The Study on the Influence of Antiseptic Mouthwash on Microtensile Bond Strength of Resin Composite to Dentin: A Pilot Study

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Abstract

This research aimed to investigate the effect of different types of antiseptic mouthwash on the microtensile bond strength (µTBS) of a self-etch adhesive system to dentin. Flat dentin surfaces were prepared from twenty extracted sound human third molar teeth. They were divided into four groups according to different mouthwashes (artificial saliva (control), 1% hydrogen peroxide (H_2O_2), 0.2% povidone-iodine (PVP-I), and 0.12% chlorhexidine (CHX)). The specimens were immersed in antiseptic mouthwash for 30s. The adhesive application and restoration were applied using a self-etch adhesive system and a nano-filled resin composite. The specimens were serially sectioned into 1 mm^2 resin slabs using a low-speed diamond saw. Four slabs were obtained from each tooth; therefore, twenty specimens were used in each group (n=20). Each specimen was assessed by a µTBS test at 0.5 mm/min using a universal testing machine. The µTBS values were in megapascal (MPa). The failure mode was examined using a stereomicroscope. The data were analyzed by one-way analysis of variance (ANOVA), Welch's test, and Games-Howell for the post hoc test.

The data from the microtensile bond strength test indicated that the control (26.04±4.54 Mpa) had the significantly highest µTBS values among the other groups ($p<0.05$). The hydrogen peroxide (22.01±4.14 Mpa), povidone-iodine (21.97±5.12 Mpa), and chlorhexidine (22.87±6.36 Mpa) were significantly lower than the control ($p<0.05$) but had no significant differences between mouthwash groups ($p>0.05$). From the failure mode analysis, the predominant failure mode was the adhesive failure for every mouthwash group. This study indicated that all mouthwash groups affect the microtensile bond strength to dentin which results in significantly lower µTBS values than the control.

Keywords: *Antiseptic Mouthwash, Chlorhexidine, Hydrogen Peroxide, Microtensile Bond Strength, Povidone-Iodine, Self-Etch Adhesive*

1. Introduction

In order to reduce bacterial load in oral cavity and minimize the risk of infection during dental procedures, using antiseptic mouthwash is recommended before dental treatment. American Dental Association (ADA) and the United States' Centers for Disease Control and Prevention (CDC) recommend 1% hydrogen peroxide mouthwash and 0.2% povidone-iodine before starting dental treatment. In addition, the New Zealand Dental Association (NZDA) suggests 1% hydrogen peroxide, 0.2% povidone-iodine, and 0.12% chlorhexidine mouthwash for 30 seconds before procedures (Jamal et al., 2020).

However, using mouthwash before dental treatment may cause residual liquid covering enamel or exposed dentin surfaces if there is inadequate cleaning and may affect surface properties, especially regarding the bond strength between enamel/dentin and dental adhesive. On enamel, there has been a reported study indicating that the bond strength of the universal adhesive in etch and rinse mode is reduced by the application of hydrogen peroxide and povidone-iodine (Özduman et al., 2021). Similar to chlorhexidine, there was a study that showed the results in reducing the bond strength of orthodontic brackets to enamel surfaces (Singh et al., 2018). In contrast, some studies reported that povidone-iodine and chlorhexidine may have no impact on the bond strength of orthodontic composite to enamel surfaces (Demir et al., 2005). On dentin, there were limited studies that examined the effect of mouthwash on the bond strength of dental adhesive to dentin. One of them found that there were no statistical differences in the dentin shear bond strength when comparing hydrogen peroxide and povidone-iodine regardless of the application mode of a universal adhesive (Kutuk et al., 2021).

To study dentin surfaces, the self-etch adhesive system has been focused on due to their reliable clinical performance, fewer steps, less technique-sensitive, and less aggressive in dentin (Perdigão et al., 2020). In addition, to assess the bond strength of dental materials, generally, they are typically categorized in macro and micro test settings which are macro shear, macro tensile, micro shear, and micro tensile test. The microtensile bond strength test is one of the assessments of the adhesive properties that have been used worldwide and appears to be able to accurately evaluate the bonding performance with better stress distribution compared with other traditional bond strength tests (Van Meerbeek et al., 2010).

Nevertheless, there have been limited studies about the effect of mouthwashes on the microtensile bond strength of the adhesive system to dentin after rinsing and contaminating with antiseptic mouthwash. While some studies found that chlorhexidine might not affect the microtensile bond strength to dentin (Chang & Shin, 2010; Dalkilic et al., 2011), there have been limited research studies about the effect of various mouthwashes on the microtensile bond strength of the self-etch adhesive system to dentin directly.

Therefore, this *in vitro* study aims to compare the microtensile bond strength of the self-etch adhesive system to dentin after being contaminated with different types of antiseptic mouthwash. With the null hypothesis of this study, there were no statistically significant differences between the microtensile bond strength (μTBS) of a self-etch adhesive system to dentin after contamination of different types of antiseptic mouthwash.

2. Objectives

To compare the microtensile bond strength (μTBS) of the self-etch adhesive system to dentin after being contaminated with different types of antiseptic mouthwash.

3. Materials and Methods

3.1 Materials

Antiseptic mouthwashes and the materials used in this study will be shown in Table 1

Table 1 Antiseptic mouthwashes

Product names and manufacturers	Compositions
Hydrogen peroxide mouthwash (Department of Pharmacology, Faculty of Dentistry, Mahidol University, Bangkok, Thailand) Batch No. 0309	Hydrogen peroxide, stabilizer, distilled water
Betadine gargle (Thai Meiji Pharmaceutical Co., Ltd., Bangkok, Thailand) Batch No. 859262	Povidone iodine, distilled water
0.12% Chlorhexidine mouthwash (Mdent by Mahidol, Bangkok, Thailand) Batch No. 20001639	Chlorhexidine gluconate, Ethyl Alcohol

Self-etch adhesive and composite resin

Product names and manufacturers	Compositions
Clearfil™ SE Bond (Kuraray Noritake Dental Inc., Okayama, Japan) Batch No. 2A0399, 1H0769	Primer: 10-Methacryloyloxydecyl dihydrogen phosphate (MDP), HEMA, dl-Camphorquinone, Hydrophilic dimethacrylate, N,N-Diethanol-ptoluidine, Water Bonding agent: MDP, Bis-GMA, HEMA, Hydrophobic dimethacrylate, dlCamphorquinone , N,N-Diethanol-p-toluidine, Silanated colloidal silica
Filtek™Z350 XT (3M ESPE, St. Paul, MN, USA) Batch No. NE83167	Bis-GMA, UDMA, TEGDMA, Bis-EMA, ZrO2-SiO2

3.2 Methods

Ethics consideration

The study protocol was approved by the ethical committee of the Institutional Review Board from the Faculty of Dentistry/Faculty of Pharmacy, Mahidol University (COE.No.MU-DT/ PY-IRB 2022/038.1708).

Teeth preparation

Twenty extracted sound human maxillary and mandibular third molar teeth were kept in 0.1% thymol solution at room temperature within one month after extraction. The teeth were carefully selected with nearly identical size and shape to assure homogeneity and were cleaned by hand instruments to remove dental calculus and soft tissue remnants. The exclusion criteria involving the teeth with any sign of caries and cracking of enamel and dentin were then discarded.

Specimen preparation

A flat dentin surface was prepared by horizontally sectioning parallel to the occlusal surface at a level 1 mm below the dentin-enamel junction using a low-speed diamond saw (Isomet, Buehler, Lake Bluff, Illinois, USA) on a water coolant. The surfaces were wet abraded using 600-grit abrasive papers for 60 seconds (Saikaew et al., 2016) on a grinding machine (Struers, Rotopol-21, Cleveland, OH, USA).

Study design

The specimens were then randomly organized into four groups. Each group is composed of five teeth, according to the different types of antiseptic mouthwash. The dentin surfaces were immersed with each type of mouthwash for 30 seconds in plastic vials**.** Each group was gently dried with mild airflow using a triple syringe for 10 seconds before the application of adhesive systems.

Group I: artificial saliva (Control)
Group II: 1% hydrogen peroxide (H_2O_2)
Group III: 0.2% povidone-iodine (PVP-I)
Group IV: 0.12% chlorhexidine (CHX)

Adhesive application and restoration

After drying the specimens, the self-etch adhesive bonding (Clearfil™ SE Bond, Kuraray Noritake Dental Inc., Okayama, Japan) was applied according to the provided information by the manufacturer. The primer was applied to the prepared surface for 20 seconds and dried with mild airflow. The adhesive was applied and distributed evenly with mild airflow, then light-cured for 10 seconds. For each specimen, a composite core build-up was made in two increments with 1.5 mm thickness, using nano-filled resin composite (Filtek™Z350 XT, 3M ESPE, St. Paul, MN, USA). A resin composite was built up with a plastic instrument (Hu Friedy, USA) and ball-burnisher (Hu Friedy, USA), and then light-cured with light emitting diode curing device (LED; Bluephase G2®, Ivoclar Vivadent, Schaan, Liechtenstein) for 40 seconds with a light intensity 1,100 mW/cm^2. After that, all specimens were stored in distilled water at 37°C for 24 hours in an incubator for a complete set of restoration.

Microtensile bond strength (µTBS) test

The restored specimens were then serially sectioned into resin slabs measuring 1 mm in thickness. After a 90° rotation, the slabs were subsequently re-sectioned again to produce resin-dentin sticks with a rectangular cross-sectional area measuring 1 mm^2 and a length of 6 mm using a low-speed diamond saw (Isomet, Buehler, Lake Bluff, Illinois, USA) in a water coolant (Omar et al., 2007; Sano et al., 2020). Four sticks were obtained from each tooth. Thereby, twenty specimens were used in each group (n=20). The bonded surface area was measured before each test using a digital caliper (Mitutoyo, Tokyo, Japan). The ends of the sticks were attached to a universal testing machine (Type 5500R, Instron, Canton, MA, USA) using cyanoacrylate glue (Zapit, Dental Ventures of America, Corona, CA, USA). The specimen was subjected to load until failure using crosshead speed at 0.5 mm/min. The µTBS values were determined in megapascal (MPa) calculated from peak load at failure (N) divided by the bonded surface area (mm^2).

Failure mode analysis

The fractured specimens were mounted on an aluminum stub and subsequently coated with palladium for 150 seconds. The failure modes were examined using a stereomicroscope (SMZ-2T, Nikon, Tokyo, Japan) at an accelerating voltage of 15 kV and magnifications of 40x to identify the failure patterns which were classified into three types: (Kutuk et al., 2021).

(1) adhesive failure

(with over 80% of the failure occurring within the adhesive or at the tooth-adhesive interface)

(2) cohesive failure within substrates
(with over 80% of the failure in dentin or resin composite)
(3) mixed failure
(involving a combination of adhesive and cohesive failure)

Statistical Analysis

Microtensile bond strength (µTBS)

Statistical analysis for µTBS data was conducted by statistical analysis software SPSS® Statistic (SPSS Inc., IBM Corporation, NY, USA) version 28.0. Normality was tested by Shapiro-Wilk test and variance homogeneity was measured by Levene's test. Since the data was normality and variance homogeneity, a one-way analysis of variance (ANOVA) was made to compare differences, and multiple comparisons were performed using Welch's test and Games-Howell's post hoc test. The results were reported as a mean ± standard deviation (SD) and the statistical tests were performed with a significant level of 0.05.

4. Results and Discussion

4.1 Results

Microtensile bond strength (µTBS) test

The results of the microtensile bond strength values of all tested groups are presented in Table 2.

Table 2 Mean microtensile bond strength ± standard deviation (SD) in MPa of all tested groups (n=20).

Groups	**µTBS** (MPa)
Control	26.04 ± 4.54^{a}
H_2O_2	22.01 ± 4.14^{b}
PVP-I	21.97 ± 5.12^{b}
CHX	22.87 ± 6.36^{b}

Different superscript letters on the vertical column indicate statistical differences ($p<0.05$).

The data of µTBS indicated that the control (26.04±4.54 Mpa) had the significantly highest µTBS values among the other groups ($p<0.05$). The hydrogen peroxide (22.01±4.14 Mpa), povidone-iodine (21.97±5.12 Mpa), and chlorhexidine (22.87±6.36 Mpa) were significantly lower than the control ($p<0.05$) but had no significant differences between groups ($p>0.05$).

The povidone-iodine had the significantly lowest µTBS values compared with the control ($p<0.05$) but no significant differences compared with any other mouthwashes used ($p>0.05$).

Failure mode analysis

The distribution of the failure mode of all tested groups is presented in Table 3.

Table 3 Distribution of the failure mode (Ad/Co/Mix) percentage (%) of all tested groups.

Groups	**The failure mode (%)**		
	Ad	**Co**	**Mix**
Control	95	5	0
H_2O_2	90	5	5
PVP-I	90	10	0
CHX	90	10	0

Abbreviation: Ad: adhesive failure, Co: cohesive failure within substrates (dentin or resin composite), Mix: mixed failure.

From this study, the predominant failure mode observed was the adhesive failure for every mouthwash group. The second observed failure was the cohesive failure within substrates of both dentin and resin composite. The failure mode that was hardly ever detected in this study was the mixed failures that researchers only detected in the hydrogen peroxide group.

4.2 Discussion

The antiseptic mouthwashes used in this study were derived from dental practitioners providing patients with preprocedural mouth rinsing before dental treatments. According to the recommendation, the type and concentration of antiseptic mouthwashes that the researcher selected to use in this study were 1% hydrogen peroxide, 0.2% povidone-iodine, and 0.12% chlorhexidine. Meanwhile, using mouthwash before any dental procedure may still have a residual liquid or composition covering the enamel and/or the exposed dentin if there is inadequate cleaning. This situation may also influence the properties and qualities of restorative materials used. For example, in a clinical case of a non-carious cervical lesion that was a wedge-shaped lesion or even a margin of the cavity was in the subgingival area. The mouthwash that is rinsed into the oral cavity may still have a residual liquid or composition covering the enamel and/or the exposed dentin and may affect surface properties, especially regarding the bond strength of the dental material.

Moreover, the researcher also considered the situation that the mouthwash can be permeable and percolate into the exposed dentin, leading to penetration into the deepened depth of the cavity. Furthermore, water coolant and/or cavity preparation may still not be enough to clean the residual or remaining mouthwash. Consequently, these might affect the bonding performance of adhesive and restorative materials used.

The adhesive or bonding system that the researcher used in this study was a mild self-etching system due to its long history and plenty of research results that proved their long-term clinical outcome in non-carious cervical lesions. Adhesives that do not use a separate etching step are known as self-etch adhesives system. This is because they contain acidic monomers that simultaneously condition and prime the dental substrate. Accordingly, this approach has been claimed for its ease of use, shorter application time, and fewer procedure steps, as well as being less technique-sensitive, omitting wet bonding and simplifying the drying process, resulting in a reliable clinical performance, even though outcomes can vary depending on the specific product.

Self-etch adhesives are available in both two-step and one-step formulations, depending on whether a self-etching primer and mostly solvent-free adhesive resin are provided separately or combined into a single solution. One-step adhesives offer a simpler application process compared to two-step self-etch adhesives. However, they involve intricate mixes of hydrophilic and hydrophobic components. These complex mixtures are currently regarded as compromise materials with documented complexities. Typically, they reduced the immediate bond strength in comparison with multi-step adhesives. Furthermore, any kind of aging reveals lower long-term bonding effectiveness and an increased occurrence of interfacial nano-leakage (Van Landuyt et al., 2009).

In addition, the efficacy of two-step self-etch adhesives has resulted in long-term clinical outcomes, particularly in managing non-carious cervical lesions. This adhesive partially decalcifies dentin to a depth of less than 1 µm, leaving hydroxyapatite crystals and smear layer remnants within the resulting sub-micron hybrid layer. This is also considered as the gold standard in dentin bonding systems (Van Meerbeek et al., 2011; Peumans et al., 2015). Therefore, the researcher decided to use the two-step self-etch adhesive system in this laboratory experiment. The restoration used in this research was a nano-filled resin composite filling material which has been commonly and widely used in clinical restorative dentistry nowadays.

According to the results of this study, the types of antiseptic mouthwash were affecting the microtensile bond strengths (µTBS). This study indicated that all mouthwash groups, which are hydrogen peroxide, povidone-iodine, and chlorhexidine, affect the microtensile bond strength which resulted in a significant difference in lower µTBS compared with the control group, but had no significant differences among any antiseptic mouthwash groups. The results of this study are quite similar to the previous studies (Shirani et al, 2022; Kutuk et al, 2021). However, the control group presented no significant differences from other groups in their studies, which differed from this study. They used distilled water as a control while this project used artificial saliva as a control group.

Therefore, the experimental null hypothesis that there were no statistically significant differences between the microtensile bond strength (µTBS) of a self-etch adhesive system to dentin after contamination of different types of antiseptic mouthwash was rejected.

This present study showed that there was a significant difference between the antiseptic mouthwash groups which are 1% hydrogen peroxide, 0.2% povidone-iodine, and 0.12% chlorhexidine compared with the control group resulting in significantly lower microtensile bond strength values than the control group ($p<0.05$).

From the results of 1% hydrogen peroxide, the microtensile bond strength (µTBS) had significantly lower µTBS compared with the control ($p>0.05$). For hydrogen peroxide, the previous study by Kutuk et al. (2021) conducted with 1.5% hydrogen peroxide with universal adhesive in self-etch and etch and rinse mode indicated that 1.5% hydrogen peroxide had no impact on the dentin shear bond strength. The concentration of the hydrogen peroxide mouthwash that is mostly used is 1-1.5% in concentration. From the past literature review, a lack of an adverse soft tissue effect has resulted when utilizing a daily rinse of 1%–1.5% hydrogen peroxide during a two-year follow-up (Gusberti et al.,1988). More concentrations of hydrogen peroxide used in the oral cavity such as 3% hydrogen peroxide mostly used in terms of cavity disinfectant, not as a mouthwash.

According to this research finding, the application of 1% hydrogen peroxide may have had an impact on the bond strength in comparison to the control group. However, the cause for the lower bond strength can be ascribed to the presence of oxygen ions. It was determined that the existence of residual oxygen jeopardized the polymerization of resin (Özduman et al., 2021). Accordingly, it could be suggested that these might be tested in any other laboratory instruments such as surface properties and penetration for furthermore investigation.

For 0.2% povidone-iodine, the results of microtensile bond strength were significantly lower than control ($p<0.05$) and were the lowest microtensile bond strength values among any other groups. Based on our results, this may be related to the study by Limsirivong et al. (2023) that was performed to assess the effects of mouthwashes on the shear bond strength of resin-matrix ceramic (RMCs) materials repaired with resin composites. The results found that the aged RMCs that were immersed in 0.2% povidone-iodine for 30 seconds had the lowest repaired bond strength.

This is also probably related to the recent study from Tanthanuch et al. (2023) that focused on the effect of various types of mouthwash on the surface characteristics of bulk-fill and conventional resin composites. Following immersion of the mouthwashes, all groups adversely affected the restoration properties, resulting in significantly reduced surface hardness and increased roughness and color values across all tested resin composites. Notably, the most substantial degradation occurred with the immersion in 0.2% povidone-iodine. The study observed that the mean pH of 0.2% povidone-iodine was the lowest, and in terms of titratable acidity, this solution exhibited the highest volume of sodium hydroxide. To clarify, titratable acidity is assessed through titration with standard alkaline solutions or sodium hydroxide, with greater use of sodium hydroxide solution indicating higher acidity levels.

Regarding 0.12% chlorhexidine, prior studies examined the impact of chlorhexidine on the microtensile bond strength to dentin, considering various concentrations and application methods. They found that 0.12% and 2% chlorhexidine pretreatment did not affect the microtensile bond strength of specimens tested. (Chang, & Shin, 2010; Dalkilic et al., 2011). The results from this study showed that 0.12% chlorhexidine has a statistically significant difference lower in microtensile bond strength value compared with the control and may affect the bond strength of the adhesive and restoration.

However, in previous systematic review from Coelho et al. (2020) reported the influence of chlorhexidine as a cavity disinfectant which was mostly found at a 2% concentration enabling maintenance, greater bond strength values, and also found in a lower bond strength value when using chlorhexidine.

For the failure mode analysis, the predominant failure found in this study was the adhesive failure for every experimental group. This result might be due to the self-etch adhesive system used in this study and the procedure of microtensile testing. For the reason of self-etch adhesive system, this result findings are probably related to the difficulty of the bonding agent to infiltrate into the exposed collagen dentin completely and decreased mechanical properties of the dentin. The self-etch adhesive system depends on acidic functional monomers presented in both two-step and one-step application systems. Consequently, both systems can mildly etch the tooth structure and incorporate the smear layer within the adhesive layer form to create a hybridized smear layer. This hybridized latter network is regarded as a weak link in the adhesive interface which potentially contributes to the adhesive failure (Kusumasari et al., 2021).

Additionally, the procedure of microtensile testing exhibits better force distribution between interfaces. The specimen preparation for the bonded interface of small (1 mm^2) specimens for the microtensile test has a better stress distribution during loading. These result in fewer cohesive failures in dentin compared with other conventional bond strength testing. This is considered an attribute to a reduction in flaw density.

Moreover, the application of this method often yields higher apparent bond strengths at failure than those observed with larger specimens (Van Meerbeek et al., 2010).

Within the limitations of this laboratory-based investigation, the results are from a simulated condition of the preprocedural use of mouthwash prevention before dental procedures. However, in a real-world clinical scenario, restorative materials are exposed to the oral environment, subject to temperature and pH changes that may influence their properties. Consequently, further studies might be conducted in the areas that are close to the actual oral condition, employing diverse antiseptic mouthwash types and concentrations, various adhesive systems and restorative materials, varying application durations, and delving into surface properties and qualifications. These efforts aim to provide a more comprehensive understanding of the impact of antiseptic mouthwashes on dentin bond strength.

5. Conclusion

This study indicated that all mouthwash groups which are hydrogen peroxide, povidone-iodine, and chlorhexidine, affect the microtensile bond strength (μTBS) to dentin which results in significantly lower microtensile bond strength values than the control. Therefore, the rinsing process should be meticulously done after preoperative antiseptic mouthwash is used regarding the exposed dentin cavity before any restorative procedures.

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