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**RESEARCH ARTICLE**

# Effects of different cavity disinfectant procedures on microtensile bond strength of permanent teeth

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ABSTRACT

**Objectives:** The aim of this study was to compare the effects of different cavity disinfectants and KTP laser applied before/after acid-etching on microtensile bond strength of a composite resin to permanent teeth. The selection of the right cavity disinfectants may reduce the residual bacteria after cavity preparation.

**Materials and Methods:** Chlorhexidine (CHX), propolis(PRO), ozonated water(OW), gaseous ozone(OG) and KTP laser were used for this purpose. Twenty two third molar teeth were divided into two groups (n=12,n=10). In the first group, the cavity disinfectants were used before acid-etching (n=12) and in the second group, cavity disinfectants were used after acid-etching(n=10). One group received no pre-treatment and was set as control(CONT). After sample preparation, composite crowns were built up and sticks were obtained from these samples. The sticks were then stressed in tension until failure using a universal testing machine and the data were recorded. The sticks were analyzed under SEM.

**Results:** The μTBS values of the disinfectants applied before acid-etching were KTP>CONT>OW>PRO>CHX>OG and the μTBS values when disinfectant solutions were applied after acid-etching were KTP>CONT>PRO>OW>CHX>OG, respectively.SEM analyses showed cohesive fractures in increased μTBS.

**Conclusions:** The cavity disinfectants should be used before acid-etching and the results of this study revealed that KTP and OW might be safely used as cavity disinfectants.

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## INTRODUCTION

Dental caries is a microbially mediated disease characterized by demineralization of the tooth and the goal of restorative treatments is removing the infected enamel and dentin and filling the area with a suitable restorative material[1,2]. However, still, no criteria are available to judge whether the carious tissue is completely removed. The excavation of the colored and softened dentin cannot remove all the bacteria from the cavity during preparation but histobacteriologic studies showed that 15-25% of the teeth still contained bacteria at the deepest portion of the carious lesions even after removal of the stained dentin[1,3]. The remaining bacteria proliferate in time and may lead sensitivity, secondary caries, loss of restorative material and pulp damage[1,4,5]. For these reasons elimination of bacteria from cavity surfaces is of major importance and to remove all the bacteria from the cavity preparation and to reduce the potential for residual caries, the use of antibacterial solutions has been suggested in addition to the physical removal of carious dentin for the disinfection of dentinal cavities.[1,4-9] Today the application of antibacterial solutions could be beneficial and solve the problem of residual bacteria therefore several antibacterial agents were used for this purpose. The selection of the disinfectant compatible with the bonding system is of great importance as any positive benefits would be negated if the antimicrobial action deteriorated the bonding efficacy.[10]

Chlorhexidine (CHX) is widely used in dentistry as an antimicrobial agent. CHX not only has an antibacterial effect, but also has effects on improving the integrity of the hybrid layer. In addition, it has a rewetting capacity and a strong affinity to tooth structure.[7,8,11]

The use of ozone in dentistry thought that ozonated water (OW) and gaseous ozone (OG) can be used as antibacterial agents for this purpose. Ozone has been used for many years for different purposes such as killing bacteria in the water industry, decontamination of hospital side rooms, in gum infections, during surgery, for failed implant cases, root caries, root canal treatment and for its potential application in reducing bacterial counts in dental unit water delivery systems[12-15]. Ozone (O3) in a gaseous or aqueous phase has been shown to be a powerful and reliable antimicrobial agent against bacteria, fungi, protozoa, and viruses[15,16]. It forms oxidated radicals in the presence of water that penetrate and act on cell membranes, affecting the osmotic stability, promoting the oxidation of amino acids and nucleic acids, and causing cellular lysis depending on the reaction's extension[17].

Propolis (PRO) is a natural flavanoid-rich resinous product of honeybees, which is known for its biological properties, including antibacterial, antifungal, healing properties and antiinflammatory, anesthetic, and antimicrobial activities[7,18-22]. There are no data available on the effect of propolis on the microtensile bond strength of restorative materials.

Lasers are alternative treatments to antibacterial agents. As they are used for many purposes in dentistry, they are also used as cavity disinfectants[6]. A frequency-doubled Nd:YAG laser was introduced that is called potassium titanyl phosphate (KTP) laser. The KTP laser is a solid-state laser designed to pass an Nd:YAG incident beam of 1064μm through a KTP crystal to produce an intense visible green laser light of 532nm. This process causes 1064 μm wavelength to be shortened by half while doubling the beam's frequency[14,23]. KTP laser has similar characteristics to Nd: YAG. While it has a few more unique characteristics; the green visible light of KTP laser is absorbed well by melanin and hemoglobin, but it is not absorbed well in water. This feature allows the KTP laser to penetrate dentin with less damage, since water is the major component of dentin. KTP lasers have high energy photons which are well-known for having shorter wavelength allowing chemical and photodynamic reactions to progress efficiently with less damage to both hard and soft tissues of dental pulp[24].

The ideal dentin disinfectant should combine the possession of a potent antimicrobial action and should not interfere with the bonding of the subsequently applied adhesive system. In the literature there isn't a consensus how these disinfectant effects -enhancing, negating, impairing- the dentin bonding process[10]. Therefore, the objective of this study was to compare the effects of different cavity disinfectant approaches; CHX, OG, OW, PRO and KTP laser on microtensile bond strength (μTBS) of composite resin on permanent teeth when applied to cavity surfaces before and after acid-etching. The tested null hypothesis was that different cavity disinfectants or applying a disinfectant before or after acid-etch do not affect μTBS of composite resin on dentin.

## MATERIALS AND METHODS

This study was approved by the Ethical Committee of the Cumhuriyet University permission 2012-09/09. Twenty two third molar teeth used in this study and the teeth were free of caries which were collected from the patients between 20-24 aged. Recently extracted teeth were stored in distilled water and used within one month.

### Specimen preparation

One third of the crown was removed using Isomet low speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA). A stereomicroscope was used to check for the absence of enamel and pulp tissue on the resultant substrate. For the first group, the cavity disinfectants were applied to the surface before applying acid-etching, dentin bonding and composite resin.

For the second group, cavity disinfectants were applied to the surface after applying acid-etching to surface followed by the application of dentin bonding and composite resin to the surface.

### Test groups

*Chlorhexidine gluconate group*
A 2% CHX (Drogsan, Ankara, Turkey) solution was applied to dentin for 20 s with a cotton pellet. The cavity surfaces of the teeth were then dried with air for 10 s.

*Propolis group*
Propolis samples were collected from three different localities in Turkey, Zara/Sivas (Middle Anatolia). Hand collected propolis were kept desiccated and in the dark up to their processing. Propolis samples were ground with an ultra centrifugal mill (Retsch, Haan, Germany), and 25 g powder was dissolved in 50 mL dimethyl sulfoxide (DMSO) (100%, w/v) by magnetic mixer for 24 h at 37°C. Working solutions at concentrations of 10% were then prepared in sterile saline solution. The dentin surfaces were treated with a 30% propolis solution for 20 s. The cavity surfaces of the teeth were then dried with air for 10 s.

*Gaseous ozone group*
Gaseous ozone was applied with an ozone generator KaVo HealozoneTM 2130C (KaVo Dental, Biberach, Germany) to the dentin for 30 seconds using a handpiece and silicone caps (for sealing).

*Ozonated water groups*
The ozonated water was freshly prepared using a custom-made ozone generator (TeknO3zone, Izmir/Turkey) produced by the manufacturer. The amount of aqueous ozone was measured with the help of the eprobe, which was in the reaction tank connected to the generator. The ozone density of the

distilled water in the reactor tank was shown by the digital indicator on the generator. The concentration of ozonated water used for this study was between 3.5 ppm and 4 ppm. The ozonated water was used within 5 minutes after its preparation and applied to the dentin surface for 30 s.

*KTP laser groups*
The KTP Laser (Smartlite D, Deka, Calenzano Firenze, Italy) was applied to dentine surface with a wavelength of 532nm, with a non-contact mode for four times, applying 10 s with waiting 5 s for 1 min, at 1W energy output with a pulsed mode (Ton: 10,Toff:50) and focal distance of 1mm.

*Acid-etching*
37% phosphoric acid (FineEtch 37, Spident Co., Ltd, Korea) was applied for 15 s, rinsed for 20 s and dried.

*Bonding Procedures*
Prime&Bond NT (Dentsply Detrey, Konstanz, Germany) was applied to dentin for 20 s and light-cured with a LED curing light (Bluephase, Ivoclar Vivadent) for 15 s.

*Composite Resin*
Resin composite (Tetric N-Ceram, Ivoclar Vivadent, Liechtenstein) was built up in 1mm increments up to 4 mm and light cured for 20s for each increment.

After applying composite to dentin, the teeth were stored in distilled water for 24 h. At the end of 24 h, the teeth were longitudinally sectioned in both “x” and “y” directions with a slow-speed saw under water cooling to obtain bonded sticks with a cross-sectional area between 0.7 $mm^2$-1 $mm^2$. For each group, ten sticks were obtained. The sticks were stored in distilled water for 24h. Then the sticks were fixed to the universal testing machine with cyonoacrylate adhesive plus an accelerator (Zapit, Dental Ventures of America, Corona, CA, USA). The specimens were stressed in tension until failure using a universal testing machine (LF Plus, LLOYD Instruments, Ametek, Inc., England) at a crosshead speed of 0.5 mm/min, and the microtensile bond strength (μTBS) was calculated and expressed in MPa.

The sticks were analyzed under scanning electron microscope (SEM) and they were classified by adhesive, mix and cohesive failure. Failure modes were classified as Adhesive: where the fracture site was located between the adhesive and the dentin, or mixed: Where the fracture site included both adhesive failure at the interface and cohesive failure within the resin composite.

## Statistical Analysis

After recording the data, the results were subjected to statistical analysis using the software Statistical Packages for Social Sciences for Windows 15.0 (SPSS, Inc., Chicago, IL, USA). μTBS data were analyzed using Two-way ANOVA and Bonferroni correction tests were made at a p level of $p<0.05$.

## RESULTS

The mean μTBS values of permanent teeth and the differences of the groups were shown in tables (Table 1, 2). The ranking of μTBS values of the disinfectants applied before acid-etching were KTP> CONT> OW> PRO> CHX> OG, respectively. KTP laser showed significantly increased values when applied before acid-etching ($p<0.05$). The μTBS values when disinfectants were applied after acid-etching were KTP> CONT> PRO> OW> CHX> OG, respectively. There were no significant differences between KTP laser, PRO and CONT groups ($p>0.05$).

When the effects of disinfectants applied before acid-etching or after acid etching were compared, there were significant decreases in μTBS values of OW, KTP laser and OG groups ($p<0.05$). There were no changes in μTBS values of PRO and an

**Table 1.** The means and standard deviations of the groups

| Group | Application | n | Mean (MPa)+ Std deviation |
|---|---|---|---|
| Control | | 10 | 17,75±5,29 |
| CHX | Before | 10 | 11,12±2,46 |
| | After | 10 | 11,71±3,28 |
| O3 water | Before | 10 | 17,00±5,76 |
| | After | 10 | 12,59±3,35 |
| O3 gas | Before | 10 | 10,63±3,49 |
| | After | 10 | 7,74±2,24 |
| Propolis | Before | 10 | 15,02±5,04 |
| | After | 10 | 15,03±2,92 |
| KTP | Before | 10 | 24,75±5,41 |
| | After | 10 | 18,28±6,58 |
| Total | Before | 10 | 16,04±6,57 |
| | After | 10 | 13,07±5,20 |

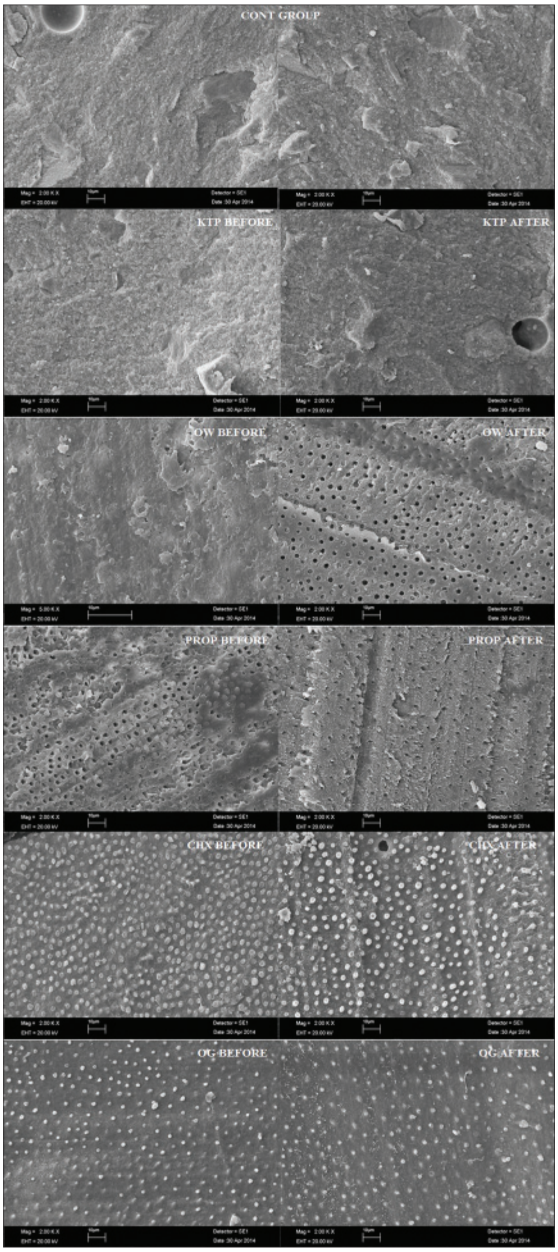


**Fig 1.** SEM images of the disinfectant applied before and after acid-etching

increase in CHX group was observed, but they were not significant (p>0.05).

Cohesive fractures were predominantly seen both in KTP and CONT groups (Figure 1). OW groups showed cohesive failures when it was applied before acid-etching and showed mix failures when it was applied after acid-etching while PROP groups showed mix failures both in after and before acid-etching. CHX and OG groups resulted adhesive failures both in after and before acid-etching.

## DISCUSSION

According to the results of this study the null hypothesis was rejected. The disinfectants have effects on µTBS. The effects of the application time of disinfectants varied according to the groups. The use of cavity disinfectants may reduce or completely remove the bacteria from tubules and this may reduce secondary caries, damage to pulp or restoration failure. Dental professionals could avoid from these problems with the use of cavity disinfectants. SEM images of the groups showed that cohesive and mixed failure types showed increased bond strengths.

**Table 2.** Intercomparisons of the groups (p<0.05)

| Before | | | After | | |
|---|---|---|---|---|---|
| Group | Group | Significance | Group | Group | Significance |
| Control | CHX | ,001* | Control | CHX | ,003* |
| | O3 water | ,707 | | O3 water | ,012* |
| | O3 gas | ,000* | | O3 gas | ,000* |
| | Propolis | ,169 | | Propolis | ,178 |
| | KTP | ,001* | | KTP | ,791 |
| CHX | Control | ,001* | CHX | Control | ,003 |
| | O3 water | ,004* | | O3 water | ,655 |
| | O3 gas | ,806 | | O3 gas | ,046* |
| | Propolis | ,051 | | Propolis | ,096 |
| | KTP | ,000* | | KTP | ,001* |
| O3 water | Control | ,707 | O3 water | Control | ,012* |
| | CHX | ,004* | | CHX | ,655 |
| | O3 gas | ,002* | | O3 gas | ,015* |
| | Propolis | ,315 | | Propolis | ,220 |
| | KTP | ,000* | | KTP | ,005* |
| O3 gas | Control | ,000* | O3 gas | Control | ,000* |
| | CHX | ,806 | | CHX | ,046* |
| | O3 water | ,002* | | O3 water | ,015* |
| | Propolis | ,028* | | Propolis | ,000* |
| | KTP | ,000* | | KTP | ,000* |
| Propolis | Control | ,169 | Propolis | Control | ,012 |
| | CHX | ,051 | | CHX | ,096 |
| | O3 water | ,315 | | O3 water | ,220 |
| | O3 gas | ,028* | | O3 gas | ,000* |
| | KTP | ,000* | | KTP | ,102 |
| KTP | Control | ,001* | KTP | Control | ,791 |
| | CHX | ,000* | | CHX | ,001* |
| | O3 water | ,000* | | O3 water | ,005* |
| | O3 gas | ,000* | | O3 gas | ,000* |
| | Propolis | ,000* | | Propolis | ,102 |

CHX has been used in dentistry for many purposes. It has been used as a root canal irrigant, disinfectant or mouthwash in dentistry. It has rewetting capacity and a great affinity to tooth structure.[6,8] Many authors assumed that CHX can improve dentin bond strength because of its antibacterial effects but the results are controversial. The previous studies showed that CHX had no adverse effects on bond strength[1,2,25-28], while the others[29-31] have reported that CHX improved bond strength. Contrary to these results, CHX showed significantly decreased μTBS values when compared to control group's values both in two groups. The authors are in agreement with the authors that when CHX was applied to the cavity surface, μTBS values decreased.[5,32-34] The reason of decreased μTBS could probably be explained by inadequacy of CHX in effective removal of smear layer[5], effects of the macro molecules of CHX and reducing the potential for chemical bonding with the cements[33]. Furthermore, it is speculated that CHX inhibits the dentin MMPs and decelerates the loss of resin-dentin bonds[4]. The manufacturers recommend the use of disinfectants after acid-etching dentin, followed by the application of total-etch adhesive systems[5] and according to the results of this study, there were no significant differences between the application times of CHX.

Propolis has been studied as a root canal medicament extensively, but to the authors' knowledge, there is no other study on its effects on microtensile bond strength. The PRO group showed significantly decreased μTBS values than KTP and increased μTBS values than OG group when applied before acid-etching and significantly increased values than OG group when applied after acid-etching. Decreased μTBS were seen than control group but they were not significant. And also there were no significant differences when it was applied after or before acid-etch. To the authors' knowledge, this is the first bond strength study with propolis and the results revealed that it may be used as a cavity disinfectant when compared to control group.

KTP laser group showed increased μTBS values than the other groups regardless of the application times. In the present study, KTP laser showed superior effects when compared to the other groups. Schoop et al[35] found that the KTP laser obviously causes melting and recrystallization of the surface, thus partly obliterating the dentinal tubules. The increased bond strength may probably be affected by the recrystallization of the surface.

Application of OG appears attractive for cavitated lesions with its strong oxidizing activity. In the present study, OG showed less μTBS values than the remaining groups. Cadenaro et al[36] and Gerspach et al[2] showed that there were no statistical differences when OG applied to dentin surface but in this study decreased values were seen. When applied after acid-etching, OG showed decreased μTBS values and results of this study were in agreement with Schmidlin et al[37] that decreased bond strength was seen when compared with the other groups.

OW has been used as an irrigation solution in endodontic therapies and found effective on microorganisms. There is not enough information about OW's effect on bond strength. In this study, OW showed significantly increased μTBS values than OG and CHX. As it showed significantly decreased values than KTP group, there was no significant difference between control groups when applied before acid-etching. It was shown that OW was able to open the tubular structure by removing organic debris[38] and this may provide the increased μTBS values. To authors' knowledge there are few studies on ozonated water on bond strength and this study showed that OW has no deleterious effects on the bond strength.

## CONCLUSION

Within the limits of this study, it could be concluded that the cavity disinfectants could be used before an acid-etching for improving the bond strength and the results of the present study revealed that KTP laser and ozonated water might be safely used as cavity disinfectants. Further studies are necessary to compare the effectiveness of KTP laser and ozonated water on the bond strength values of various restorative materials.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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