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Research Article

Effect of 2% Chlorhexidine on the Gingival Micro leakage of Composite Restorations Using 5th, 6th, 7th and Universal Generation of Dentine Bonding Agents

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INTRODUCTION

The development of the primary bonding systems began in 1950s with the introduction of cavity seal materials by Hagger and introduction of “surface-active comonomer” by Bowen, and Bunnocore suggested use of 85% phosphoric acid on enamel to change it for penetration of acrylic resin (1, 2). After that, several bonding systems were introduced to make chemical bond between restorative materials and tooth, which they have been classified into various generations based on their constituents. The first generation of them was based on the Bowen introduced composition which, they had very poor clinical performance (2). The second generation of bonding systems was introduced in the early 1980’s that had dentin bond strength about 1-10 MPa, which it was less than to resist against resin composite shrinkage stress. It seemed that this poor performance was due to that they supposedly interaction with the smear layer rather than dentin (1, 2). Third generation of bonding systems could eliminate smear layer in modified or complete way (1) and while they had a better performance than the second generation, they were still unpredictable even at in vitro (2). Fourth generation of bonding systems were introduced in the 1990’s which, they were used generally in the form of total etch along with phosphoric acid, they are available today and could establish acceptable and predictable bond with enamel surface and dentin structure (1-3). Fourth generation bonding systems are generally known as multipurpose adhesive systems because of that they can be used in mixed cavities to bond with both enamel and dentin structure and some of them have this capability to bond with different materials such as amalgam alloy and metals along with composites (1). In order to simplify bonding procedure, fifth generation adhesive systems were produced, which separate acid etching still required. After that, by using acidic primers, self-etch adhesives were presented to dentistry world(4). Therefore, there was no need to separate etching step. Self-etch adhesive system are user friendly and less technique-sensitive than total-etch adhesive (4). Among self-etch adhesive systems, 6-generation bonding systems had 2 bottles, one bottle contain acidic primer that performs simultaneously etching and priming dentin and enamel, and another bottle containing bond(4). In the trend toward more simplification, 7-generation bonding system came to markets which, has only one bottle that incorporate etching , priming and bonding in to the one step (4). At the ongoing trend between manufacturers to simplifying bonding technology , new bonding system was produced which named as universal bonding system (5, 6). Universal bonding systems are able to use by both etch-and-rinse and self-etch technique with the same single bottle of adhesive system(6). Some of the universal bonding systems contain components that enable them to bond with zirconia and silica-based glass ceramics without the use of additional priming agents (6). For all of the adhesive systems, the quality of bond to the tooth structures always has been an important factor. Many efforts have been made to enhance the durability of tooth-colored materials to dental structure so far and low durability of resin –dentin bond compared to resin- enamel bond is one of the possible reasons for shorter lifetime of tooth-colored restorations (3). Loss of the bond between the tooth and restorative materials causes gap, which it might lead to marginal microleakage, post-operative sensitivity, marginal discoloration, pain, and recurrent carries. Therefore, a bonding procedure that could provide proper strength and longer clinical durability is essential for the success of the direct restorations with resin composites (7). Progressive loss of resin-dentin bond integrity was attributed partly to the hydrophilic nature of the adhesives systems that causes unwanted water absorption, phase separation, resin leaching and the endogenous collagenolytic enzymes that can slowly hydrolyze collagen structure of hybrid layer (8). Endogenous matrix metalloproteinase (MMPs) is a family of enzymes with 26 known members, involved on the loss of hybrid layer over time (9). Some members of the MMP's family have been seen so far in pulp-dentin complex, which include MMP-9 (gelatinase), MMP-2(gelatinase), MMP-8 (collagenase), MMP-13 (collagenase) and MMP-3 (Stroelysin), MMP-14 (MT1-MMP) and MMP-20 (Enamelysin) and they are known as main MMPs in the pulp, odontoblasts, and pre-dentin / dentin (10, 11). The accurate performance of these enzymes in the dentin-pulp complex is not clear yet, but probably they play important role in the formation of the dentin matrix, the progression of caries lesions, secondary dentin formation, and stability and loss of the bond (10, 12). Extracellular matrix (ECM) of dentin includes mainly (90%) collagen type 1, and collagen type 3 and 5 to a lesser extent (1-3%) (2). The ECM can be damaged by a variety of mechanisms, including of 1: releasing of enzymes by the host (for example MMPs) and bacteria 2: phagocytosis of matrix components 3: releasing of active hydrogen particles 4: releasing of cytokines, inflammatory mediators, and apoptotic proteins (10, 12).

In caries lesions, collagen is initially decomposed by MMP-8 into the smaller pieces and then this decomposition continues by MMP-2 and MMP-9 (13). On the other hand, etch-and-rinse and self-etch adhesives can activate MMPs during bonding procedure by reducing environment PH (3, 13, 14). It seems that MMPs performance can be affected by chlorhexidine (15, 16).Chlorhexidine was introduced in 1940s, and it was used commonly as disinfectant in the 1950's (17), and in 1969, Schroeder showed that this material could be used as plaque controller in dentistry (18). It has been specified that chlorhexidine additionally has this ability to inhibit the activity of protease enzymes derived from the host (19), This could justify the use of chlorhexidine in the process of bonding to reduce MMPs activity. So far, different studies achieved contradictory results about the performance of chlorhexidine at the bonding systems. Some studies, such as the study of Gunaydin et al (1997) and Carriliho et al (2007) have referred to the positive effect of chlorhexidine on the bond durability (3, 20), and some studies such as the studies conducted by Kapdan et al. (2016) stated that the use of chlorhexidine not only has no positive effect it also reducing bond durability (21). Therefore, debates on the effect of chlorhexidine not yet have been led to a definitive result. In this following, at this study, we tried to examine the effect of the chlorhexidine on the dentinal microleakage of tooth-colored class V restorations.

MATERIALS AND METHODS

Tooth preparation

40 extracted non-carious human molar teeth without obvious decay or anatomical defects were selected for this study. After extraction, the teeth were cleaned of soft tissue and debris. Then, the teeth were immersed in 0/05% thymol solution for 24 hours at the room temperature (22). On the buccal and lingual surface of each tooth, class V cavities were prepared in such a way that gingival wall placed 1mm below the CEJ with 2 mm occlusogingival height, 3mm mesiodistal width at the top, 2 mm mesiodistal width at the bottom and 1.5 mm depth. Cavity preparation was performed by 008 fissure bur (Tizkavan, Tehran, Iran) and a high-speed water-cooled handpiece; burs were changed after every 5 cavity preparation.

Bonding application procedure

Teeth were randomly divided into eight groups of 5 teeth for each group and assigned to one of the following groups (Table 1 shows composition of used materials):

Group I: In this group, cavities were etched with 37% phosphoric acid (3M ESPE, MN, USA) for 30s in enamel and 15s in dentin. After that cavities were washed and gently air dried. Then Adper Single Bond 2 was applied on the prepared cavities by micro-brush in two layers and light cured for 10s with Valo LED curing device (Ultradent, USA) with the intensity of 1000 mW / cm2. After that composite filling was done incrementally with A2 shade of Filtek Z250 (3M ESPE, USA), which each layer was cured for 20s.

Group II: In first step, enamel wall of cavities were etched for 15 s. Then, cavities washed and gently dried for 5s. SE Primer (Kuraray, Japan) was applied with a microbrush and air dried after 20s. SE Bond was placed and uniformly distributed with gentle air pressure and cured for 10s. Filling was done the same as former group.

Group III: The enamel walls of cavities were etched for 15s, then washed and air dried. At the next step, Clearfil S3 Bond (Kuraray, Japan) was applied and dried with air flow after 20 s and light cured for 10 seconds. The cavities were restored with a composite resin such as former groups.

Group IV: At first according to manufacturer's instructions, enamel walls of cavities were etched for 15 s. Then, single bond universal was placed for 20s and dried with air flow and cured for 10s. Then the cavities were restored with Filtek Z250 as former groups.

Group V: As Group 1, Single Bond 2 adhesive system was used, with the difference that after the cavities were etched, 2% chlorhexidine (Clorhexidina s / FGM, SC, brazil) was placed with a micro-brush on the cavities and after 60 seconds, using a new micro-brush, additional amounts of chlorhexidine were removed from the cavities. at next, cavities were dried by air spray until that they do not lose their surface shining (23). At the continuation cavities were prepared and restored such as was performed for Group I.

Group VI: In this group as Group II, Clearfil SE Bond was used, with the difference that after enamel etching step, 2% chlorhexidine was used such as group V. Next steps were done the same as group II.

Group VII: Like Group III, Clearfil S3 Bond was used, with the difference that 2% chlorhexidine were used after etch step and before adhesive application. Next steps were done such as group III.

Group VIII: At this group, all steps were done like group IV but with this difference that after etching step, 2% chlorhexidine was used such as group IV. Other steps were done with no changes such as group IV.

Microleakage test

In the following, restorations were polished under running water by 600, 800, 1000, 1200 grits silicon carbide papers. After that samples were kept for 24 hours in a moist environment at 37° C. Then, they underwent aging process by thermocycling machine (each 30 seconds / 5^0- 55^0 / 30s dwelling / 5000 cycles / NEMO Thermocycling Machine, Mashhad, Iran) (24, 25). After thermocycling, teeth were dried and then all surfaces of teeth were sealed with 2 layers of nail polish except for 1 mm wide zone around the restorations. Moreover, at the apex of teeth, sticky wax was used. Then, samples were immersed for 2 hours in a dark room in silver nitrate solution (50% by weight). At next , the samples were washed and placed for 4 hours in radiology developing solution under the fluorescence light, then they were washed again and stored in normal saline solution (26). After 24 hours, teeth were sectioned Buccolingually by the cutting machine saw (NEMO multi saw, Mashhad, Iran) in approximate center of restoration then, samples were assessed for microleakage by stereomicroscope (SMZ800, Nikon, Tokyo, Japan). Depth of the microleakage was assessed by the using following scale system(27):

Code 0: No dye penetration

Code 1: dye penetrated less than $\frac{1}{2}$ of gingival wall (from margin to gingival half-wall of restoration)

Code 2: dye penetrated more than $\frac{1}{2}$ of gingival wall (after gingival half-wall without reaching to axial wall)

Code 3: Penetration of dye to axial wall

STATISTICAL ANALYSIS

The Sample Kolmogorov-Smirnov Test, Kruskal-Wallis and Mann-Whitney test were used for statistical analyses. The level of statistical significance was set at $p<0.05$.

Table 1: Composition and application technique of the Materials

Materials	Manufacture	Composition	Mode of Application
Adper Single Bond 2	3M ESPE, MN, USA	Ethyl alcohol, Bis-GMA, silane-treated silica, 2-hydroxyethyl methacrylate (HEMA), glycerol 1,3-dimethacrylate, diurethane dimethacrylate, copolymer of acrylic and itaconic acids	Adhesive: Using a fully saturated brush tip for each coat, apply two consecutive coats of Adper Single Bond adhesive to etched enamel and dentin. Dry gently for 2-5 s. Light cure for 10 s
Clearfil SE Bond	Kuraray Medical Inc, Okayama, Japan	Primer: MDP, HEMA, dimethacrylate monomer, water, catalyst Bond: MDP, HEMA, dimethacrylate monomer, microfiller, catalyst	Prime for 20 s (no mixing required) Apply Bond and light-cure for 10 s.
Clearfil S3	Kuraray Medical Inc, Okayama, Japan	MDP, Bis-GMA, HEMA, camphoroquinone, ethanol, water, colloidal silica	Apply bond and wait 10 s. Dry with high-pressure air for 5 s. Light-cure for 10 s.
Single bond universal	3M ESPE, MN, USA	MDP Phosphate Monomer, Dimethacrylate resins, HEMA, Vitrebond™ Copolymer, Filler, Ethanol, water, initiators, silane	Apply bond and wait 20 s. Dry with high-pressure air for 5 s. Light-cure for 10 s.
Filtek Z250	3M ESPE, MN, USA	Bis-GMA, UDMA, Bis-EMA resin, zirconium, silica	Insert incrementally in 2-mm increments. Light-cure for 40 s
Clorhexidina s	FGM,sc,brasil	Chlorhexidine digluconate at 2%deionized water, volatile surfactant	Apply for 60s after etch then additional solution removed by a clean microbrush
Etch-Rite 38% phosphoric acid	Pulpdent, USA	Phosphoric acid, amorphous fumed silica	15 s etching 10 s rinsing and drying
Silver nitrate powder	Penta, Czech	Silver nitrate powder	*Explained in the text
Abbreviations: Bis-GMA, bis-phenol A diglycidylmethacrylate; MDP, 10-methacryloyloxydecyl dihydrogen phosphate; UDMA, urethane dimethacrylate; BisEMA, ethoxylated bisphenol-A-glycidyl methacrylate;			

RESULTS

Table 2 shows the score related to dentin microleakage of bonding system when they used according to manufacturer's instructions.

Table 3 shows the score related to dentin microleakage of bonding systems when they used with 2% chlorhexidine.
In front of each group in table 2 and 3, results of One-Sample Kolmogorov-Smirnov Test (KS Test) have been shown. P<0.05 for all groups that indicates non-normal distribution of the variables, Therefore, to analyze data, non-parametric tests (Kruskal-Wallis and Mann-Whitney U) were used.

TABLE 2: Microleakage scores and Kolmogorov-Smirnov Test results of groups 1 to 4

Groups	Bonding system	Score 0	Score 1	Score 2	Score 3	**P value**
Group I	Adper Single Bond 2	8	2	0	0	**.000**
Group II	Clearfil SE Bond	5	3	0	2	**.032**
Group III	Clearfil S3	7	0	0	3	**.000**
Group IV	Single bond universal	5	2	0	3	**.004**

Krusksal-Wallis test results for Group 1 to 4 represent that the P=0.395 and as P>0.05, so significant difference was not observed between the performance of bonding systems of groups 1 to 4.

TABLE 3: Microleakage scores and Kolmogorov-Smirnov Test results of groups 5 to 6

Groups	Bonding system	Score 0	Score 1	Score 2	Score 3	**P value**
Group V	Adper Single Bond 2 + CHX	2	0	2	6	**.002**
Group VI	Clearfil SE Bond + CHX	7	2	1	0	**.000**
Group VII	Clearfil S3 + CHX	5	2	3	0	**.008**
Group VIII	Single bond universal + CHX	9	1	0	0	**.000**

The result of the Kruskal-Wallis test for Group 5 to 8 represents that the P=0.001 and as P<0.05, therefor there were statically significant differences between groups 5 to 8. As a result, for microleakage comparison of groups 5 to 8, Post Hoc Paired Comparison was used, which its results are showed in Table 4. These results showed that there was difference between groups 5 and 6 as well as between groups 5 and 8 which, this difference was due to increase in the microleakage of Group 5.

TABLE 4: Post Hoc Paired Comparison for groups 5 to 8

Groups	P value
Group V vs Group VI	.012
Group V vs Group VII	.145
Group V vs Group VIII	.001
Group VI vs Group VII	1.000
Group VI Vs Group VIII	1.000
Group VII Vs Group VIII	.729

To compare the effect of chlorhexidine on microleakage of bonding systems, Mann-Whitney U test was used which, its data are showed in table 5. According to results obtained, the use of chlorhexidine causes significant change in the Adper Single Bond 2, while chlorhexidine was not caused statistically significant changes in other groups.

TABLE 5: Mann-Whitney U test results

Groups	P value
Adper Single Bond 2 (ASB2) VS. Adper Single Bond + CHX	0.003
Clearfil SE Bond (CSB) VS. Clearfil SE Bond + CHX	0.393
Clearfil S3 Bond (C3B) VS. Clearfil S3 + CHX	0.853
Single bond universal (SBU) VS. Single bond universal + CHX	0.105

DISCUSSION

Analysis of the data of this study shows that there is no significant difference in the microleakage of different generations of bonding systems in vitro when used in accordance with the manufacturer's instruction after 5000 cycles of thermocycling. In addition, results of this study state that use of 2% chlorhexidine after enamel etching and before bonding application can have different effects on the dentinal microleakage. In present study use of 2% chlorhexidine before self-etch bonding systems (Adper single bond universal, Clearfil S3 bond, Clearfil SE bond) had no negative effect on the dentinal microleakage. On the other hand, chlorhexidine significantly increased the gingival microleakage in Etch-and-Rinse bonding systems (Adper Single Bond 2). Siso et al (2009) examined the effect of different methods of preparation of class V cavities include using the SE Bond alone and in the presence of chlorhexidine 2% on microleakage of composite restorations. They concluded that the use of chlorhexidine before bonding system has no significant effect on the microleakage of composite restorations after 500 cycles of thermocycling (28).as well as Geraldo-martinez et al. (2007) examined the effect of 2% chlorhexidine on microleakage of SE Bond bonding system after thermocycling. They concluded that chlorhexidine has no effect on the microleakage of SE Bond bonding system (29). Kapdan et al (2015) investigated the effect of chlorhexidine 2% on microleakage of composite restorations using Prime and bond NT bonding system, which is one of the 5-generation bonding systems, after 500 cycles of thermocycling. They concluded that chlorhexidine 2% used in cavities before bonding application increases the dentin microleakage, but this increase is not statistically significant (21), which these results are somewhat in line with the results of the current study. However, in some other studies, researchers reported that using the chlorhexidine is ineffective on the microleakage of restorations done with Etch-and-Rinse bonding system(33-30) .

In the contradictory, other studies reported desired effect of chlorhexidine on adhesive systems, for example, the study was conducted by Saffarpour et al. reported that application of 2% chlorhexidine before bonding system did not effected immediate microleakage of Single Bond 2 adhesive system, but after 10000 cycles of thermocycling, chlorhexidine reduced dentinal microleakage (34). On the other hand, studies that measured bond strength to examine chlorhexidine effect on the adhesive systems, reached to different results too, such as Gunaydin et al, they examined the effect of chlorhexidine on the Single Bond 2, SE Bond, and Prompt-L- Pop bonding systems. At that research, for aging at the in vitro, 5000 cycles of thermocycling were used, and in vivo section, restorations remained in patients mouth for 6 months; assessment results showed that chlorhexidine decreased immediate bond strength, but after the aging procedure, whether in vivo and in vitro, better bond strength was reported for experimental groups in compare to control groups (3).By analysis of the results of the previous researches, it was found that this difference does not limited only to bond durability after using chlorhexidine and there were also different results for immediate effect of chlorhexidine on created bond. However, in this study, as the potential effect of chlorhexidine on MMPs and the durability of bond were considered, no test was done to investigate the immediate bond.

We faced with different and contradictory results from several studies that have been evaluated the effect of chlorhexidine on the bond durability of bonding systems from positive (3, 16, 20, 35), to negative (28, 36) or neutral (23, 27, 37, 38) . As a result, there was no consensus on the effect of the chlorhexidine on the durability of the adhesive systems. Chlorhexidine is an inhibitor protease (3), which we referred to its initial application as antimicrobial agent , Due to having a possible role in inhibiting the activity of MMPs, it was examined in this study. In addition, it should be noted that chlorhexidine could increase the Free Surface Energy of dentin (39) and affect the created bond by this property. When the results of the various investigations and this study were considered meticulous, there were factors that have been different in studies such as bonding system, the storage solution that teeth before cavity preparation or before doing tests were kept on it, the company manufactured chlorhexidine, different density of chlorhexidine, the duration of time that chlorhexidine applied on the cavity which includes the range of 10 seconds to 2 minutes, and different aging methods (3, 21, 27, 28, 31-34, 40) .

In studies that used microleakage test to assess the effect of chlorhexidine, different dying methods were used, such as fuchsin or methylene blue dye. In this study, silver nitrate dye was used to investigate the role of chlorhexidine on microleakage of bonding systems. This dying method can be a desired specific test to examine the marginal seal (41). Silver infiltration is naturally diffusion dependent, factors like time samples have been exposed to the silver nitrate, the size of the specimens (or length of the diffusion path), the nature and depth of dentin, etc., have a significant effect on the appearance of microleakage (42). At some studies microleakage were evaluated by dye materials methods to specified the effect of chlorhexidine (21, 27, 28, 31, 32), our ability to reduce microleakage is one of the signs of the success of treatment. Furthermore, other methods such as measuring the shear bond strength, microtensile bond strength, TEM and SEM were used (3, 7, 16, 20, 23, 35-38, 43-45). (28) As the objective of this study was evaluating the effect of chlorhexidine on MMP activity and thus the durability of bond, samples underwent aging procedure. The most common artificial aging technique is long-term storage in water(46), and other method is thermocycling that ISO organization stated that using this technique with 500 cycles in distilled water with temperature of 5 to 55 ° c is the proper method for aging (47). Many studies have proven the effectiveness of this method using more cycles for evaluation of long-term durability of bond as essential matter (46, 48). As the long-term durability in composite restorations is very important, in this study, teeth underwent thermocycling procedure for 5000 cycles, while researchers such as Deng (25) and kayo Saito (24) said increasing in the cycles of thermocycling is ineffective to made more aging samples. However, in the case of the results of the in vivo and in vitro research, this point should also be considered that there is no evidence to show that aging methods like thermocycling can reconstruct the in vivo experiments (3). In addition, the minimum density of chlorhexidine to stop the performance of the MMPs is different for each specific MMP(19). In investigations, it has been found that inhibition of the activity of MMP-2 is possible by the 0.0001% density of chlorhexidine, while concentration greater than 0.02% is required to stop the activity of MMP-8 and required density to inhibit MMP-9 activity has been reported 0.002 % (19, 49), while used density in this research and the studies were reviewed above, were more than reported value for inhibit MMPs activities.

However, after applying bonding system, chlorhexidine density might be reduced to less value than that could able to inhibit MMPs activity. In addition, the results of the SEM investigation conducted by Perdigao showed remaining particles of chlorhexidine on the surface and among dentinal tubules (50). It is possible that the remained particles cause decrease in surface wettability and reduce the impregnate ability of the tooth surface in some bonding systems (32). More investigations in this area are needed to achieve a unified view about the influence of chlorhexidine on the bonding system.

CONCLUSION

Within the limitation of this study, the following conclusions can be drawn:

1. Using 2% chlorhexidine has no negative effect on the dentin microleakage of Self-Etch bonding systems after 5000 cycles of thermocycling.
2. Using 2% chlorhexidine caused an increase in the dentin microleakage of Single Bond 2 (Etch-and-Rinse bonding systems) after 5000 cycles of thermocycling.
3. There is no difference for gingival microleakage of 5-generation (Single Bond 2), 6-generation (SE Bond), 7-generation (S3 Bond) and universal (single Bond Universal) bonding systems .

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REFERENCE

1. Setcos J, Staninec M, Wilson N. Restorative dentistry: the development of resin-bonding for amalgam restorations. British dental journal. 1999;186(7):328-32.
2. Swift Jr EJ. Literature Review Bonding systems for restorative materials-a comprehensive review. Pediatric dentistry. 1998;20(2).
3. Gunaydin Z, Yazici A, Cehreli Z. In Vivo and In Vitro Effects of Chlorhexidine Pretreatment on Immediate and Aged Dentin Bond Strengths. Operative Dentistry. 2016;41(3):258-67.
4. Heymann H, Edward Swift J, Ritter A. STURDEVANT’S ART & SCIENCE OF OPERATIVE DENTISTRY. 5 ed. St. Louis, Missouri: Mosby Inc; 2006. 568 p.
5. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, Van Landuyt K. State of the art of self-etch adhesives. Dental materials. 2011;27(1):17-28.
6. Chen C, Niu L-N, Xie H, Zhang Z-Y, Zhou L-Q, Jiao K, et al. Bonding of universal adhesives to dentine–Old wine in new bottles? Journal of dentistry. 2015;43(5):525-36.
7. Silva EMd, Glir DH, Gill AWMC, Giovanini AF, Furuse AY, Gonzaga CC. Effect of Chlorhexidine on Dentin Bond Strength of Two Adhesive Systems after Storage in Different Media. Brazilian Dental Journal. 2015;26(6):7.
8. Tezvergil-Mutluay A, Pashley D, Mutluay M. Long-term durability of dental adhesives. Current Oral Health Reports. 2015;2(4):174-81.
9. Hannas AR, Pereira JC, Granjeiro JM, Tjaderhane L. The role of matrix metalloproteinases in the oral environment. Acta odontologica Scandinavica. 2007;65(1):1-13.
10. Jain A, Bahuguna R. Role of matrix metalloproteinases in dental caries, pulp and periapical inflammation: An overview. Journal of oral biology and craniofacial research. 2015;5(3):212-8.
11. Summit JB, Robins JW, Hilton TJ, Schwartz RS. Fundamentals of Operative Dentistry: A Contemporary Approach. Fundamentals of Operative Dentistry: A Contemporary Approach: Quintessence Publishing Co Inc.,U.S.; 3rd Revised edition edition (Feb. 2006); 2006. p. 230,1,41,42.
12. Fanchon S, Bourd K, Septier D, Everts V, Beertsen W, Menashi S, et al. Involvement of matrix metalloproteinases in the onset of dentin mineralization. European journal of oral sciences. 2004;112(2):171-6.
13. Moon PC, Weaver J, Brooks CN. Review of Matrix Metalloproteinases’ Effect on the Hybrid Dentin Bond Layer Stability and

Chlorhexidine Clinical Use to Prevent Bond Failure. The Open Dentistry Journal. 2010;4:147-52.

14. Mazzoni A, Pashley DH, Nishitani Y, Breschi L, Mannello F, Tjäderhane L, et al. Reactivation of inactivated endogenous proteolytic activities in phosphoric acid-etched dentine by etch-and-rinse adhesives. Biomaterials. 2006;27(25):4470-6.
15. Pashley DH, Tay FR, Yiu C, Hashimoto M, Breschi L, Carvalho RM, et al. Collagen Degradation by Host-derived Enzymes during Aging. Journal of Dental Research. 2004;83(3):216-21.
16. Brackett MG, Tay FR, Brackett WW, Dib A, Dipp FA, Mai S, et al. In Vivo Chlorhexidine Stabilization of Hybrid Layers of an Acetone-based Dentin Adhesive. Operative Dentistry. 2009;34(4):379-83.
17. Balagopal S, Arjunkumar R. Chlorhexidine: The gold standard antiplaque agent. J Pharm Sci Res. 2013;5(12):270-4.
18. Souza M, Cecchin D, Farina AP, Leite CE, Cruz FF, da Cunha Pereira C, et al. Evaluation of chlorhexidine substantivity on human dentin: a chemical analysis. Journal of endodontics. 2012;38(9):1249-52.
19. Kim D-S, Kim J, Choi K-K, Kim S-Y. The influence of chlorhexidine on the remineralization of demineralized dentine. Journal of Dentistry. 2011;39(12):855-62.
20. Carrilho M, Carvalho R, De Goes M, Di Hipolito V, Geraldeli S, Tay F, et al. Chlorhexidine preserves dentin bond in vitro. Journal of Dental Research. 2007;86(1):90-4.
21. Kapdan A, Öztaş N. Effects of chlorhexidine and gaseous ozone on microleakage and on the bond strength of dentin bonding agents with compomer restoration on primary teeth. Journal of Dental Sciences. 2015;10(1):46-54.
22. Carvalho AA, Moreira FC, Cunha LM, de Moura SM, Souza JBd, Estrela C, et al. Marginal microleakage of class II composite resin restorations due to restorative techniques. Revista Odonto Ciência. 2010;25(2):165-9.
23. Brackett WW, Tay FR, Brackett MG, Dib A, Sword RJ, Pashley DH. The Effect of Chlorhexidine on Dentin Hybrid Layers In Vivo. Operative Dentistry. 2007;32(2):107-11.
24. Saito K, Sirirungrojying S, Meguro D, Hayakawa T, Kasai K. Bonding Durability of Using Self-Etching Primer with 4-META/MMA-TBB Resin Cement to Bond Orthodontic Brackets. The Angle Orthodontist. 2005;75(2):260-5.
25. Deng D, Huang X, Huang C, Yang T, Du X, Wang Y, et al. Effects of chlorhexidine on bonding durability of different adhesive systems using a novel thermocycling method. Australian dental journal. 2013;58(2):148-55.
26. Mousavinasab SM, Atai M, Alavi B. To compare the microleakage among experimental adhesives containing nanoclay fillers after the storages of 24 hours and 6 months. The open dentistry journal. 2011;5(1).
27. Alaghemand H, Abolghasemzadeh F, Pakdel F, Chelan RJ. Comparison of microleakage and thickness of resin cement in ceramic inlays with various temperatures. Journal of dental research, dental clinics, dental prospects. 2014;8(1):45.
28. Siso HS, Kustarci A, Goktolga EG. Microleakage in resin composite restorations after antimicrobial pre-treatments: effect of KTP laser, chlorhexidine gluconate and Clearfil Protect Bond. Oper Dent. 2009;34(3):321-7.
29. Geraldo-Martins VR, Robles F, Matos AB. Chlorhexidine's effect on sealing ability of composite restorations following Er: YAG laser cavity preparation. J Contemp Dent Pract. 2007;8(5):26-33.
30. Meiers JC, Kresin JC. Cavity disinfectants and dentin bonding. Oper Dent. 1996;21(4):153-9.
31. Sung EC, Tai ET, Chen T, Caputo AA. Effect of irrigation solutions on dentin bonding agents and restorative shear bond strength. The Journal of Prosthetic Dentistry. 2002;87(6):628-32.
32. Darabi F, Eftekhari M. Effect of chlorhexidine on microleakage of composite. Journal of Dentistry of Tehran University of Medical Sciences. 2009;6(1):16-22.

33. Alaghemand H, Esmaeili B, Firouz P, Soltani MH, Rouhaninasab M, Bijani A. A comparative study of the preventive effect of chlorhexidine o. 12% and nano zinc oxide particles on the distraction of collagen scaffolding of the hybrid layer by two immunohistochemistry and microleakage tests. Dentistry and Medical Research. 2014;2(2):33.
34. Saffarpour A, Saffarpour A, Kharazifard MJ, Rad AE. Effect of Chlorhexidine Application Protocol on Durability of Marginal Seal of Class V Restorations. Journal of Dentistry (Tehran, Iran). 2016;13(4):231-7.
35. Leitune VCB, Portella FF, Bohn PV, Collares FM, Samuel SMW. Influence of chlorhexidine application on longitudinal adhesive bond strength in deciduous teeth. Brazilian oral research. 2011;25(5):388-92.
36. Boruziniat A, Babazadeh M, Gifani M. Effect of chlorhexidine application on bond durability of a filled-adhesive system. Journal of Dental Materials and Techniques. 2013;2(1):6-10.
37. de Castro FA, de Andrade MF, Junior S, Vaz LG, Ahid FM. Effect of 2% chlorhexidine on microtensile bond strength of composite to dentin. Journal of Adhesive Dentistry. 2003;5:129-38.
38. Chaharom MEE, Ajami AA, Kimyai S, Abbasi A. Effect of chlorhexidine on the shear bond strength of self-etch adhesives to dentin. African Journal of Biotechnology. 2011;10(49):10054-7.
39. Perdok JF, van der Mei HC, Genet MJ, Rouxhet PG, Busscher HJ. Elemental surface concentration ratios and surface free energies of human enamel after application of chlorhexidine and adsorption of salivary constituents. Caries research. 1989;23(5):297-302.
40. Singla M, Aggarwal V, Kumar N. Effect of chlorhexidine cavity disinfection on microleakage in cavities restored with composite using a self-etching single bottle adhesive. Journal of Conservative Dentistry. 2011;14(4):374.
41. Perdigao J. Dentin bonding-variables related to the clinical situation and the substrate treatment. Dental materials : official publication of the Academy of Dental Materials. 2010;26(2):e24-37.
42. Van Meerbeek B. The "myth" of nanoleakage. The journal of adhesive dentistry. 2007;9(6):491-2.
43. Soares C, Pereira C, Pereira J, Santana F, Prado C. Effect of chlorhexidine application on microtensile bond strength to dentin. Operative Dentistry. 2008;33(2):183-8.
44. Jang S-H, Hur B, Kim H-C, Kwon Y-H, Park J-K. Effect of 2% chlorhexidine application on microtensile bond strength of resin composite to dentin using one-step self-etch adhesives. Journal of Korean Academy of Conservative Dentistry. 2010;35(6):486-91.
45. Zhou J, Tan J, Yang X, Cheng C, Wang X, Chen L. Effect of chlorhexidine application in a self-etching adhesive on the immediate resin-dentin bond strength. Journal of adhesive dentistry. 2010;12(1).
46. Yuasa T, Iijima M, Ito S, Muguruma T, Saito T, Mizoguchi I. Effects of long-term storage and thermocycling on bond strength of two self-etching primer adhesive systems. European journal of orthodontics. 2010;32(3):285-90.
47. Standardization IOf. Dental materials-guidance on testing of adhesion to tooth structure: International Organization for Standardization; 1994.
48. Mohammed-Salih HS. The effect of thermocycling and debonding time on the shear bond strength of different orthodontic brackets bonded with light-emitting diode adhesive (In vitro study). Journal of Baghdad College of Dentistry. 2013;25(1):139-45.
49. Gendron R, Grenier D, Sorsa T, Mayrand D. Inhibition of the activities of matrix metalloproteinases 2, 8, and 9 by chlorhexidine. Clinical and diagnostic laboratory immunology. 1999;6(3):437-9.
50. Perdigao J, Denehy GE, Swift EJ, Jr. Effects of chlorhexidine on dentin surfaces and shear bond strengths. Am J Dent. 1994;7(2):81-4.