Original Article

Effect of Chlorhexidine and Green Tea Extract Application on The Microtensile Bond Strength and Durability of Etch-and-Rinse Adhesives

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Received 28 July 2021 and Accepted 27 April 2022

Abstract

Introduction: Despite improvements in dentin bonding, the bond will be demolished over time and decreased by water and matrix metalloproteinase (MMP). This study aimed to evaluate the effects of the combination of different methods of MMP inhibition and detect the possible synergic effect on strength and bond durability. **Methods:** Thirty human third molar teeth without any cracks or decay were used in this study. The occlusal surface of teeth were removed, and dentine surfaces were flattened with silicon carbide plates. After preparing for restoration, teeth were divided into 4 groups, including 1- the control group, 2- the chlorhexidine (CHX) 2% group, 3- the green tea (GT) extract 15%+rinse+CHX 2% in 30 sec, and 4- GT extract15%+rinse+CHX 2% in 60 sec for the application time of GT. Composite samples were divided into two groups, and microtensile bond strength was evaluated immediately and 3 months after storage in water. The fracture type of samples was then evaluated using a stereomicroscope. **Results**: The immediate bond strength had no significant difference in experimental groups. The mean bond strength for GT+CHX in 30 and 60 sec after a 3-month storage had a significant difference with the 3-month storage control group, however, not significant with the immediate control group. **Conclusion**: The use of GT extract with CHX led to bond durability after a 3-month storage. Further studies are required for investing the *in vivo* effect of this combination.

Keywords: Chlorhexidine, Grape seed extract, Green tea extract, Matrix metalloproteinase, Microtensile bond strength

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Ahmadi Shadmeri M, Boruziniat A, ChalakiNia H. Effect of chlorhexidine and green tea extract application on the microtensile bond strength and durability of etch-and-rinse adhesives. J Dent Mater Tech. 2022; 11(4): 205-212.

Introduction

Hybrid layer degradation has a consequential effect on the durability of the bond to dentin. This phenomenon can occur due to extrinsic or intrinsic factors, such as hydrolysis by water/oral fluid sorption, thermal expansion, fatigue forces, and matrix metalloproteinase (MMP) enzymes (1-3).

Exposed collagen fibrils at the base of the hybrid layer may be degraded by the collagenolytic and gelatinolytic activity of bacterial or host-derived MMP enzymes; this is due to the discrepancy between the depth of acid etching and resin infiltration (4, 5). These enzymes exist in dentin inactively. Matrix metalloproteinases may be activated by heat, chemical agents, reactive oxygen, free radicals, and acidic pH caused by phosphoric acid etching (6, 7).

The main methods adopted for the inhibition of MMPs' activity are the application of specific or non-specific inhibitors (4, 8, 9), use of cross-linker agents (10-12), and elimination of water, which is a critical factor for the activity of MMPs (13, 14).

Chlorhexidine (CHX) is the most important MMPs inhibitor used in different studies, which can inhibit the binding of essential ions (Ca and Zn) to MMP enzymes (1, 4, 9).

Cross-linkers, such as grape seed and glutaraldehyde, bind to collagen fibrils and prevent their attachment to MMPs (10, 11). Although, these agents may improve the mechanical properties of dentin; however, their application time is not clinically acceptable (12, 15).

The presence of water is a critical factor for the activation of MMP enzymes. Water removal by the application of alcohol or acetone may improve the bond durability via inhibiting MMPs enzymes (13, 14).

This study hypothesized that the combination of cross-linking agents, MMPs inhibitors, and drying agents might have a synergistic effect that could probably reduce the application time.

Materials and Methods

Tooth preparation

Thirty intact, noncarious human third molars, were obtained through atraumatic extraction in patients whose treatment plan entailed extraction. After debridement, the teeth were disinfected with 1% chloramine-T and were stored in saline for 2 weeks, which was replaced every week.

The teeth were mounted to 1 mm below the cementoenamel junction in acrylic resin, so that the long axis was perpendicular to the floor. Enamel surfaces were removed using a slow-speed saw with a diamond-coated disk (Isomet, Buehler, Lake Bluff, IL, USA), and dentin surfaces were ground with 400- and 600-grit silicon carbide papers under running water to produce a standard smear layer. One tooth was excluded from the study due to early fracture during the initial preparation and another one was excluded because of a fracture which occurred during the process of tooth sectioning for obtaining beams.

Pilot study

To achieve an appropriate solution consisting of MMPs inhibitors, cross-linkers, and drying agents, different solutions were produced, as described below:

15% grape seed extract (GSE) alcoholic solution and 2% CHX were mixed for 1 h in a magnetic stirrer, however, precipitated so the mixture was inapplicable. The same process happened for a mixture of 15% green tea alcoholic solution and 2% CHX.

This may be due to the interaction between the cationic group of CHX and the anionic groups of grape seed and green tea.

Dentin surfaces were etched with 35% phosphoric acid for 15 sec, washed with water for 10 sec, and gently air-dried. The specimens were divided into 4 groups of 7 teeth according to the solution used before bonding application. The groups included:

1- Control group: two consecutive layers of Single Bond Adper Adhesive were applied, gently air-dried, and light-cured for 20 sec.

2- Group 2: 2% CHX solution was applied for 30 sec, slightly air-dried, and followed by the application of the adhesive in the same manner as the control group.

3- Group 3: alcoholic solution of 15% green tea extract (GTE) was applied for 30 sec, then rinsed and air-dried, followed by the use of 2% CHX for 30 sec and the application of the adhesive in the same way as the control group.

4- Group 4: solution of GTE and 2% CHX was used for 60 sec, followed by the application of the adhesive in the same way as the control group.

After bonding application, resin composite build-up was accomplished using double 2-mm-thick increments, with each increment being light-cured for 40 sec. Specimens were post-cured for 40 sec all around. The prepared specimens were randomly divided into two subgroups. In the first subgroup, the samples were stored in 100% humidity for 24 h, and then microtensile bond strength was evaluated. In the second group, to evaluate short-term bond durability, the samples were stored in distilled water for 3 months. The distilled water was refreshed on a weekly basis.

Microtensile bond strength evaluation

Specimens were serially sectioned perpendicular to the adhesive interface to obtain slices with a cross-sectional area of approximately 1 mm^2. Three to five intact beams were obtained from each tooth. The microtensile bond test was performed by a universal testing machine at a cross-head speed of 1 mm/min. The failure modes were checked out at 40X magnification using a stereomicroscope (LEO, 1450 UP, Zeiss; Oberkochen, Germany) and recorded as adhesive, cohesive in dentin or composite, and mixed.

Statistical analysis

The data were processed using SPSS software version 16 (SPSS Inc. Chicago, IL). The normal distribution of the data was confirmed using the Kolmogorov-Smirnov test, and the data were analyzed using one-way ANOVA and Tukey's post-hoc tests with a significance level of 0.5.

Results

The mean values of bond strengths in the experimental groups are presented in Table II. The one-way ANOVA test indicated a statistically significant difference between and within the experimental groups ($P<0.001$) (Table III).

According to Tukey's post hoc test (Table II), no statistically significant difference was observed between the immediate bond strength of the experimental groups and the immediate bond strength of the control group. After 3 months of storage, although the bond strengths of all of the experimental groups were higher than that of the control group, the difference between the recorded values for the control and CHX group were not proven to be statistically significant.

Green tea extract-CHX 30 sec and 60 sec had a significant difference after 3 months with the three-month control group. Nevertheless, no significant difference was observed with the immediate bond strength of the control group.

The most frequently observed failure mode in all experimental groups were reported to be adhesive failure, mixed and cohesive fracture in composite resin, in descending order of frequency.

Table I. Devices and materials used in this study

Device/material	Manufacturer/country	Device/material	Manufacturer/country
Filtek Z250 A2 universal restorative	3M ESPE/USA	Bluephase Curing Light	IvoclarVivadent/USA
Adper Single Bond 2	3M ESPE/USA	Consepsis Chlorhexidine 2%	Ultradent/USA
35% phosphoric acid etch gel	Ultradent/USA	MITREAPEL super CA Glue	Beta Kimya/Turkey
Grape seed extract powder	Purebulk Inc./USA	Alcohol 70%	Bidestan/Iran
Green tea extract powder	Purebulk Inc./USA		

Table II. Effect of pretreatments on dentin mu TBS (mean and standard deviation) for different treatment groups and different duration

	After 24 hours			After 3 months		
Treatment	n	Mean	SD	n	Mean	SD
Control	20	23.83 aA*	4.77	17	12.94 bA	4.59
CHX 2%	19	20.88 abA	5.78	16	17.98 abA	5.42
GT+CHX (30 sec)	18	23.65 aA	9.53	17	22.29 abA	5.82
GT+CHX (60 sec)	15	22.58 aA	9.80	16	20.15 abA	5.89

mu TBS: microtensile bonding strength , CHX: Chlorhexidine; GT: Green tea
*Different letters denote statistically significant differences at p=0.001. Small letters refer to the horizontal comparison between two time points. Capital letters refer to comparison among the groups (Tukey's post hoc test).

Table III: Results of one-way ANOVA test

mTBS	Sum of squares	df	Mean square	F	Sig.
Between groups	1594.687	7	227.812	5.118	0.000
Within groups	5786.929	130	44.515		
Total	7381.616	137			

Discussion

The degradation of the hybrid layer is one of the important factors that affects bond durability (16). Instead of forming an environment for enzymatic actions, water will cause bond degradation by restricting the complete curing of resin monomers and plasticizing the resin components of the adhesive. Furthermore, it seems that the incomplete penetration of adhesive resins enables and facilitates the penetration of biologic liquids to the interface layer (17-19).

Based on the results of a study by Carrilho et al. (20) collagen fibrils demolition did not happen with mineral oils used for sample storage instead of water. However, the effect of MMPs on bond durability cannot be accepted without the effect of water presence as they need water for their activation. Another study aimed to decrease the effect of water on bond durability, solutions were prepared using alcohols to eliminate water on dentin surfaces (14).

However, the samples in the current study were stored in water for 3 months due to the important role of water in *in vivo* conditions. That could be a valid means to simulate the aging of resin dentin restorations. The results of this study showed that bond strength after 3 months of storage in water was significantly lower than bond strength after 24 h in the control group. In another study by Kitasako et al. (21), the results indicated that replacing the storage water daily in aims of restricting microbial growth, could weaken the resin-dentin interface. Considering this, storage water was replaced weekly in the present study.

Among other factors contributing to bond attenuation, there is the inconstancy of the demineralized dentinal collagen matrix, which is due to MMPs host-derived enzyme activation that was first reported by Verma (22). Three methods are used for MMPs inhibition: water elimination, MMP inhibitors and using cross-linkers. In this study different types of MMP inhibition methods were used in combination to achieve a probable synergic effect and consequently a reduced application time. Chlorhexidine and GTE from the MMP inhibitors group, GSE from cross-linkers, and alcohols as drying agents were employed.

At first, as a pilot study to choose the final solutions, 15% alcoholic solute of GSE and GTE was admixed with CHX; however, both of them immediately sedimented and made a clot; therefore, the solution was unusable.

In another attempt, each solution was used in separate stages; nevertheless, despite the removal of CHX moisture and thinning it on dentin, the same sedimented layer was formed after the application of GTE and GSE, which prohibited the infiltration of adhesive and weakened the bond as the whole samples fractured even before the test.

A possible reason for the sedimentation may be the formation of the hydrogenic bonds of H+ groups in CHX and OH- groups in GSE and GTE.

As a final approach to prevent the sedimentation, the dentin surface was rinsed with water after the application of GTE or GSE for certain times (10 sec). Likewise, in other studies, the dentin surface was rinsed after using cross-linker agents (23).

In the GSE group, the bond strength was so weak that all samples failed before the test, and the microtensile bond strength (mu TBS) was significantly lower than the control group; consequently, this group was excluded from the study.

Based on the results, the 24-hour CHX group was similar to the 24-hour control group, indicating the lack of negative effect in initial bond strength as discussed in other studies (24-27). It was found that mu TBS in the CHX group had no significant difference after 3 months of storage with the 24-hour control and 24-hour CHX group, showing the CHX ability to maintain bond durability, which was consistent with the results of studies conducted by Zhou et al., Hiraishi et al., and Armstrong et al. (28-30). On the other hand, the mu TBS in the 3-month CHX group was higher than in the 3-month control group; however, it was not significant, indicating the low effect of CHX on bond durability.

The findings of studies have shown that the effect of CHX on bond strength is mostly due to the inhibition of MMP enzymes (31). Since water has an important role in bond degradation, even though CHX inhibits MMP activity, it contains water that may enhance hydrolytic degradation (32, 33). In a study by Ozelin et al. (23), GTE was used for 15, 30, and 60 min, which is not a clinical time for GTE application; therefore, in the current study, GTE was applied for 30 and 60 sec along with the use of CHX.

According to the results of the current research, mu TBS had no significant difference between 24-hour GTE+CHX (30- and 60-sec) and the control group. Moreover, the 30-sec and 60-sec did not differ from each other, showing any negative effect of using GTE at both times.

Monteiro et al. (34) conducted a study with similar conditions and obtained the same results for GTE and CHX separately. The findings of another study by da Fonseca et al. (35) showed no difference between CHX 2% and GTE 1% for 30 sec with the control group; nonetheless, mu TBS was a slightly higher in the GTE groups than in other groups.

The recorded mu TBS in GTE 30-60-seconds group, after 3 months of storage showed a significant difference with the 3-month control group, however, no difference with the 24-hour control group. This finding indicated the maintenance of bond strength and enhancement of the bond durability, compared to the control group or CHX group, which could be due to the synergic effect of the combined use of these two materials, each one acting its own function (36). No difference was detected between the 30 and 60-second GTE groups.

Green tea extract can be used as an enhancer for bond durability in acid-etched dentin due to its polyphenols, especially epigallocatechin gallate (EGCG). Epigallocatechin gallate has an MMP inhibitory effect and can induce hydrogen bonding and hydrophobic interactions with collagenase that can alter their structure and their enzymatic activity (37). Similar results were reported in a study conducted by Du et al. (37) using EGCG for immediate and after 6 months mu TBS. This protection provided by EGCG against the degradation of composite-dentin bonds can be the result of suppressing the denaturing effect of etching on dentine collagen.

Fracture type was evaluated after the mu TBS test was carried out. Adhesive fracture in the dentin-resin interface was the most frequently encountered fracture in all groups. Despite the significant difference between the groups regarding mu TBS values, no significant difference in terms of the type of fracture was identified. The same results were reported in studies conducted by El-Din et al. and Gurgan et al.(38, 39), showing no relationship between mu TBS and fracture type. The null hypothesis of this study, which was the possible synergic effect of the combination of cross-linking agents, MMP inhibitors, and drying agents, was accepted. Further investigations can be held to measure the *in vivo* factors, such as thermal, chemical, and tensional stress factors.

Conflicts of interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that was presented in this article.

Acknowledgment

This study was extracted from an undergraduate thesis (no: 2720). The authors extend their gratitude to the Research Deputy of Mashhad University of Medical Sciences, Mashhad, Iran, for their financial support towards this research project (grant number: 910969).

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