Antibacterial capacity of cavity disinfectants against *Streptococcus mutans* and their effects on shear bond strength of a self-etch adhesive

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We evaluated the antibacterial properties of three disinfectants [2% chlorhexidine (CHX), 6% sodium hypochlorite (NaOCl), and 0.01% urushiol] against *Streptococcus mutans* and their effects on bond strength of Scotchbond™ Universal. The reduction in bacterial growth was evaluated by the colony counting method. Total 105 specimens were assigned to seven groups, according to surface pretreatment: control group (C) without pretreatment; chlorhexidine gluconate with rinse (CR) or without rinse (CD); NaOCl with rinse (NR) or without rinse (ND); and urushiol with rinse (UR) or without rinse (UD). The shear bond test was performed at a cross-head speed of 0.5 mm/min. None of the disinfected specimens had viable microbes after a 30 min incubation. The control group exhibited the strongest bond; however, no significant difference was detected with the disinfectant-treated groups, except weak bonding with ND group. These findings suggest that all disinfectants tested had strong antibacterial capacity and may better be rinsed away.

Keywords: Antibacterial capacity, Shear bond strength, Chlorhexidine, Sodium hypochlorite, Urushiol

INTRODUCTION

Demineralization of hard tooth tissues is caused by bacterial biofilms that produce acid as a result of carbohydrate fermentation¹. Heterogeneous oral biofilms consist of various types of bacteria embedded in an extracellular matrix. Cariogenic bacteria, such as *Streptococcus mutans* and *Lactobacilli* in the biofilm, are major pathogens of caries that metabolize carbohydrates to acids².

Advanced dental caries that cannot be remineralized should be eradicated by surgical intervention with a bur or other instrument. Under these circumstances, smear layers composed of residual organic and inorganic components are always produced along tooth cavity walls³ and they are usually contaminated with saliva, blood, or microorganisms. Residual bacteria may proliferate in the smear layer beneath a restoration⁴. Therefore, pretreating cavity surfaces with an antibacterial agent is invaluable for eliminating the harmful effects of residual bacteria or their toxins⁵. Branstrom reported that the smear layer should be removed to eliminate microbes⁶.

Chlorhexidine (CHX) has frequently been applied to tooth cavities^{7,8} prior to placing a restoration because of its broad spectrum antibacterial activity and substantivity (48 h⁹, 4 weeks¹⁰, and 12 weeks¹¹). CHX-pretreated dentin has higher shear bond strength after removing the smear layer and smear plugs¹². CHX also minimizes convective and evaporative water fluxes from underlying dentin; thus, enhancing bond strength of self-etch adhesives¹³. Furthermore, CHX acts as matrix metalloproteinase (MMP) inhibitor; thus, it preserves dentin bond strength.

Sodium hypochlorite (NaOCl) is often used as

a chemomechanical agent to remove caries and is used during dentin bonding procedures because of its antibacterial and tissue dissolving properties¹⁴. It is presumed that NaOCl reduces compactness of the smear layer because it eliminates the collagen phase, which increases the bond strength of the self-etching adhesive, as it may enhance diffusivity of the acidic monomers, through water-filled channels between smear layer particles, which increase their size to reach and interact with the underlying dentin surface¹³. However, there are controversies about the use of these antibacterial agents as cavity disinfectants because of several positive and negative reports about their effects on adhesive bond strength^{12,15,16}.

Urushiol is a cavity disinfectant candidate and a major component of the lacquer tree, which has been used as a folk remedy to relieve abdominal discomfort in the form of boiled chicken soup in Korea. Urushiol has both antibacterial and antioxidative activities^{17,18}. Urushiol consists of a catechol substituted with a 15 or 17 carbon alkyl chain (Fig. 1). The antibacterial characteristics of urushiol are thought to be dependent on the degree of unsaturation of the alkyl chain¹⁷. However, no study has investigated using urushiol as a cavity disinfectant, despite its well-known antibacterial effect.

Therefore, the antibacterial properties of these three agents against *S. mutans* and their effects on self-etching adhesive bond strength to dentin were examined in this study. The null hypotheses tested were (1) no difference in the antibacterial capacity against *S. mutans* among the disinfectants and (2) no reduction in dentin bond strength when the cavity was pretreated with the disinfectants whether they were rinsed off or not.

Fig. 1 Structure of typical urushiol.

MATERIALS AND METHODS

Bacterial strains and culture conditions

Streptococcus mutans (ATCC 25175) was used to measure the antibacterial activities of 2% solution of chlorhexidine digluconate (CAVITY CLEANSER™, Bisco, Schaumburg, IL, USA), 6% NaOCl (RC CLEANER, Ilchung Dental, Seoul, South Korea), and 0.01% urushiol (lacquer tree extract from Japan). The bacterial strain was cultured in brain-heart infusion (BHI) broth alone or in BHI broth containing 1.5% (w/v) agar at 37°C.

Antibacterial activity assay

Streptococcus mutans was grown in BHI broth alone or in BHI broth containing 2% CHX, 6% NaOCl, or 0.01% urushiol at 37°C. The reduction in bacterial growth was evaluated using the colony counting method. Briefly, a single colony of bacteria was inoculated in 5 mL BHI medium and incubated for 24 h at 37°C with shaking at 180 rpm. The bacterial culture was diluted 1:100 in fresh medium and incubated at 37°C until the late-log phase. Approximately 6×10^7 bacterial cells were inoculated into 1 mL BHI medium containing each of the disinfectants. At 0, 30, 60, and 90 min after inoculating the bacteria, the number of viable bacterial cells in the supernatant was determined by counting colony forming units (CFU) on the BHI agar plates. This analysis was repeated at least three times.

Shear bond strength analysis

A total of 105 extracted human molars were used in this study. This study was approved by Dankook University, College of Dentistry, South Korea (H-1503/003/006), and all patients provided prior consent. The extracted teeth were stored in thymol solution at 4°C and used within 2 weeks. The teeth, including the roots, were embedded in an octagonal pillar-shaped mold filled with self-cure acrylic resin (Ortho-Jet, Lang Dental Mfg, Wheeling, IL, USA) after scaling and cleansing. After the acrylic resin polymerized, the acrylic blocks were removed from the molds, and the occlusal surface was ground perpendicular to the long axis of the teeth with a low-speed diamond disk saw in order to expose a mid-coronal dentin. The dentin surface was polished

with #600-grit silicon carbide paper for 60 s to unify the smear layer.

The specimens were assigned randomly to one of seven main groups ($n=15$), according to the proposed surface pretreatment: control group without pretreatment (C); 2% CHX pretreatment without rinse (CD); 6% NaOCl pretreatment without rinse (ND); 0.01% urushiol pretreatment without rinse (UD); 2% CHX pretreatment with rinse (CR); 6% NaOCl pretreatment with rinse (NR); and 0.01% urushiol pretreatment with rinse (UR).

The disinfectant was applied using a disposable brush tip, and left undisturbed for 20 s. Then, the teeth in the CR, NR, and UR groups were rinsed with water for 10 s, and dried gently with oil-free air for another 10 s. Teeth in the CD, ND, and UD groups were dried without rinse. Scotchbond™ Universal adhesive (3M ESPE, St. Paul, MN, USA) was applied and brushed on for 20 s using a fully saturated brush tip and then lightly air-dried for 5 s. The surface was light-cured for 20 s with a LED curing unit (Elipar Freelight 2, 3M ESPE) with a light output of 750 mW/cm².

Composite resin (Filtek Z350^{XT}, 3M ESPE) was applied carefully to the treated dentin surface by placing the material into a cylindrically shaped split Teflon mold with an internal diameter of 4 mm and height of 3 mm. The mold was filled incrementally in two 1.5 mm layers, and each layer was light cured for 20 s with the same curing unit. The shear test was performed with a compressive load applied at the resin-tooth interface using a mono-beveled chisel-shaped metallic rod at a cross-head speed of 0.5 mm/min.

Field emission scanning electron microscopic (FE-SEM) analysis

The mid-coronal occlusal dentin of seven molars was used as specimen to examine the effect of the disinfectants on shear bond strength. The dentin surfaces were similarly treated as the shear bond strength specimens. They were mounted on aluminum stubs, sputter-coated with gold/palladium, and examined using a FE-SEM (S-3000H, Hitachi, Tokyo, Japan) operating at 5 kV.

Statistical analysis

Two-way analysis of variance (ANOVA) was used to examine the effects of the disinfectants and their mode of application on dentin bond strength. The independent *t*-test was performed to detect significant differences between subgroups (mode of application; rinsing off or drying only after applying the disinfectants). One-way ANOVA and Tukey's HSD *post-hoc* tests were used to examine differences in bond strength and antibacterial capacity among the groups. A $p < 0.05$ was considered significant. All analyses were carried out with SPSS 19.0 software (SPSS, Chicago, IL, USA).

RESULTS

No viable microbes were detected after a 30 min incubation with three of the disinfectants, indicating

that they had a similar potent antibacterial effect against *S. mutans* when they were used at standard concentrations (2% CHX, 6% NaOCl, and 0.01%

Fig. 2 The numbers of CFUs according to incubation time.
ND: not detected, CFU: colony forming unit

urushiol) (Fig. 2).

Mean and standard deviation bond strength values of all groups are shown in Table 1. A two-way ANOVA indicated no differences for the factor “disinfectants” ($p=0.136$) or for the interaction between factors ($p=0.712$). However, the factor “mode of application” was significantly different among the groups ($p=0.033$) (Table 2). The control group exhibited the highest bond strength; however, no significant difference was detected with the disinfectant-treated groups, except the ND group. Among the disinfectant-treated groups, the CR group had the highest bond strength value and the ND group exhibited the lowest value. Groups in which the disinfectants were rinsed off tended to show better shear bond strength than those in which the disinfectants were not rinsed, especially the NaOCl treatment. The ND group showed significantly lower strength than that of the NR group ($p<0.05$).

The FE-SEM examination showed that the smear layer and smear plugs covered the dentin surface in the control group (Fig. 3A). However, the three disinfectants removed overlying contaminants at different degrees when they were rinsed off (Figs. 3B–D). On the other hand, some residual contaminants covered the dentin surfaces when the disinfectants remained without rinsing (Figs. 3E–G).

Table 1 Shear bond strength values (MPa)

Group	<i>n</i>	Mean (S. D.)
Control	15	16.74 (5.14) ^a
CR	15	15.07 (4.86) ^{a,b}
NR	15	13.45 (3.50) ^{a,b}
UR	15	14.84 (4.12) ^{a,b}
CD	15	14.10 (5.56) ^{a,b}
ND	15	10.96 (4.57) ^b
UD	15	12.06 (4.47) ^{a,b}

Same superscripts indicate mean values with no statistically significant difference ($p>0.05$).

Table 2 Two-way analysis of variance of shear bond strength

Source	Type III Sum of Squares	df	Mean Square	<i>F</i>	Sig.
Corrected model	196.38 ^a	5	39.28	1.89	0.104
Intercept	16,192.51	1	16,192.51	779.80	0.000
Disinfectants (A)	84.85	2	42.42	2.04	0.136
Mode of application (B)	97.37	1	97.37	4.69	0.033
A * B	14.16	2	7.08	0.34	0.712
Error	1,744.25	84	20.77	—	—
Total	18,133.14	90	—	—	—
Corrected Total	1,940.63	89	—	—	—

a. R Squared=0.101 (Adjusted R Squared=0.048)

Fig. 3 FE-SEM images ($\times 500$): (A) Control group, (B) CR group, (C) NR group, (D) UR group, (E) CD group, (F) ND group, (G) UD group.

DISCUSSION

Pulp damage after restoration is thought to be caused by bacteria passing between the cavity walls and the filling material or through microleakage¹⁹. A dentin adhesive with long-lasting antibacterial activity against diverse microbes should prevent bacteria from passing toward the pulp. Moreover, if the microbes remaining after preparing the tooth could be removed by disinfecting the cavity before restoration, it would help reduce damage by obtaining relatively clean cavity walls.

Streptococcus mutans was chosen as the target

microorganism because it is a well known major pathogen of caries²⁰. These bacteria are small enough to move rapidly and penetrate easily into the dentinal tubules²¹, which may lead to pulp damage. The antibacterial capacity of three disinfectants against *S. mutans* was examined in this study by counting colony forming units. No *S. mutans* was detected after the 30 min incubation with the three disinfectants (2% CHX, 6% NaOCl, and 0.01% urushiol). Therefore, they all seemed to have very potent antibacterial characteristics. Based on the result that urushiol was bactericidal at a very low concentration (0.01 wt%), it may be useful to apply this solution to the cavity walls as a cavity

disinfectant. Thus, our first hypothesis was accepted.

Each disinfectant has its own antibacterial mechanism. CHX is a positively charged hydrophobic and lipophilic molecule that interacts with phospholipids and lipopolysaccharides in the bacterial cell membrane and enters the cell²². CHX gluconate is a stable water-soluble salt that readily dissociates and releases a positively charged CHX component²³. CHX (2%) is bactericidal by precipitating cytoplasmic contents, leading to cell death²⁴. NaOCl alters cellular metabolism and destroys phospholipids. It also promotes formation of chloramines, which have an oxidative action that irreversibly inactivates bacterial enzymes²⁵. Urushiol disrupts the bacterial cell membrane²⁶. It promotes bleb formation and lysis of *Helicobacter pylori*. This action is so quick that most *H. pylori* lysis occurs within 10 min. One study reported that urushiol can be used safely as an antimicrobial agent without systemic complications²⁷.

Although these disinfectants have potent antibacterial capacity, it is useless if they negatively impact the bond strength of a restoration. The mode of applying the disinfectant to the cavity may also affect bond strength, such as rinsing off or drying only without rinsing. The influence of these two factors on shear bond strength of a self-etching adhesive was examined in this study.

The control group exhibited the strongest bond strength, and the groups pretreated with a disinfectant were not different, except weak bonding with ND group. No significant differences in bond strength were detected among the groups treated with the three disinfectants.

Our CHX results were in agreement with those of other studies, reporting that CHX does not affect shear bond strength to dentin^{28,29}. However, one study showed that varied concentrations of chlorine ions and crystal-shaped precipitates were formed on the CHX treated dentin surface, this resulted in significantly lower bond strength¹⁵. It has also been reported that CHX reduces bond strength due to its binding to loose apatite remnants within the smear layer, resulting in more pronounced nanoleakage of a self-etching adhesive to dentin³⁰. On the other hand, other studies have reported that CHX-treated dentin results in higher shear bond strength than that of an untreated control group. The authors attributed the improved bond strength to removal of the smear layer and smear plugs, which prevented direct contact of the self-etching adhesive with dentin; consequently, removing the smear layer facilitates formation of a stronger and more homogeneous hybrid layer^{31,32}.

Applying NaOCl in this study also resulted in no difference in bond strength with the control group after it was rinsed off, which agrees with a previous study reporting that NaOCl does not influence bond strength to dentin³³. However, we observed significantly lower shear bond strength when NaOCl remained without rinsing. Previous studies have reported the same results like this study, that is, better or worse bond strength using NaOCl. Significantly increased bond strength was observed and was attributed to eliminating the collagen

layer, leading to better penetration of the adhesive into intertubular dentin and possible removal of the smear layer^{34,35}. On the other hand, significantly decreased dentin bond strength was reported and was attributed to organic monomers in the adhesive system that could not sufficiently penetrate into the demineralized dentin because of damage to the organic component of dentin³⁶.

Urushiol, like CHX, recorded similar shear bond strength to that of the control group regardless of rinsing. Urushiol is a very good candidate cavity cleanser because of its excellent antibacterial capacity and little change in dentin bond strength at a very low concentration.

A two-way ANOVA revealed that the factor “mode of application” resulted in a significant difference in shear bond strength. Although application mode did not influence bond strength when CHX and urushiol were tested, significantly lower bond strength was detected in the ND group compared to that in the NR group. Therefore, the second hypothesis was partly rejected. We conclude that rinsing the disinfectant away is the best method to clean the cavity.

The FE-SEM examination of the dentin surfaces revealed that the smear layer and smear plugs remained in the control group (Fig. 3A). Smear layers were removed at different degrees in the groups treated with the disinfectants when they were rinsed off (Figs. 3B–D). However, some residual contaminants covered the dentin surfaces when the disinfectants remained without rinsing (Figs. 3E–G). These residual contaminants may hinder direct contact with resin components and tooth substances. These kinds of dentin surface changes have been reported previously³⁷. Although we have provided clear evidence that the dentin surfaces were altered similarly with those of previous studies, the bond strength results varied. These bond strength discrepancies among studies may be attributed to differences in sample preparation, application mode, or time. Thus, the results of these studies should be analyzed with caution.

One possible shortcoming of the study is that *Streptococcus mutans* was sole target microorganism used in this study because of its well-known pathogenicity in dental caries. However, dental plaque is a complicated ecosystem of approximately 1,000 bacterial species³⁸. Therefore, use of a microcosm model in future studies would be preferable³⁹. There are many factors affecting dentin bond strength. Therefore, further studies must consider these factors.

CONCLUSION

Under the limitations of this study, we conclude that 0.01% urushiol had strong antibacterial capacity against *S. mutans* as well-known cavity disinfectants tested (2% CHX and 6% NaOCl) and that the disinfectants may better be rinsed away when they are used to clean the cavity walls to achieve optimum bonding performance.

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