurrent caries is the main problem with the use of fissure sealants. [2] Antibacterial adhesive systems under the sealant may be useful in preventing caries formation after microleakage or partial loss of the sealant. The release of these antibacterial agents depends on the PH and the chemical structure of the materials. A low PH of the self-etching systems may reduce the activity of the antibacterial agents. [7] Therefore, it is recommended to disinfect the cavity and tooth surfaces prior to the bonding agent application with antibacterial agents such as chlorhexidine solution before applying the bonding agent. Depending on the type of antibacterial agents and adhesive systems, the function of the antibacterial agents and the bond strength of the adhesive systems may change. [13, 14] The aim of this study was to evaluate the microleakage of the sealants after the use of antibacterial chlorhexidine solution on the etched enamel with and without the use of fifth- and sixth-generation adhesive systems.

Chlorhexidine is a broad-spectrum antibacterial agent that is effective against plaque and gingivitis as well as caries. It has also been offered as a matrix metalloproteinase inhibitor in recent years. [24] Furthermore, it may have a stabilizing effect on the smear layer, transforming it from a semi-permeable, loosely bonded layer to a more impermeable, tightly bonded layer. [13] This effect may be beneficial in self-etch dentin bonding systems. These systems have a mild acidic monomeric primer that is applied to the smear layer without rinsing. It is therefore necessary to disinfect the smear layer before the acid primer. [13]

One of the most important properties of chlorhexidine that is desirable in this study is its cationic charge. It bonds to the surface of amino acids and hydroxyapatite and is released over time, exerting a long-lasting antibacterial effect. [25] Because of this strong positive charge, it binds readily to phosphate groups and increases the surface free energy of enamel and can increase bond strength, especially when hydrophilic bonding agents are used. [13]

Doman et al. (1997) showed that the shear bond strength of brackets was not significantly affected after treatment of etched enamel with Transbond XT, which contained chlorhexidine varnish in its primer. [17] This means that the chlorhexidine does not affect the wettability of adhesive systems such as Transbond XT used in bonding the brackets. [16-21] The use of chlorhexidine varnish in the primer of adhesive systems or chlorhexidine solution on the etched enamel may be acceptable application methods. [17-22] However, the application of the chlorhexidine varnish as a separate layer or the chlorhexidine gel has resulted in a reduction of shear bond strength. [18, 22] In the studies by Haidari et al. [26] and Saffarpour et al., [27] chlorhexidine rinse 2% was used and it did not have any negative effects on enamel microleakage of composite restorations in different bonding systems.

In this study, the chlorhexidine solution was applied to the etched enamel during fissure sealing, and the results indicated that the use of chlorhexidine solution increased the microleakage of the sealant. Two different generations of adhesive systems (total-etch adhesive system and self-etch adhesive system with acid PH) were used after applying the chlorhexidine layer. None of the adhesive systems used in this study reduced the microleakage to the level of the control group. In prepared cavities that extend to the dentin, chlorhexidine does not affect the function of Single Bond and Clearfil SE Bond. [13]

It seems that different methods of chlorhexidine application may affect its function. The chlorhexidine substance on the enamel surface decreases the ability of the resin to impregnate the micropores of the enamel. SEM seems to require investigation to clarify this hypothesis. In addition, there may be some negative interactions between chlorhexidine and adhesive systems. The use of sealants with chlorhexidine varnish incorporated into the sealant resins or bonding agents could result in less microleakage. It is proposed to investigate the use of chlorhexidine varnish in combination with adhesive systems to seal the fissures in a further study.

Conclusion

The use of chlorhexidine solution on the etched enamel without rinsing leads to an increase in the rate of microleakage of the fissure sealant. The use of Single Bond and Clearfil SE Bond bonding systems cannot neutralize the negative effects of chlorhexidine mouth rinse on the rate of microleakage of the fissure sealant. Therefore, it cannot be used as one of the steps in the application of the sealant to reduce the colonization of bacteria around the fissure sealant.

Funding

This study was a research project (No: 2212) supported and funded by Zahedan University of Medical Sciences.

Conflicts of Interest

There is no conflict of interest to declare.

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[DOR: 20.1001.1.22519890.2023.12.2.1.6]

[DOI: 10.22088/cjdr.12.2.50]