Original Article

The Effect of Disinfection with Chlorhexidine on the Shear Bond Strength of Equia Resin-Modified Glass Ionomer Cement to Dentin in Permanent Teeth after Two Thermocycling Protocols

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KEY WORDS
Chlorhexidine;
Dental Cavity Preparation;
Disinfection;
Dentition;
Permanent;
Glass Ionomer Cement;
Shear Strength;

Received June 2016;
Received in Revised form January 2017;
Accepted March 2017;

ABSTRACT

Statement of the Problem: There are some concerns regarding the effect of chlorhexidine (CHX) applied for cavity disinfection on the bond strength of adhesive restorations to dentin.

Purpose: This study sought to assess the effect of CHX on the shear bond strength (SBS) of Equia resin-modified glass ionomer cement (RMGIC) to dentin in permanent teeth.

Materials and Method: In this experimental study, the buccal surface of 84 freshly extracted human premolars was ground to expose the flat dentin. The samples were randomly assigned to four groups (n=21). The steps were as following in the group I_a: conditioning, Equia RMGIC, 500 thermal cycles; group I_b: conditioning, Equia RMGIC, 6000 thermal cycles; group II_a: conditioning, CHX, Equia RMGIC, 500 thermal cycles, and group II_b: conditioning, CHX, Equia RMGIC, and 6000 thermal cycles. Twenty samples from each group were subjected to SBS test and one sample was inspected under a scanning electron microscope. Data were analyzed using two-way ANOVA and t-test.

Results: The SBS was significantly different among the groups (p= 0.007). The groups subjected to 500 thermal cycles showed significantly higher SBS to dentin when they were treated with CHX (p= 0.000). There was no significant difference between samples thermocycled for 6000 cycles with and without application of CHX (p= 0.269). The SBS in the groups that were thermocycled for 6000 cycles (I_b and II_b) was lower than those thermocycled for 500 cycles (I_a and II_a). This difference between II_a and II_b was statistically significant (p= 0.007).

Conclusion: Chlorhexidine can positively affect the short term SBS of Equia RMGIC to dentin.

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Cite this article as: Sheikh Hasani Y., Paryab M., Saffarpour A., Kharazifard MJ., Shahrabi M. The Effect of Disinfection with Chlorhexidine on the Shear Bond Strength of Equia Resin-Modified Glass Ionomer Cement to Dentin in Permanent Teeth after Two Thermocycling Protocols. *J Dent Shiraz Univ Med Sci.,* 2017 December; 18(4): 265-271.

Introduction

After cavity preparation and caries removal, microorganisms remain on dentinal surfaces, which may cause postoperative sensitivity and secondary caries. [1] Therefore, rinsing the prepared cavity with disinfectants is recommended prior to restoration. [2] Chlorhexidine gluconate (CHX) is one of the most effective antimicrobial agents against *Streptococcus mutans.* [3] Bonding to the surface of bacteria, amino acids, and hydroxyapatite, CHX exerts its prolonged antimicrobial effect. [4] It is recommended to avoid rinsing the cavity after CHX application in order to benefit from the maximum substantivity of CHX. However, the potential interference of CHX with bond of adhesive restorations to the tooth structure is a concern.

Despite the limited negative results, [5] most studies on the effect of CHX on composite resin bond strength to dentin have revealed that CHX not only has no negative effect on the bond strength of composite resin to dentin, [6] but also may prevent (or delay) the interfacial degradation of dentin-resin bond. This is due to its inhibitory effect on matrix metalloproteinases, which consequently increases the durability of the bond. [7-8] However, it should be noted that the effect of CHX on bond strength depends on the type of bonding agent that is used. [9]

Resin-modified glass ionomer cements (RMGICs) are new glass ionomer-based adhesive restorative materials with improved physical properties compared with the conventional glass ionomers (GIs). [10] These new materials are recommended for restoration of deciduous teeth, permanent teeth in areas under lower occlusal function, and the teeth prepared with atraumatic restorative treatment technique (ART). [10-12] Uncertain evidence exists regarding the antibacterial activity of GI-based materials. [13-14] Hence, it is recommended to disinfect the cavity walls before applying the GI-based materials like other restorative materials.

In comparison with composite resins, RMGICs self-adhere to hard tissue, thanks to the micromechanical interlocking of their constituents. Moreover, their mechanism of attachment to dentin is somewhat different; so that they attach to the dentin through a chelation reaction, followed by metal ion exchange, and formation of a layer between the GI and tooth structure. [15-17] Yet, it is still unclear how CHX may affect the quality of GI-tooth structure interactions.

In a study on Vitremer, 2% CHX did not interfere with the microtensile bond strength of RMGI to the primary tooth dentin. [18] Few studies on Fuji II LC restorative material showed that disinfection with CHX had no negative effect on its bond strength to permanent tooth dentin after 24 hours. [19- 20] Yet, the long-term bond strength between this material and dentin was reported to have significantly decreased. [20]

Wandenya *et al*. [21] evaluated the shear bond strength (SBS) of Fuji IX GI restorative material to CHX-treated dentin after 24 hours. Statistically insignificant superiority was observed in the bond strength values of CHX-treated samples compared with the non-disinfected controls. However, they were not concerned with assessing the long-term effect of CHX on the bond strength of Fuji IX restorative material.

The present study was performed to check the effect of cavity disinfection with CHX on the SBS of Equia RMGIC to the dentin of permanent teeth after two thermocycling protocols simulating the short-term and long-term clinical service. Based on the null hypothesis of the study, disinfection of the tooth surfaces with CHX would have no effect on the bond strength of RMGIC to dentin even in long-term.

Materials and Method

A total of 84 human premolars, freshly extracted for orthodontic purposes, were collected over 6 months. They were disinfected with 0.5% chloramine T solution for one week. The samples had no cracks, fissures, or caries on clinical examination. The patients' age range was 15-30 years. To remove any residues and debris, the teeth surfaces were cleaned with water/pumice slurry.

A guiding groove of 2-mm depth was created in the buccal surface of each tooth by using a fissure diamond bur (#008; Teeskavan, Tehran, Iran). The surface enamel was removed by using a 120-grit silicon carbide disc to expose a flat dentin surface. Next, the dentin surfaces were sequentially polished with 240, 400 and 600-grit silicon carbide papers. The teeth were randomly divided into four equal groups (n=21).

Group I_a

First, cavity conditioner containing polyalkenoic acid (GC Company; Tokyo, Japan) was applied on the dentin surface for 10 seconds, rinsed with water for 10 seconds, and gently air dried for five seconds. Then, Equia RMGIC (GC Company; Tokyo, Japan) was injected into a plastic cylindrical mold of 3×3mm (internal diameter×height) and attached to the dentin surface. A glass slide was gently pressed on top of the mold for the excess material to leak out. The mold was cut and removed following the initial setting of material (about 6 minutes). The surfaces of samples were immediately coated with Fuji Coat LC (GC Company;

Tokyo, Japan) and light-cured for 10 seconds. According to the ISO/TR 11405 standardization, [22] the samples were stored in distilled water at 37°C for 24 hours and then underwent 500 thermal cycles (TC 300; Vafaee Company, Tehran, Iran).

Group I_b

The process of dentin conditioning, RMGIC application, and storage of samples were performed as in the group I_a. To determine the long-term SBS of material to dentin, the samples were subjected to 6000 thermal cycles. [23]

Group II_a

The process of dentin conditioning was performed as described for previous groups. Next, 2% CHX solution (Consepsis; Ultradent Products Inc., South Jordan, UT, USA) was applied to the prepared surfaces with its applicator for 60 seconds and gently air-dried for 10 seconds according to the manufacturer's instructions. Afterwards, the RMGIC application and storage of samples were performed in a similar fashion as in the group I_a. Finally, the samples were thermocycled for 500 cycles.

Group II_b

Dentin conditioning, disinfection with CHX, RMGIC application and storage of samples were performed as in the group II_a. Next, the samples were thermocycled for 6000 cycles.

The thermocycling process was performed between 5-55°C, with 50-second dwell time. All thermocycled samples were stored in distilled water at 37°C for 24 hours before the shear test and scanning electron microscopy (SEM). In each group, 20 samples were considered to undergo the SBS test and one for SEM evaluation.

Shear bond strength measurement

To perform the SBS test, the prepared samples were mounted on the center of cold-cure acrylic resin blocks. The SBS of RMGIC was measured by using a universal testing machine (Z050; Zwick Roell, Ulm, Germany). The acrylic blocks fitted into a metal ring and the blade was placed on the dentin–material interface, along the long axis of each sample. Progressively increasing load was applied with a crosshead speed of 0.5 mm/minute until dislodgement occurred. The load resulting in the dislodgement of the restoration was recorded in Newtons. It was then divided by the cross-sectional area of the bonding interface to calculate the SBS in megapascals (MPa).

Statistical analysis

Data were analysed using SPSS version 20 (SPSS Inc.; IL, USA). The distribution of data was assessed through the Kolmogorov-Smirnov test. The mean SBS was analyzed and compared among the four experimental groups by using two-way ANOVA and t-test. Pairwise comparisons were also performed. *p* Values less than 0.05 were considered statistically significant.

Mode of fracture

Two calibrated blind observers examined the fracture surfaces under a light microscope at 10x magnification and determined three modes of fracture; type 1 was cohesive failure in GI, type 2 was adhesive failure at the GI-dentin interface, and type 3 was mixed adhesive and GI cohesive failure.

Preparation of teeth for SEM analysis

The remaining one sample in each group was prepared for SEM assessment. They were mounted in acrylic resin blocks and placed in a cutting machine. A 1-mm thick section was cut in mesiodistal direction from the middle part of the RMGIC cylinder including the RMGIC-tooth interface. The sections were conditioned with polyalkenoic acid (Cavity conditioner; GC Company, Tokyo, Japan) for 10 seconds to remove the smear layer produced through the cutting process and dried in a vacuum system (EM3200, KYKY) up to 200 gauges. Then, they were sputter coated with 20-30-nm thick particles of gold in a sputter coater (SBC-12, KYKY) with 10-20 mA flow and 10^{-1} $pa/_{mm\ Hg}$ air pressure. Examination of the prepared surfaces was performed through SEM (EM3200; Cayman Island) at 40x and 250x magnifications at an accelerating voltage of 26 KV.

Results

Shear bond strength

Eighty samples were assessed for the SBS. Table 1 shows the minimum, maximum and the mean SBS values in each group. In all groups except group II_a, some samples were debonded prior to increasing the load (3 samples in the group I_a and 2 samples in the groups I_b and II_b). The results of two-way ANOVA revealed statistically significant differences among the groups (p= 0.007). Comparison of the two groups samp-

Table 1: The minimum, maximum, mean and standard deviation of the shear bond strength of Equia RMGIC to dentin in permanent teeth in the four study groups

Groups	Minimum-Maximum	Mean (Standard deviation)
I_a	0.00- 4.88	2.33 (1.60)
I_b	0.00- 4.40	1.03 (1.22)
II_a	2.34- 6.35	4.24 (1.44)
II_b	0.00- 3.60	1.47 (1.26)

Group I_a: Conditioning, Equia RMGIC, thermocycling for 500 cycles
Group I_b: Conditioning, Equia RMGIC, thermocycling for 6000 cycles
Group II_a: Conditioning, application of CHX, Equia RMGIC, thermocycling for 500 cycles
Group II_b: Conditioning, application of CHX, Equia RMGIC, thermocycling for 6000 cycles

les in the group II_a which were disinfected with CHX provided significantly higher bond strength to dentin than the samples in the group I_a, which were not disinfected (p= 0.000). Comparison of the SBS of samples thermocycled for 6000 cycles (groups I_b and II_b) revealed no statistically significant difference between the samples with and without application of CHX on dentin (p= 0.269). Moreover, comparison of the samples with different number of thermal cycles showed that the groups subjected to 6000 thermal cycles (I_b and II_b) had lower SBS than those thermocycled for 500 cycles (I_a and II_a); this difference was statistically significant between groups II_a and II_b (p= 0.007).

Fracture mode assessment

Table 2 shows the fracture modes in samples. Groups I_a and II_a in which the samples underwent 500 thermal cycles mostly showed mixed fracture; while, groups I_b and II_b in which the samples were subjected to 6000 thermal cycles predominantly showed adhesive mode of fracture.

Table 2: The fracture modes of Equia RMGIC bond to dentin in the four study groups

Study Groups	Cohesive in GI	Adhesive	Mixed
I_a	4	3	10
I_b	0	12	6
II_a	5	5	10
II_b	0	12	6

Group I_a: Conditioning, Equia RMGIC, thermocycling for 500 cycles
Group I_b: Conditioning, Equia RMGIC, thermocycling for 6000 cycles
Group II_a: Conditioning, application of CHX, Equia RMGIC, thermocycling for 500 cycles
Group II_b: Conditioning, application of CHX, Equia RMGIC, thermocycling for 6000 cycles

The SEM analysis

The Equia RMGIC-dentin interface was scanned and inspected at 40x and 250x magnifications. Appropriate sealing with no evident gap at the Equia RMGIC-dentin interface was observed in the group II_a in which the conditioned dentin was disinfected with CHX prior to the application of Equia and thermocycled for 500 cycles (Figure 1).

Discussion

Owing to adhesive restorative materials, currently tooth preparation is more conservatively done in the new dental restorative procedures. The RMGICs are a new generation of adhesive restorative materials with several positive points like fluoride releasing potential and improved marginal seal. The two constituent parts of GIs are composite resin and glass ionomer. They are reported to form an amorphous zone like the hybrid layer formed by composite resins. The RMGICs also create a chemical bond to the tooth structure through the ionic interactions similar to GI. [15]

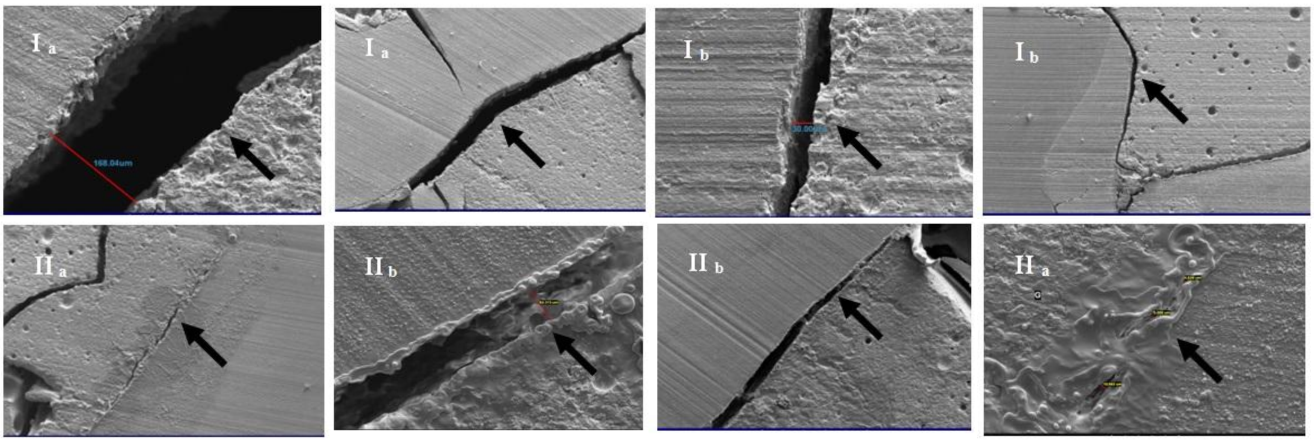

Figure 1: The SEM micrographs at 40x (top images) and 250x magnifications (below images).
Group I_a: Conditioning, Equia RMGIC, thermocycling for 500 cycles
Group I_b: Conditioning, Equia RMGIC, thermocycling for 6000 cycles
Group II_a: Conditioning, application of CHX, Equia RMGIC, thermocycling for 500 cycles
Group II_b: Conditioning, application of CHX, Equia RMGIC, thermocycling for 6000 cycles

In the first phase of bonding, hydrogen bonds are created as the result of chelation reactions. As the time passes, bond is created between the Calcium and Aluminum ions of the cement and the negative hydroxyl ions of hydroxyapatite crystals; it subsequently increases the cross-link density and compressive strength of RMGI material. [16] The second phase of setting reaction of RMGICs continues with creation of bond between the carboxyl ions and positively charged calcium (CA) and aluminum (Al) ions in hydroxyapatite crystals. The process ends up with the formation of an ion-enriched layer on the cement-tooth interface. [17] These materials owe their great sealing ability and microleakage resistance to such chemical bonds. [17]

Limited number of studies has evaluated the effect of CHX molecules on the interaction between RMGIs and tooth structure. [18-20] It was hypothesized that the dentinal tubules are likely to be physically occupied and occluded by CHX molecules. [24] Meanwhile, they have a strong cationic charge, which tends to compete with Ca and Al ions in reacting with anionic carboxyl groups; and thus, they may interfere with the maturation reaction of RMGICs. The present study assessed the effect of CHX application on the shear bond strength of Equia RMGIC to dentin after two thermocycling protocols.

The mean SBS of Equia RMGIC to dentin in experimental groups was in the range of 1.03-4.24 MPa. It was different from the previously reported values for RMGIC, which were 6-9 MPa in permanent teeth and 6.5 MPa in primary teeth. [21] The various SBS values found in different investigations can be possibly attributed to several factors such as the type of material, application method, tooth preparation methods, and number of thermal cycles.

The present study analyzed the SBS data based on CHX application and number of thermal cycles. The SBS was higher in those samples, which were disinfected with CHX prior to application of Equia RMGIC and thermocycled for 500 cycles (group II_a); therefore, the null hypothesis regarding the short-term SBS was accepted. No sample in the group II_a experienced debonding of restoration prior to loading in shear test. They were predominantly fractured in mixed mode.

According to the SEM micrographs, the minimum gap and maximum sealing at the Equia-dentin interface was observed in the group II_a. It means that the CHX deposits on the surface and within dentinal tubules not only had no negative effect on the SBS, but also increased it. Having not been reported so far, this phenomenon can be explained as the maximum contact between an adhesive material and the tooth surfaces in order to maximize the chemical and micro-mechanical adhesion under ideal conditions. Contact between the restorative material and tooth surface highly depends on the water content, roughness, and chemical composition of the tooth surface. [25]

The higher SBS of Equia RMGIC after application of CHX on conditioned dentin could be due to the fact that CHX caused chemical changes and increased the surface energy of dentin, particularly after removing the smear layer. Increased surface energy could result in higher wettability by the restorative material. Thus, disinfection of the dentin surface with CHX before application of RMGIC may be considered as an essential step in treatment.

Some studies found association between the increased tooth surface energy and wettability of the restorative materials after application of CHX. [26-29] This result was consistent with Wadenya *et al.*'s findings [21] who reported an improvement in the SBS of Fuji IX GI to CHX-treated dentin. Although the efficacy was not statistically significant, CHX application was recommended as a step of treatment. In similar studies on Fuji II LC, Photac Fil and Vitmer, no increase in bond strength was noted after the application of CHX. [18-20] The difference could be explained by the difference in depth of dentin, material composition, and water content, concentration of CHX solution and method of CHX application in these studies.

Comparison of samples thermocycled for 6000 cycles revealed no significant difference between the samples with and without CHX application. In other view, the reduction noted in the SBS of RMGIC to dentin in both groups thermocycled for 6000 cycles in comparison with the groups undergoing 500 thermal cycles was significant in samples disinfected with CHX (II_a and II_b). It indicates that the long-term SBS of Equia RMGIC to dentin is negatively influenced by CHX solution. It confirmed what was found by Dursun *et al*. [20] who reported that the long-term SBS of Fuji II LC to CHX-treated dentin did not increase over time. Dursun *et al*. [20] stated that CHX, which has strong cationic properties, could react with anionic groups and interfere with the chemical adhesion and maturation reaction of RMGIC. They also measured the SBS of a different material and assessed the long-term SBS after six months of storage. [20]

It is noteworthy that in the present study the higher number of thermal cycles was considered as effective as long-term storage for the evaluation of durability of bond strength. Since the reduction was observed in all samples thermocycled for 6000 cycles, the possibility of a thermal shock in the first steps of RMGIC setting would be considered. Thus, it is recommended to evaluate the long-term shear bond strength of RMGIC to dentin after several months of storage in conditions simulating the oral environment.

Among the limitation of this *in vitro* study was the inability to simulate the biologic changes such as masticatory forces and chemical attacks by acids and enzymes, which challenge the durability of restoration in the oral cavity. Conduction of more laboratory and clinical studies are recommended to assess the effect of CHX on the bond strength of RMGIC to dentin in similar conditions.

Conclusion

The conclusion can be drawn that chlorhexidine has a positive effect on the bond strength of Equia RMGIC to dentin, which can be due to the increased wettability of tooth surface. Moreover, it can be claimed that the possible thermal shock, which is caused by the high number of thermal cycles, can decrease the shear bond strength of RMGIC to dentin. Thus, to evaluate the long-term effects of CHX on the SBS of RMGIC to dentin, storage in conditions simulating the oral environment should be considered.

Acknowledgment

This article was based on an undergraduate thesis submitted to the dental faculty of Tehran University of Medical Sciences. The authors would like to thank the staff of the Department of Pediatric Dentistry for their cooperation in conduction of this project.

Conflict of Interest

The authors disclose no potential conflicts of interest.

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