Original Article

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Effect of 2% Chlorhexidine on the Enamel Microleakage of Composite Restorations Using 5th, 6th, 7th and Universal Generation of Dentine Bonding Agents (In Vitro)

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ABSTRACT

BACKGROUND AND OBJECTIVE: The matrix metalloproteinase plays a significant role in the bonding stability of the composite-tooth. Chlorhexidine has been offered as matrix metalloproteinase inhibitor in current years. The aim of this study was to investigation of effect of 2% chlorhexidine on enamel microleakage of composite fillings in different bonding systems.

METHODS: In this in vitro study, class V cavities were prepared on the lingual and buccal surface of forty extracted molar teeth. The cavities were etched and divided randomly to 8 groups: 1: Adper single Bond 2 (ASB), 2: Clearfil SE Bond (CSE), 3: Clearfil S3 Bond (CS3), 4: Single Bond Universal (SBU), 5: ASB + 2% Chlorhexidine (CHX), 6: CSE +2%CHX, 7: CS3 +2% CHX, 8: SBU + 2%CHX. After bonding application, the cavities were restored with Z250 composite and thermocycling procedure (5 to 55 C, 5000 cycles) was done. Enamel microleakage was measured by dye penetration test. The teeth were sectioned buccolingually and microleakage was recorded based on a 0–3 scoring system under a stereomicroscope (Motic Micro Optic, industrial group Co, LTD, Japan) at 40x magnification. Data analysis was done.

FINDINGS: In ASB, CSE, CS3 and SBU bonding systems, Percent of samples without microleakage before use CHX was 80%, 90%, 90%and 80% and after it was 100%, 80%, 80% and 90 % respectively.

CONCLUSION: Use of 2% chlorhexidine has not adverse effect on enamel microleakage of composite restorations in different bonding systems.

KEY WORDS: *Chlorhexidine, Bonding agents, Leakage.*

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Introduction

The use of tooth-colored restorations has become popular in recent years. The dental-restoration interface, which includes the dentin organic matrix, is the hydroxyapatite residue and adhesive resin, is the weakest part of any composite repair. The loss of bonding between the composite and the tooth leads to micro leakage, marginal color change, post-surgery sensitivity and secondary caries (1, 2).

Researchers have used various methods to measure the micro leakage of composites, including the "penetration" method. This technique is performed with various colored materials, including methylene blue, foscin and silver nitrate. This test is very sensitive. The reason for using this test is the size of the silver nitrate nanoparticles compared to other micro leakage dyes because the size of the silver ion particles (0.059 nm) is much smaller compared to the bacterial size (1.05 nm).

Therefore, it is likely that any repair that prevents silver ion leakage is impenetrable to bacteria (3) creating a durable bond between composite and tooth for the clinical success of repair, is necessary. In fact, the bonding of composite to dentin gradually decreases, due to water absorption by the adhesive system and monomer exit (4, 5).

On the other hand, endogenous collagen lithic enzymes called matrix metalloproteinase can degrade gradually the collagen structure in the hybrid layer . The matrix metalloproteinase enzyme is a family of enzymes with 26 members known to exist in the pulp, odontoblast, and dentin/pre-dentin. The precise performance of these enzymes is unclear, but it is likely to play a role in the formation of dentin matrix, progression of caries, create secondary dentin and loss of dentin bonding. In this case, the metallic enzymes of metalloproteinase 2 and 9 play an important role. The activation of these enzymes by etch & rinse and self-etch adhesives occurs during the bonding process by reducing the peripheral pH (6-9).

Efforts have been made to increase the durability of the composite bond to the teeth and it has been shown that chlorhexidine can inhibit the activity of the host protease enzymes and reduce the activity of the matrix metalloproteinase enzyme. In addition, chlorhexidine is a substance with a broad spectrum of antibacterial and low toxicity, and has a significant reduction effect on the Streptococcus mutans, which is used in many studies as a disinfectant after the preparation of the cavity and before putting the restoration into operation. (10,11). On the other hand, in self-etch adhesives that do not require a separate etch step, the smearer and the bacteria remain in the cavity and can penetrate the pulp and cause the pulp to irritate and inflammate. Many studies have been done on the effect of chlorhexidine on the performance of dentinal bonding systems. Researchers have found positive effects of chlorhexidine on the durability of dentin bonding (1,12-14).

The bond to the enamel due to its structure is a stable bond, due to the fact that during application of chlorhexidine to dentin, enamel is also impregnated, but few studies have been done on the effect of using chlorhexidine on the bond to the enamel. Therefore, the purpose of this study was to investigate the effect of 2% chlorhexidine on enamel micro leakage of composite restorations in 5, 6, 7 generation bond systems and universal bond systems.

Methods

In this experimental study, after approval by the ethics committee of Babol University of Medical Sciences with the code of ethics 241.1395mubabol.rec. 40 healthy human molars with no decay or anatomical defects were selected for testing, and teeth with caries or cracks and other defects were excluded from the study .The connected tissues were removed slowly after being pulled off with Kort and then teeth were immersed in 0.05% Thymol solution (21).

On the lingual and buccal surfaces of each tooth, a class V cavity with dimensions of 2×2×3 was shaved using a turbine of high speed and with air-water spray, by a fissure mill 008 (Teeskavan, Tehran, Iran) that gingival margin of the cavity is 1 mm lower than the CEJ and its occlusal edge is in the enamel. The list and chemical composition of the consumables in this study are presented in Table 1. The teeth were randomly divided into 8 groups.

Group 1: Cavities were etched for 30 seconds in enamel and 15 seconds in dentin with phosphoric acid 38% (Pulpdent, USA). Then the cavities were washed and dried with gentle air pressure. Continuous 2bonding, Single Bond (3M ESPE, USA) was placed in two consecutive layers by micro brush according to the manufacturer's instruction, and cured for 10 seconds by light core valo (Ultradent, USA) with an intensity of 21,000 mW / cm and the cavities were restored with A2 composite film Z250 Filtek (M, USA3), and each layer was cured for 20 seconds.

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Table1. List and chemical composition of consumables in this study

Materials	Chemical mixture	Manufacturer
Adper Single Bond 2	Ethyl alcohol, Bis-GMA, silane-treated silica, 2-hydroxyethyl methacrylate (HEMA), glycerol 1,3-dimethacrylate, diurethane dimethacrylate, copolymer of acrylic and itaconic acids	3M ESPE, MN, USA
Clearfil SE Bond	Primer: MDP, HEMA, dimethacrylate monomer, water, catalyst Bond: MDP, HEMA, dimethacrylate monomer, microfiller, catalyst	Kuraray, Japan
Clearfil S3 Bond	MDP, Bis-GMA, HEMA, camphoroquinone, ethanol, water, colloidal silica	Kuraray, Japan
Single bond universal	MDP Phosphate Monomer, Dimethacrylate resins, HEMA, Vitrebond™ Copolymer, Filler, Ethanol, water, initiators, silane	3M ESPE, USA
Filtek Z250	Bis-GMA, UDMA, Bis-EMA resin, zirconium, silica	3M ESPE, USA
Clorhexidina s	Chlorhexidine digluconate at 2%,deionized water, volatile surfactant	FGM, brazil
Etch-Rite 38% phosphoric acid	Phosphoric acid, amorphous fumed silica	Pulpdent, USA

Group 2: The enamel wall of the cavity was etched for 15 seconds. After washing and drying the cavities, the CLEARFIL SE BOND (Kuraray, Japan) bonding system was used according to factory orders. First, the primer was applied to the surface of the cavities for 20 seconds and dried with gentle air pressure. Then SE Bond was used and was coated with Light Core Valo for 10 seconds. Composite repair is done as in Group1.

Group 3: The enamel wall of the cavity was etched for 15 seconds. After washing and drying the cavities, the CLEARFIL S3 BOND (Kuraray, Japan) bonding system was used according to factory orders. Bonding was applied to the cavity for 10 seconds and cured for 10 seconds, and then the composite repair was performed similar to the previous ones.

Group 4: The enamel wall of the cavities was etched for 15 seconds. After washing and drying the cavities, Single Bond Universal Bonding System (3M ESPE, USA) was used according to factory orders. Bonding was applied to the cavity for 20 seconds and cured for 10 seconds, and then the composite repair was performed similar to the previous ones.

Group 5: Cavities were eched for 30 seconds in enamel and 15 seconds in dentin. Then it was washed and dried with mild air pressure and a 2% chlorhexidine solution was used for 60 seconds using micro brush on the walls of the cavities followed by another clean micro brush, an excess of chlorhexidine was removed from the tooth surface, and the tooth was dried by air so that it did not lose its superficial luster (22). In the following, Single Bond 2 bonding and composite restorations were performed as in Group 1.

Group 6: The enamel wall of the cavity was etched for 15 seconds. After washing and drying, the cavity was prepared with a 2% chlorhexidine solution for 60 seconds and then placing the primer and the CLEARFIL SE BOND and composite restorations as in group 2.

Group 7: The enamel wall of the cavity was etched for 15 seconds. After washing and drying, the cavity was prepared with 2% chlorhexidine for 60 seconds, and then the CLEARFIL S3 BOND bonding and composite restorations were continued as in Group 3.

Group 8: The enamel wall of the cavity was etched for 15 seconds. After washing and drying the cavities, 2% chlorhexidine was used for 60 seconds, after which Single Bond Universal bonding and composite repair were performed as in Group 4.

Micro leakage test: The restoration was polished for 30 seconds under the water using sanding silicon carbide papers 600, 800, 1000, 1200 and 2000 grate. After completing the polishing process, the samples were kept at 37 °C for 24 hours in a humid environment, and then aging was carried out by Thermocycling Machine, mashhad Nemo under 5000 thermal cycles (5-55).

After thermocycling, all teeth were dried and then 2 layers of nail polish were applied to all teeth surfaces except 1 mm around the restoration, and in the apex, in addition to nail polish, wax adhesive was used for dental seal. Subsequently, the specimens were immersed in a dark room in a silver nitrate solution (50 wt.%) For 2 hours and then washed and incubated for 4 hours in the radiotherapy solution under fluorescence light, washed again and were saved in a normal saline solution (25).

24 hours later, they were cut with a cutting machine and the teeth were cut by a Baku-Lingual

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cutter using chisel blade. They were evaluated and were micro leakage recorded (SMZ800, Nikon, Japan) in the composite enamel with the following grading system (15): Code zero: no penetration of color, code 1: less than occlusal wall, code 2: more than occlusal wall (without reaching the wall of the axial), code 3: penetration into the wall of the axial. For statistical analysis of the samples, Kruskal Wallis and Mann-Whitney tests were used and p<0.05 was considered significant.

Results

In Adper Single Bond 2, Clearfil SE Bond, Clearfil S3 Bond and Single bond Universal bonding systems, the percentage of non-micro leakage samples before the use of chlorhexidine was 80%, 90%, 90% and 80%, respectively. Table 2 shows the frequency of enamel micro leakage codes of the above systems when used without chlorhexidine. Statistical comparison of different groups by Kruskal-Wallis test showed no significant difference in the rate of micro leakage of different bondings.

After applying chlorhexidine, the percentage of non-micro leakage samples in Adper Single Bond 2, Clearfil SE Bond, Clearfil S3 Bond and Single bond Universal bonding systems was 100%, 80%, 80% and 90%, respectively. Table 3 shows the enamel micro leakage codes of the above bonding systems when used with chlorhexidine.

The results of Kruskal-Wallis test for these groups indicate no significant difference between enamel micro leakage of bonding agents in the case of chlorhexidine. The effect of chlorhexidine on any bonding showed that chlorhexidine did not produc a significant statistical change in the enamel micro leakage of different bonding systems (Table 4)

Table2. Frequency and percentage table of micro leakage codes in different bonding systems

Group	Bonding type	0 N(%)	1 N(%)	2 N(%)	3 N(%)
1	Adper Single Bond	8(80)	1(10)	1(10)	0
2	Clearfil SE Bond	9(90)	1(10)	0	0
3	Clearfil S3	9(90)	1(10)	0	0
4	Single bond universal	8(80)	1(10)	1(10)	0

Table 3. Frequency and percentage table of micro leakage codes in different bonding systems with chlorhexidine

Group	Bonding type	0 N(%)	1 N(%)	2 N(%)	3S N(%)
5	Adper Single Bond + CHX	10(100)	0	0	0
6	Clearfil SE Bond + CHX	8(80)	2(20)	0	0
7	Clearfil S3+CHX	8(80)	0	2(20)	0
8	Single bond universal + CHX	9(90)	0	1(10)	0

Table4. Comparison of different bonding microleakages with and without chlorhexidine

Bonding systems	P-value
Adper Single Bond ***VS.*** Adper Single Bond+CHX	0.481
Clearfil SE Bond ***VS.*** Clearfil SE Bond+CHX	0.739
Clearfil S3 ***VS.*** Clearfil S3 + CHX	0.684
Single bond universal ***VS.*** Single bond universal+CHX	0.971

Discussion

In this study, the effect of chlorhexidine on the enamel micro leakage of composite restorations was similar in 5, 6, 7 generations and universal bondings. Meanwhile, the use of chlorhexidine did not have a significant effect on the micro leakage of 5, 6, 7 generations, and universal bonding after 5,000 cycles. The results of this study are consistent with the results of Kapdan et al, and Siso et al, and Saffarpour et al. (16-18). In the study of Siso et al., Clearfil SE bond (self-etching bonding) and Kapdan et al. and Saffarpour et al. Prime & Bond NT and single bond 2 (total bonding), were used respectively.

In the studies, chlorhexidine had no effect on enamel micro leakage of composite restorations. Perhaps the most important cause of micro leakage is contraction caused by polymerization. This force generates a stress of about MPa13 between the composite and the tooth structure. These stresses severely affect the interface and lead to a gap. Chlorhexidine is an antiseptic agent that has bactericidal and bacteriostatic properties and absorbs the enamel and dentin (19). Some suggest the use of chlorhexidine after cutting the cavity and before bonding, and some recommend it after etching. The

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use of chlorhexidine after etching seems to be a more logical approach, since the major part of the smear layer of bacteria is eliminated from the cavity and the chlorhexidine solution neutralizes the microorganisms and their toxins (20).

Chlorhexidine has a positive ionic charge that easily absorbs phosphate ions and increases the free energy of the enamel and dentin (21). The results of Alikhani et al. study differ with the results of this study and show an increase in enamel micro leakage after application of chlorhexidine. In the study, Clearfil SE bond was used (22). The reason for the difference in results can be the same in the etch. Due to the absence of collagen lysis in the enamel, chlorhexidine plays no role in the enamel band. Long-term storage of samples in water for 6 months can lead to chlorhexidine dissolution and increased micro leakage (22).

Meiers et al. and Kresin et al. stated that the chlorhexidine residues used as disinfectants can prevent the effect of phosphoric acid on the tooth and prevent resin penetration (20, 23), but in this study chlorhexidine in all groups were used after the etching stage.Perhaps because of this, the use of chlorhexidine did not affect the micro leakage of enamel interfacing. Today, self-adhesive etching bonds are very popular due to the simplicity of the process, but the performance of the enamel bond in these systems is doubtful. That's why enamel etching is recommended. Clearfil SE bond is a mild self-etching adhesive with pH of about 2. This bonding agent contains MDP monomer with two hydroxyl groups, which can be bonded to calcium (17). This monomer is also available in CLEARFIL S3 BOND and Single Bond Universal, and in addition to etching enamel, it can be due to the same micro leakage in different groups. Perdigao et al. also argued that the use of chlorhexidine after etching does not affect the bond strength (24), and is consistent with the results of this study. Since the purpose of this study was to evaluate the effect of chlorhexidine on the bands' durability, the samples were subjected to thermocycling. The most commonly used synthetic aging method is long-term storage in water (25).

Another method is thermocycling, which is a suitable method based on the ISO instruction of 500 cycles in distilled water between 5 and 55 degrees Celsius. In several studies, the application of more thermal cycles has been proven to examine the long-term evaluation of the bond (26). In this study, samples were subjected to 5000 cycles. The results of this study showed that chlorhexidine 2% had no negative effect on enamel micro leakage of total etching and self-etch bonding. Considering the importance of micro leakage of composite restorations, further studies are needed in this regard. It is suggested that the effect of the use of chlorhexidine on enamel micro leakage of composite restorations in self-etching bondings without etching of enamel and under occlusal forces should be investigated.

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