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Original Article

# The Effect of Chlorhexidine Application on the Microtensile Bond Strength and Durability of a Total-Etch Adhesive

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## Abstract

**Aim:** The aim of this study was to evaluate the effect of chlorhexidine (CHX) on resin–dentin microtensile bond strength (μTBS) after 6-month aging and to compare with sodium hypochlorite. **Materials and Methods:** A total of 40 extracted human third molar teeth were mounted in the acrylic resin. Flat occlusal surfaces of dentin were exposed, and after acid etching, the samples were divided randomly into four groups as follows: (a) Control group: Single Bond adhesive resin was applied. (b) The dentin surfaces were exposed to 2% CHX, and then, Single Bond was applied. (c) Dentin surfaces were treated by 5.25% sodium hypochlorite; after rinsing and drying, Single Bond was applied. (d) At first, 5.25% sodium hypochlorite was applied for 30 s, and then, 2% CHX and Single Bond adhesive were applied. Finally, Filtek P60 composite was bonded on the dentin surface. The samples of each group were divided into two subgroups of 24 h and 6 months. μTBS tests were performed using universal testing machine. Afterward, modes of failures were investigated. The statistical analyses were carried out using ANOVA, *t*-test, and Dunnett's test. **Results:** The mean of μTBS for the 24-h and 6-month groups was 15.19 and 10.99 MPa, respectively. Bond strength of all groups except Group D decreased after 6 months, and this bond strength reduction in Group C was more than other groups. Most failure modes were in adhesive type. **Conclusions:** The use of CHX did not have better preservation of μTBS when compared to control group. Use of hypochlorite is not recommended.

**Keywords:** Chlorhexidine, matrix metalloproteinase, microtensile bond strength, sodium hypochlorite

## Introduction

Despite technical and chemical improvements in dental materials, microleakage is yet a problem that influences resin–dentin interfaces. Microleakage is mostly attributed to polymerization shrinkage, which causes postoperative sensitivity, secondary caries, and pulpal injuries.[1]

The destruction of the hybrid layer at the resin–dentin interface could compromise the entire restoration, thereby lead to restoration failure. Hybrid layer degradation could occur for various reasons, including water absorption and hydrolysis, fatigue forces, thermal expansion, and matrix metalloproteinase enzymes (MMPs). MMPs-2, 8, and 9 are known as active proteases in the oral cavity that can degrade collagen fibers in the hybrid layer.[2] These enzymes are present in the dentin and are secreted by odontoblasts in the form of a proenzyme, which is an inactive form of the enzyme and requires extracellular activation. Acid etching and self-etching adhesive systems activate these enzymes.[3] In addition, the presence of acidic environments or a temperature increase activates dentin MMPs.[4] There are some inhibitory factors which prevent the activity of MMPs; for instance, chlorhexidine (CHX) digluconate has been known to be an inhibitor of MMPs-2, 8, and 9.[5]

In addition, problems associated with microleakage could be exacerbated by the microbial contamination of the cavity. This would be due to the incomplete caries removal

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and the presence of bacteria in the cavity. These bacteria grow, and their toxins penetrate into the pulp and cause irritation and inflammation even with the perfect seal of the cavity.[6] One way of eliminating or reducing bacteria from the cavity is the use of antiseptic solutions. Some studies have suggested the use of antibacterial agents such as CHX after cavity preparation and before restoration.[6,7] CHX is a broad-spectrum antimicrobial agent with cationic properties, and its antibacterial performance is comparable to that of sodium hypochlorite.[8] The minimum concentration of CHX inhibiting the function of MMP-9 is 0.002%. However, the function of the more sensitive MMP-2 enzyme is inhibited at a concentration of 0.0001% CHX. MMP-8 is also inhibited at a concentration of 0.02% CHX.[5]

The application of cavity disinfectants with adhesive resins could change the ability of adhesives to seal the dentin. In addition, cavity disinfectants could exert a negative effect on the bond strength of restorative materials to dentin structures.[9] Some studies have examined the effects of cavity disinfectants on the bond strength of resin composites to the dentin and reported varying amounts of bond strength, depending on the active components in these materials and the types of adhesive systems.[1,8] Inconsistencies in findings could be attributed to the limitations of bond strength tests. If cavity disinfectants interfere with the wettability of hydrophilic resins, the use of these materials could be problematic. In addition, it has been shown that cavity disinfectants, acting as a wetting agent before the use of adhesives, could improve adhesion to dentin and enamel.[10]

Given the desired properties of CHX in inhibiting MMPs enzymes, this study was done aimed at evaluating the effects of protease inhibition by CHX on the resin–dentin microtensile bond strength (μTBS) after 6 months of aging and comparing it with sodium hypochlorite. Our null hypothesis was that protease inhibition by CHX would not affect the μTBS after aging.

## Materials and Methods

In this study, 40 extracted caries-free human third molar teeth were used. Written informed consent was obtained from all patients. After calculus and debris removal, the teeth were stored in the thymol solution at 4°C and used within 2 months of extraction. After mounting the teeth in the acrylic resin, each sample was cut using a low-speed diamond saw (IsoMet 4000, Buehler, USA). The sections were in such a way that occlusal enamel surfaces were removed, and dentin surfaces were remained 1 mm more inside than dentin-enamel junction, perpendicular to the longitudinal axis of the tooth. Next, the sample surfaces were polished by silicon carbide 400, 600, 800, and 1000 grits. Dentin surfaces were etched by 35% phosphoric acid (3M ESPE/Scotchbond™ Etchant, Dental Products, St. Paul, USA) for 15 s, after rinsing for 10 s; they were then dried with gentle air spray (before the complete drying of the dentin surfaces). Afterward, the samples were divided randomly into four groups of ten as follows:

a. Control group: Adper Single Bond adhesive resin (3M ESPE, Adhesive, St. Paul, USA) was applied to the dentin surface, according to the manufacturer's instructions
b. The dentin surfaces were exposed to 2% CHX (Consepsis, Ultradent, USA). Next, they were dried slightly with cotton, and the Adper Single Bond adhesive resin was applied
c. For 30 s, 5.25% sodium hypochlorite was applied to the dentin surfaces. Afterward, the Single Bond adhesive resin was applied after rinsing and drying with cotton
d. At first, 5.25% sodium hypochlorite was applied to the dentin surface for 30 s; it was then rinsed and rehydrated by 2% CHX for 30 s. In the end, the Single Bond adhesive resin was applied.

In all groups, after removing the adhesive excess and solvent vapors, the adhesive was cured by a light cure device (Bluephase, Ivoclar Vivadent, Austria) for 20 s, using a soft start pattern. The teeth were surrounded by toffel myer transparent matrix strips, and then, two layers of the composite resin Filtek P60 (3M ESPE, Dental Products, ST. Paul, USA) with a 3-mm height were placed on them; next, each layer was cured for 40 s, according to the manufacturer's instructions.

Each group was divided randomly into two subgroups as follows:

- Group A in which the μ-TBS test was performed 24 h after the preparation
- The samples in Group B were stored for 6 months at 100% humidity and 37°C, and then, the μ-TBS test was performed.

After a 24-h storage period in distilled water, the specimens of Group A were subjected to the μTBS test, while Group B specimens were kept in distilled water for 6 months before doing the μTBS test. Next, cutoffs with a thickness of 1 mm were produced using a low-speed diamond saw. All sections were evaluated by a stereomicroscope, and samples containing bubbles or enamel remnants in the composite–dentin interface were removed. Perfect sections were mounted and cut again to provide beams with the dimensions of 1 × 1 $mm^2$ and 10 mm long. On average, 3–5 beams were obtained from each tooth. As five teeth were used in each group, 15–25 beams were evaluated in each experimental group. After examination by a stereomicroscope and the selection of healthy beams, the samples were assembled carefully using the mitreapel adhesive on a pair of resin plexiglasses to test the μTBS. The μTBS test was performed by the universal testing machine of STM device (STM 20, Santem) at a speed of 0.5 mm/min. The μTBS was calculated by dividing the debonding force by the composite–dentin beam's cross-sectional area in MPa units. After the test, failure modes were investigated by a stereomicroscope with the magnification of ×40, with failure modes classified as adhesive, cohesive, and mixed. The statistical analysis was performed by ANOVA, *t*-test, and Dunnett's test at the significant level of $\alpha = 0.05$.

## Results

The Kolmogorov–Smirnov test showed the normal distribution of the data. In a 24-h testing period, the highest and lowest μTBS were related to the control Group (A) and the sodium hypochlorite + CHX (D) group, respectively. In addition, in the 6-month testing period, Group A and chad showed the highest and lowest μTBS, respectively [Figure 1 and Table 1].

Pretreatment by sodium hypochlorite + CHX (Group D) resulted in a significantly lower bond strength in a 24-h testing period. The 6-month aging period resulted in the significant μTBS reduction of Group C. There was no significant difference between the 24-h and 6-month tested groups in failure modes, yet most of the failures were adhesives [Table 2].

## Discussion

Sodium hypochlorite is a proteolytic agent that eliminates organic compounds effectively and is used routinely for collagen fibers removal.[11] The remnants of sodium hypochlorite and its byproduct (oxygen) affect the adhesive polymerization process negatively and reduce the bond strength. This effect is maximized when sodium hypochlorite is added to the composition of total etch systems.[12] According to the results of this study, it seems that sodium hypochlorite cannot maintain bond durability as expected. Since comparisons between the 24-h and 6-month tested groups showed a significant reduction in the μTBS, the use of sodium hypochlorite is not recommended. Past research shows that this type of interference is attributed to the oxidizing effect of sodium hypochlorite and its remnants on resin polymerization.[13] The use of sodium ascorbate on dentin surfaces has been recommended for neutralizing sodium hypochlorite. Sodium ascorbate is a reducing agent that reacts with the byproducts of sodium hypochlorite and removes them, with the end products of which being oxalic acid and L-threonic acid that are both water soluble. As a result, sodium ascorbate converts the oxidized dentin into a reduced substrate, facilitates complete resin polymerization, and increases the adhesive bond strength.[14]

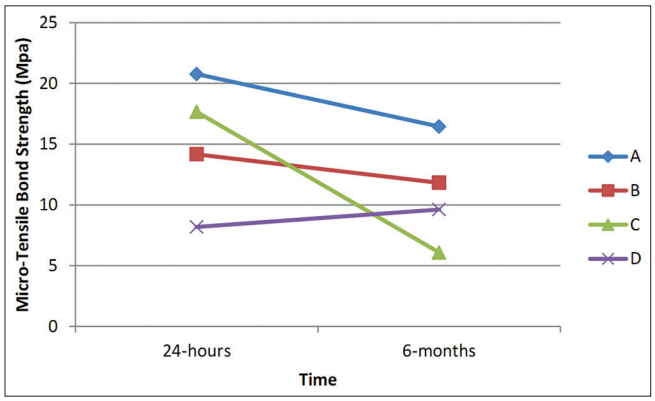


**Figure 1:** Comparing the microtensile bond strengths between groups at 24-h and 6-months testing periods (A: control group, B: 2% chlorhexidine, C: 5.25% sodium hypochlorite, and D: 2% chlorhexidine + sodium hypochlorite)

**Table 1: The microtensile bond strengths (mean±standard deviation) at 24-h and 6-month testing period**

| Time | Groups | Mean±SD |
|---|---|---|
| 24 h | A: Control group | 20.76±6.5 |
| | B: 2% CHX | 14.17±3.65 |
| | C: Sodium hypochlorite | 17.66±5.08 |
| | D: 2% CHX + sodium hypochlorite | 8.19±1.3 |
| 6 months | A: Control group | 16.45±6.74 |
| | B: 2% CHX | 11.82±4.01 |
| | C: Sodium hypochlorite | 6.08±2.71 |
| | D: 2% CHX + sodium hypochlorite | 9.62±3.11 |

CHX – Chlorhexidine, SD – Standard deviation

Another way of maintaining the bond strength is to use MMP inhibitors such as CHX. In this study, the μTBS of the CHX group had no significant difference with that of the control group at 24-h and 6-month intervals. CHX digluconate is a bis-cationic biguanide compound with antimicrobial effects on *Enterococcus faecalis*, which is recommended as a broad-spectrum disinfectant for the disinfection of dentin surfaces.[15]

The CHX release pattern is slow and continuous over a long period of time. Singh *et al*. reported that the release of CHX from the orthodontic composite resin was continuous in 60 days.[16] Graziele Magro *et al*. showed that various formulations of 2% CHX (solution, gel, CHX-Plus, Consepsis) produced more debris and smear layers than 17% ethylenediaminetetraacetic acid and 2.5% sodium hypochlorite. However, none of these formulations and depositions interfered with the push-out bond strength of sealers to the dentin.[17] In addition, CHX is an aqueous solution, and its inhibitory effect on MMPs (especially MMP 2, 8, and 9) and cysteine cathepsins lead to a significant reduction in oral microorganisms, such as streptococcus mutants in oral cavities. As a result, the past research proves the stability of the resin–dentin bond for a long time.[5]

In addition, CHX has other effects on the dentin structure. It has two strong and positive ionic charges that bond to the negative charges of phosphate groups in the mineralized dentin crystallites or carboxylate groups in the collagenous matrix; furthermore, CHX improves the bond strength by increasing the free energy of enamel and dentin surfaces. Using the inhibitory effects of MMPs, CHX causes the long-term durability of the hybrid layer and the bond strength in mineralized and demineralized dentin substrates.[18]

CHX molecules tend to get trapped under resin adhesives within the collagenous interfibrillar spaces and preserve their connection to collagenous fibrils after adhesive/primer application. In addition, the collagen fibrils treated with CHX are surrounded by adhesive monomers and can preserve CHX at the interface, with a long-term inhibitory function.[18,19]

In fact, the 2% CHX solution is composed of 2% gluconate and 98% water, which can wet the surface fully after application. Hence, the remaining humidity may decrease the functional

**Table 2: Distribution of failure modes (in percentage) between groups at 24-h and 6-month testing period**

| Time × failure mode cross tabulation | | | | |
|---|---|---|---|---|
| **Group** | **Failure mode** | | | **Total** |
| | **Adhesive** | **Cohesive** | **Mixed** | |
| A | | | | |
| Time | | | | |
| 24 h | | | | |
| Count | 7 | 2 | 2 | 11 |
| Percentage within time | 63.6 | 18.2 | 18.2 | 100.0 |
| 6 months | | | | |
| Count | 8 | 2 | 1 | 11 |
| Percentage within time | 72.7 | 18.2 | 9.1 | 100.0 |
| Total | | | | |
| Count | 15 | 4 | 3 | 22 |
| Percentage within time | 68.2 | 18.2 | 13.6 | 100.0 |
| B | | | | |
| Time | | | | |
| 24 h | | | | |
| Count | 8 | 1 | 2 | 11 |
| Percentage within time | 72.7 | 9.1 | 18.2 | 100.0 |
| 6 months | | | | |
| Count | 9 | 1 | 1 | 11 |
| Percentage within time | 81.8 | 9.1 | 9.1 | 100.0 |
| Total | | | | |
| Count | 17 | 2 | 3 | 22 |
| Percentage within time | 77.3 | 9.1 | 13.6 | 100.0 |
| C | | | | |
| Time | | | | |
| 24 h | | | | |
| Count | 10 | | 1 | 11 |
| Percentage within time | 90.9 | | 9.1 | 100.0 |
| 6 months | | | | |
| Count | 10 | | 1 | 11 |
| Percentage within time | 90.9 | | 9.1 | 100.0 |
| Total | | | | |
| Count | 20 | | 2 | 22 |
| Percentage within time | 90.9 | | 9.1 | 100.0 |
| D | | | | |
| Time | | | | |
| 24 h | | | | |
| Count | 10 | | 1 | 11 |
| Percentage within time | 90.9 | | 9.1 | 100.0 |
| 6 months | | | | |
| Count | 10 | | 1 | 11 |
| Percentage within time | 90.9 | | 9.1 | 100.0 |
| Total | | | | |
| Count | 20 | | 2 | 22 |
| Percentage within time | 90.9 | | 9.1 | 100.0 |

A: Control group, B: 2% CHX, C: Sodium hypochlorite, D: 2% CHX + sodium hypochlorite. CHX – Chlorhexidine

properties of the monomers.[20] If water is used, water hydrogen will bond to collagen fibrils instead of CHX, thereby decreasing the bond strength.[18] Therefore, in the current study, dentin surfaces were not rinsed but were just dried using mild air spraying.

Since the used CHX solution was not rinsed, and only its remnants were removed using mild air spray, there are concerns about interactions with adhesive systems. It seems that incorporating CHX into the adhesives would necessitate adding water to the formula and compromising the adhesives' effectiveness.[21] Nevertheless, different studies show that the use of CHX does not improve the bond strength and physical properties.[22,23]

Another study demonstrated that although CHX treatment interfered with the immediate bond strength (due to chemical interactions among CHX, phosphate, and deposition formation), the bond strength obtained after a 6-month period of cycling loading was stable due to the long-term integrity of the hybrid layer and the MMPs inhibitory effect of CHX. CHX could inhibit the degradation of the exposed collagen, thereby improving the longevity of the bond strength.[24,25] Loguercio *et al*. reported that stable bond strengths were maintained for 6 months after CHX treatment, regardless of the CHX application time and concentration.[26] Another study showed that even at the low concentrations of CHX and within a short time of application, CHX affected hybrid layer degradation, thereby influencing the *in vitro* bond strength positively over time.[27] In addition, Leitune *et al*. showed that CHX application was not effective in improving the bond strength of fiber posts cemented to the radicular dentin after 6 months.[22]

It has been demonstrated that CHX incorporation into the acidic conditioner can be useful in enhancing the long-term stability of collagenous fibrils in the hybrid layer against host-derived MMPs, with no need for additional steps in the bonding process.[28] When CHX was incorporated into the primer of the self-etch adhesive, CHX could preserve the dentin bond strength as long as the CHX concentration in the primer was over or equal to 0.1%.[29] In a study, Pomacóndor-Hernández *et al*. concluded that 2% CHX incorporation as a component of Adper Scotchbond SE did not influence the immediate bond strength, neither was any reduction observed in the bond strength within 3–6 months from water storage.[30]

A reason for the discrepancies between the results of different studies is that the behavior of MMPs enzymes may be different in *in vitro* and *in vivo* conditions; in addition, time plays a major role in this process. In the present study, the samples were stored for 6 months at 100% humidity and 37°C, and then, μ-TBS tests were performed. The highest μTBS were related to the control Group (A) in the 24-h and 6-month testing periods. The finding based on which the application of CHX + sodium hypochlorite (Group D) showed a higher μTBS after a 6-month storage period was unexpected compared to the 24-h period, yet the difference was statistically insignificant. The difference could have been due to the bias in testing or differences in dentin substrates. For more precise evaluations, it is recommended that the sample size be increased. It is worth noting that the μTBS test is very sensitive, and gaining precise results is affected by external factors.

In the current study, the prevalence of adhesive failure was more than other failures at the composite–dentin interface.

However, Nour El-din *et al*. and Gurgan *et al*. reported that there was no correlation between the failure mode and the bond strength value.[31,32]

Since water absorption plays an important role in the bond degradation of resin–dentin in the *in vivo* environment during the relevant time, aqueous environments are used in the studies as a reliable medium to simulate oral cavity conditions.[33] In contrast, Kitasako *et al*. and Shafiei and Memarpour reported that to prevent microorganism growth, the water storage of specimens had to be changed on a daily basis during the study; however, this daily change might damage the resin–dentin interface and decrease the bond strength.[34,35] Therefore, in the current study, the water storage medium was changed on a weekly basis.

The 6-month aging resulted in a reduction in the μTBS of all specimens, except for Group D (CHX + sodium hypochlorite). The bond strength reduction in Group C (sodium hypochlorite) was significant and more than in other groups. The 6-month water storage resulted in the least reduction in the bond strength in Group B (CHX) among other groups. Regardless of the reasons that made the CHX group obtain more stable bond strength over the 6-month period, it could be considered a good option for dentists to disinfect cavity preparations. This *in vitro* investigation was conducted under static conditions on flat dentin surfaces. Various factors, such as thermal, mechanical, and chemical factors, as well as fatigue stresses affect the bond strength. These are the limitations of the present study. Further *in vitro* researches are needed to evaluate the MMP inhibitory effect of CHX on prevention of collagen degradation and improve the bond strength longevity.

## Conclusions

- The highest bond strength was related to the control group
- The use of CHX did not have better preservation of bond strength when compared to the control group
- Bond strength of all groups except Group D (CHX + sodium hypochlorite) decreased after 6 months, and this bond strength reduction in Group C was more than other groups
- The 6-month water storage period resulted in the least bond strength reduction in Group B (CHX) when compared to other groups.

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### Conflicts of interest

There are no conflicts of interest.

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