Effect of cavity disinfectants on antibacterial activity and microtensile bond strength in class I cavity

Bo-Ram KIM¹, Man-Hwan OH² and Dong-Hoon SHIN¹

¹ Department of Conservative Dentistry, Graduate school, DankKook University, Dandaero #119, Anseo-dong, Dongnam-gu, Cheonan, Chungnam, 330-714, Korea
² Department of Nanobiomedical Science, Graduate school, DankKook University, Dandaero #119, Anseo-dong, Dongnam-gu, Cheonan, Chungnam, 330-714, Korea

Corresponding author, Dong-Hoon SHIN; E-mail: donyshin@dankook.ac.kr

This study was performed to compare the antibacterial activities of three cavity disinfectants [chlorhexidine (CHX), NaOCl, urushiol] and to evaluate their effect on the microtensile bond strength of Scotchbond Universal Adhesive (3M-ESPE, St. Paul, MN, USA) in class I cavities. In both experiments, class I cavities were prepared in dentin. After inoculation with Streptococcus mutans, the cavities of control group were rinsed and those of CHX, NaOCl and urushiol groups were treated with each disinfectant. Standardized amounts of dentin chips were collected and number of S. mutans was determined. Following the same cavity treatment, same adhesive was applied in etch-and-rinse mode. Then, microtensile bond strength was evaluated. The number of S. mutans was significantly reduced in the cavities treated with CHX, NaOCl, and urushiol compared with control group (p<0.05). However, there was a significant bond strength reduction in NaOCl group, which showed statistical difference compared to the other groups (p<0.05).

Keywords: Antibacterial activity, Microtensile bond strength, Chlorhexidine, Sodium hypochlorite, Uruishiol

INTRODUCTION

Restoration of hard tissue assumes that all infected dentin are removed with cavity preparation. However, it has been reported that it is impossible to remove all micro-organisms and that some bacteria persist even after all soft dentin is excavated. Bacterial remnants during or after cavity preparation are regarded as a major problem that can cause not only secondary caries, but also increased pulp sensitivity and pulpal inflammation.

There was a study that suggested the use of cavity disinfectants such as chlorhexidine gluconate-based solutions (CHX), sodium hypochlorite (NaOCl), hydrogen peroxide (H₂O₂), disodium ethylenediaminetetraacetic acid (EDTA), and iodine in order to remove as much bacteria as possible. However, some authors are concerned about the application of cavity disinfectants, because they may affect the sealing ability of adhesive bonding resins. The interaction between adhesive systems and cavity disinfectants is a controversial topic in restorative dentistry.

CHX is the most popular antimicrobial solution and matrix metalloproteinase (MMP) inhibitor. Many studies on CHX showed better antimicrobial activities compared to other disinfectants. Chlorhexidine gluconate in CHX binds to the amino acids in dentin and its bactericidal action lasts several hours. Multiple studies have reported that CHX significantly decreases the number of S. mutans. A 2% solution of CHX is bactericidal by precipitating cytoplasmic contents, and leading to cell death.

NaOCl is also widely used in dental procedures due to its proteolytic and disinfectant properties. NaOCl alters cellular metabolism and destroys phospholipids. It also promotes the formation of chloramines, inactivating bacterial enzymes irreversibly.

Scientific studies have been performed on the antibacterial and antioxidative efficacy of urushiol extracted from the sap of the lacquer tree, which is used as a folk remedy to relieve abdominal discomfort in the form of boiled chicken soup in Korea containing lacquer tree bark. Although it is reported that urushiol can cause allergic reactions, some studies have demonstrated that it has antimicrobial and antioxidative activities.

Jeong et al. reported that powder-type urushiol exhibits not only significant antimicrobial activity against Gram-positive and Gram-negative microorganisms but also has excellent antioxidant activity. Uruishiol consists of a catechol with an n-C15 or n-C17 alkyl side chain and the unsaturation of the alkyl chain is thought to impact its antibacterial activity. Uruishiol is also known to disrupt the bacterial cell membrane.

There was a study on the antibacterial characteristics of three cavity disinfectants (CHX, NaOCl, urushiol) and their effects on the shear bond strength of a self-etch adhesive. However, the antibacterial test was conducted in a Streptococcus mutans (S. mutans) inoculated brain-heart infusion (BHI) medium containing each disinfectant. Therefore, S. mutans in the planktonic state were the only target microorganisms. Furthermore, polymerization shrinkage stress from the restricted cavity could not be considered when estimating bond strength. Therefore, another...
study using a tooth cavity model would overcome these problems. The aim of this study was to compare the antibacterial activities of CHX, NaOCl, and urushiol disinfectants and to evaluate their dentinal microtensile bond strength in class I cavities. The null hypotheses tested were (1) having no difference in the antibacterial activity against *S. mutans* among the disinfectants and (2) observing no reduction in dentin bond strength when the cavity was treated with three disinfectants.

**MATERIALS AND METHODS**

A total of 56 human molars without caries and crack, stored in 0.1% thymol, were employed in this study. Patient consent was obtained before extraction, with the approval of Dankook University, College of Dentistry, South Korea (H-1506/006/005). After all teeth were randomly assigned, 36 teeth were used in the antibacterial activity test and 20 teeth were used in the microtensile bond strength (µTBS) test (Fig. 1).

**Antibacterial activity test**

Thirty-six extracted human non-carious molars were used. After scaling and cleansing, the whole enamel of the occlusal plane and roots were removed with a model trimmer under running water. Only one class I cavity (4×4×2 mm) was prepared in each tooth with a No. 202 diamond bur in a high-speed handpiece under copious air-water spray. All teeth were sterilized in an autoclave for 15 min at 121°C. *S. mutans* (ATCC 25175) was used to measure the antibacterial activities of the three cavity disinfectants [2% CHX (Cavity cleanser, Bisco, Schaumburg, IL, USA), 6% NaOCl (RC CLEANER, Ilchungdental, Seoul, Korea), and urushiol (extract from Japanese lacquer tree)]. The mixture (1 gm) of urushiol was diluted with 300 mL methanol (Duksan Chemical, Seoul, South Korea) and washed with 300 mL toluene (Duksan Chemical) three times. The organic layer was evaporated in vacuo to give the crude product, which was purified by column chromatography (GC-Mass, JEOL, JMS-600W, Tokyo, Japan) using ethyl acetate.

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. The reduction in the bacterial growth was evaluated using the colony counting method. The bacteria were 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. The teeth with prepared cavities were put in the 24-well plates filled with BHI broth (2 mL). Aliquots (1 mL, approximately 6×10⁷ bacterial cells) of the bacterial suspension were then transferred to 24-well plates and incubated at 37°C for 72 h. After incubation, the teeth were transferred to 24-well plates and incubated at 37°C for 72 h.

![Fig. 1 Schematic depiction of preparation for antibacterial activity and microtensile bond strength tests.](image)

(a) class I cavity formation (4×4×2 mm); (b) inoculation of *S. mutans*; (c) cavity pretreatment with disinfectant; (d) dentin chip collection with No. 8 round bur; (e) bulk filled composite build; (f) resin slabs for µTBS.

### Table 1 Groups classified according to the cavity treatment of disinfectants

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment procedure</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Rinsing without treatment</td>
<td>—</td>
</tr>
<tr>
<td>CHX</td>
<td>2% chlorhexidine gluconate treatment with rinsing</td>
<td>Cavity Cleanser, Bisco, Schaumburg, IL, USA</td>
</tr>
<tr>
<td>NaOCl</td>
<td>6% NaOCl treatment with rinsing</td>
<td>RC CLEANER, Ilchungdental, Seoul, Korea</td>
</tr>
<tr>
<td>Urushiol</td>
<td>0.01% urushiol treatment with rinsing</td>
<td>extract from Japanese lacquer tree</td>
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</table>
were taken out and dried with sterilized cotton pellets and a gentle stream of oil-free air. The teeth were randomly divided into 4 groups of 9 teeth each according to the dentin surface treatment (Table 1).

The cavities of the control group were rinsed with distilled water for 10 s and then dried with oil-free air without any disinfecting treatment. The cavities of CHX, NaOCl and urushiol groups were each treated with a given disinfectant by a disposable microbrush tip, and left undisturbed for 20 s. Then the teeth were rinsed with distilled water for 10 s and dried with oil-free air. After the treatment, all cavities were filled with sterile cotton and sealed with resin-based temporary filling material (Quicks, Denkist, Seoul, Korea), followed by light-curing for 10 s. The teeth were stored separately in sterile physiological saline (SPS) at 37°C for 72 h. The temporary filling materials and sponges were removed with a dental explorer (EXDG 16/23, Hu-Friedy, Chicago, IL, USA). Standardized amounts (50±5 mg) of dentin chips were collected from the circumferential sides of each cavity with different sterile No. 8 round burs mounted on a low-speed contra-angle hand-piece. Effort was made to collect a standard amount of dentin chips from the cavity walls by grinding 1.5 to 2 mm thickness of dentin from every cavity wall including the pulpal wall. For each cavity, a new sterile round bur kept in a freezer was used to prevent over-heating during cutting. The dentin chips were resuspended in BHI broth (1 mL), and the number of viable bacterial cells in the supernatant was determined by counting the colony forming units (CFU) on BHI agar plates. This analysis was repeated three times.

Microtensile bond strength test
The roots of the teeth were embedded in a self-cured acrylic resin (Ortho Jet Acrylic, Lang Dental, Wheeling, IL, USA) in a square shaped plastic mold. The teeth were removed from the mold after polymerization of the self-cured acrylic resin. The enamel of all the teeth was removed and class I cavities were prepared in dentin as described above for the specimens used in the antibacterial activity test. Each cavity was treated according to the corresponding group (Table 1).

The teeth of the control group were rinsed with distilled water for 10 s and dried with oil-free air without disinfection treatment. The teeth of the experimental groups were treated with the disinfectants with a disposable microbrush tip, and left undisturbed for 20 s. The teeth were then rinsed with water for 10 s and dried with oil-free air. Following the treatment, the cavities were etched for 15 s with 37% phosphoric acid gel (DenFil™ Etchant-37, Vericom, Anyang, Korea), rinsed thoroughly with water spray for 10 s and gently dried for 10 s. Scotchbond Universal Adhesive (3M-ESPE) was used as a dentin adhesive. It was applied for 20 s with gentle agitation using a fully saturated microbrush tip and gently air dried for 5 s to evaporate the solvent. Then, the cavities were light-cured for 10 s using the Elipar Freelight 2 (3M-ESPE). Composite resin (Filtek Z-350, 3M ESPE) was applied to the cavity using a bulk method and polymerized for 20 s. An additional 2 mm thick composite resin was built up and was also polymerized for 20 s to evaluate the microtensile bond strength.

After 24 h of storage in distilled water, the specimens were serially sectioned perpendicular to the bonded surface to form approximately 1×1 mm² thick resin slabs using Accutom-50 (Struers, Copenhagen, Denmark). Two to three sticks were obtained from each specimen. Therefore, 12 specimens from 5 teeth per group were used in this study. The sticks were glued to the microtensile test machine (Micro Tensile Tester, Bisco) with cyanoacrylate gel (Loctite Super Glue, Henkel, Munich, Germany) and subjected to tensile stress.

Statistical analysis
For the antibacterial activity test, averages and standard deviations of CFU were calculated from at least three independent experiments. One-way ANOVA and Fisher’s LSD post-hoc tests were used to examine the differences in antibacterial activity and microtensile bond strength among the groups. Significance was set at p<0.05. Statistical analysis was carried out with SPSS 22.0 (IBM SPSS Statistics, Amarok, NY, USA).

RESULTS
Antibacterial activity test
Figure 2 shows the mean and standard deviation values in the CFU of S. mutans. A significant decrease in the CFU was found in all disinfectant-treated groups (CHX, NaOCl, and urushiol) in comparison with the control group (p<0.05). However, there was no statistically difference between the CHX, NaOCl, and urushiol groups.

Microtensile bond strength test
The mean and standard deviation of µTBS are presented.
Table 2  Mean and standard deviation of microtensile bond strength (MPa)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of specimen</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>33.12±4.05 a</td>
</tr>
<tr>
<td>CHX</td>
<td>12</td>
<td>30.98±4.53 a</td>
</tr>
<tr>
<td>NaOCl</td>
<td>12</td>
<td>25.86±4.46 b</td>
</tr>
<tr>
<td>Urushiol</td>
<td>12</td>
<td>32.95±6.70 a</td>
</tr>
</tbody>
</table>

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

DISCUSSION

In the present study with tooth cavity model, the number of S. mutans on the cavities treated with CHX, NaOCl, and urushiol significantly decreased in comparison with the control group (p<0.05), and no significant difference was observed among the CHX, urushiol, and control groups (p>0.05).

Because of its excellent antibacterial properties, CHX is widely used. However, many researchers are suspicious of the effect of cavity disinfectants on dentin bond strength. Certain studies reported that CHX had an adverse effect on bond strength. Hassan et al.35 reported that the application of CHX before acid etching the dentin did not adversely affect the µTBS of the composite resin to dentin12-35 and these findings are consistent with the result of this study, showing no harmful effect on the dentin bond strength. Hassan et al.35 reported that the application of CHX before etching did not impact the dentin bonding strength, but using CHX after etching mostly reduced the dentin bonding strength.

In this study, urushiol treatment also showed a similar microtensile bond strength to that of the control group, which is consistent with a previous study22. That study reported urushiol would remove the overlying contaminants (smear layer and smear plugs). This phenomenon may allow intimate contact between dentin and resin-based temporary restorative material or composite resins were used11,12,23. In our pilot study, which tested composite resin and zinc oxide-based material as temporary restoratives, it was revealed that overheat generation during the removal of the former and the lack of hermetic sealing property of the latter rendered them improper as temporary filling materials. Cho and Lee24 also reported that Quicks exhibited better results than zinc oxide-based temporary restorative material with regard to microleakage.

Additional small piece of sterilized cotton was used under a temporary restorative material to prevent contact with the cavity walls. Resin-based temporary restorative material (Quicks, Denkist) was used to seal the cavities even though in other studies, zinc oxide-based temporary restorative material or composite resins were used11,12,23. In our pilot study, which tested composite resin and zinc oxide-based material as temporary restoratives, it was revealed that overheat generation during the removal of the former and the lack of hermetic sealing property of the latter rendered them improper as temporary filling materials. Cho and Lee24 also reported that Quicks exhibited better results than zinc oxide-based temporary restorative material with regard to microleakage.

As mentioned earlier, cavity disinfectants were used to remove the microbes and their toxins remained after tooth preparation, resulting in the reduction of pulpal damage. However, it is useless if disinfectants reduce the bond strength between the restorative material and the tooth structure.

The µTBS of Scotchbond Universal (SBU) without any disinfectant treatment was 33.12±4.05 MPa in this study, which is a relatively low bond strength when compared to other studies on µTBS of SBU in etch-and-rinse mode25-28. It was believed that the cavity configuration factor (C-factor) would have influenced on this low value because this study used a cavity model instead of a flat surface model. A box-like class I cavity used in this study, has the highest C factor, which implies greater stress and a potential risk of interface failure29.

Additionally, a small piece of sterilized cotton was used under a temporary restorative material to prevent contact with the cavity walls. Resin-based temporary restorative material (Quicks, Denkist) was used to seal the cavities even though in other studies, zinc oxide-based temporary restorative material or composite resins were used11,12,23. In our pilot study, which tested composite resin and zinc oxide-based material as temporary restoratives, it was revealed that overheat generation during the removal of the former and the lack of hermetic sealing property of the latter rendered them improper as temporary filling materials. Cho and Lee24 also reported that Quicks exhibited better results than zinc oxide-based temporary restorative material with regard to microleakage.

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surface and adhesives, resulting in little different bond strength with the control group.

Studies using NaOCl disinfectants have shown controversial results. Some reported that the use of NaOCl significantly increased the dentin bond strength\(^{35,38}\). On the other hand, other studies showed that the bond strength of dentin treated with NaOCl significantly decreased\(^{47-39}\), which is consistent with the current study. NaOCl treatment brought about significantly low dentin bond strength in this study. Based on the data in this study, the second hypothesis, that is having no reduction in dentin bond strength when the cavity was treated with three disinfectants was rejected.

These various results may be due to the different dentin adhesive systems used (total-etch vs. self-etch), materials (acetone-base vs. ethanol-base), concentration of NaOCl, and application time for NaOCl used in each study. For example, Hassan et al.\(^ {35}\) reported that NaOCl disinfectant increased dentin bond strength because it could increase the penetration of resin by removing the collagen layer, smear layer, and smear plug. On the other hand, there are two theories for the possible mechanisms of the reduction in bond strength with the use of NaOCl: one is that NaOCl application removes the collagen fibers and consequently prevents healthy hybrid layer creation\(^ {37}\). The other is that reactive residual free-radicals in NaOCl-treated dentin compete with propagating vinyl free-radicals during the light-curing procedure for adhesive systems, which may result in immature and incomplete polymerization\(^ {39}\).

It was proved that urushiol has antibacterial activity\(^ {29}\) and did not influence dentin bond strength when it was used as a cavity disinfectant\(^ {20}\). The results of the present study also suggest that urushiol is a good candidate for cavity disinfection. Therefore, further studies regarding the application of its antibacterial effects to various clinical situations in dentistry might be required.

**CONCLUSION**

Under the limitation of this in vitro study, all three disinfectants (CHX, NaOCl, urushiol) showed excellent antibacterial activities. However, only NaOCl negatively affected the bond strength of Scotchbond Universal in the etch-and-rinse mode. Therefore, it can be concluded that only CHX and urushiol can be recommended as cavity disinfectants because of their outstanding antibacterial effects and no harmful effects on the bond strength.

**ACKNOWLEDGMENTS**

The present research was conducted by the research fund of Dankook University in 2014.

**REFERENCES**