

# Effects of different cavity-disinfectants and potassium titanyl phosphate laser on microtensile bond strength to primary dentin

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## Abstract

**Aim:** The aim of this *in vitro* study was to compare the effects of different cavity-disinfectants and potassium titanyl phosphate (KTP) laser on microtensile bond strength to primary dentin. Chlorhexidine (CHX), propolis (PRO), ozonated water (OW), gaseous ozone (OG) and KTP laser were used for this purpose.

**Methodology:** Twelve primary molar teeth were used in this study. One-third of the teeth (from coronal portion) were removed to obtain flat surfaces. After applying the cavity-disinfectants, an adhesive (prime and bond NT) was applied to dentin surfaces, and composite crowns were built up. One group received no pretreatment and was set as a control (CONT). Ten sticks were obtained from these samples and were stressed in tension until failure using a universal testing machine and the data were recorded.

**Results:** The mean strength values (in MPa) of the sticks were OW (11.12) > KTP (9.58) > CHX (7.58) > PRO (7.42) > CONT (6.38) > OG (5.84) and OW showed significantly higher results than the other groups, except KTP group ( $P < 0.05$ ).

**Conclusions:** OW and KTP might be used safely without compromising the bond strength of restorative materials.

**Key words:** Chlorhexidine gluconate, potassium titanyl phosphate lasers, microtensile bond strength, ozone, primary dentine, propolis

**Date of Acceptance:** 25-Oct-2014

## Introduction

Incomplete removal of caries-infected enamel or dentin during cavity preparation results in the entrapment of viable bacteria, which may continue to multiply within the cavity.<sup>[1]</sup> These bacteria may produce toxins, which cause pulpal irritation and inflammation. Pretreatment of the tooth surface with an antibacterial agent is useful in eliminating the harmful effects caused by either the residual bacteria or bacterial microleakage.<sup>[2]</sup> Histological and bacteriological experiments performed to determine whether viable organisms remain on the dentinal surface at the termination of routine cavity preparation have shown that only a portion of a tooth is sterile after the preparation.<sup>[3]</sup> To remove all the bacteria from the

cavity preparation and to reduce the potential for residual caries, use of antibacterial solutions has been suggested in addition to the physical removal of carious dentin for the disinfection of dentinal cavities.<sup>[3,4]</sup>

Chlorhexidine (CHX) is one of the well-known antibacterial agent, which contains CHX gluconate that binds to the amino acids in dentin and continues to kill bacteria for several hours, thereby making it a good antibacterial agent.<sup>[3]</sup> However, using CHX as a cavity-disinfectant may be a problem for bonding procedures and may interfere with the application of adhesive resin to dentine. Various

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Website: [www.njcponline.com](http://www.njcponline.com)

DOI: 10.4103/1119-3077.151774

PMID: 25772926

studies have investigated the influence of CHX on the bond strength of various dentin bonding agents. It has been reported that 2% CHX does not influence the microtensile bond strength ( $\mu$ TBS) of single bond, prime and bond NT and clearfil SE bond adhesive resins.<sup>[5]</sup>

Propolis (PRO) is a complex mixture of substances that bees use to seal their hives. Bees collect these substances from flowers, leaves and stalks, then produce the PRO and deposit it into their hives.<sup>[6]</sup> PRO is employed in medicine and dentistry because of its antiinflammatory, antiseptic, healing and antimicrobial properties.<sup>[7]</sup>

Ozonated water (OW) and gaseous ozone (OG) can be preferred as antibacterial agents for this purpose. In general, ozone is a potent oxidant agent ( $E_0 = 2.08$  V) and has been introduced into dental practice by the development of ozone-generating devices. As this novel equipment can produce oxidants in high concentrations (ca 2100 ppm), some manufacturers have proposed the use of ozone as an antimicrobial agent.<sup>[8]</sup> Previous studies have concluded that ozone gas used as a dentin pretreatment does not jeopardize the resin dentin/enamel bond strength of two-step etch-and-rinse adhesives<sup>[8,9]</sup> self-etching adhesives,<sup>[9,10]</sup> luting cements,<sup>[11]</sup> and the mechanical properties of adhesive systems.<sup>[4]</sup>

Various types of lasers have antibacterial effects on different microorganisms. It has been reported that CO<sub>2</sub>, Nd: YAG, Er: YAG, and Er, Cr: YSGG laser irradiation are able to efficiently remove debris and the smear layer.<sup>[12]</sup> The removal of the smear layer consequently serves to eliminate microorganisms and prevent residual caries.<sup>[12]</sup> The potassium titanyl phosphate (KTP) laser, emitting at 532 nm, a new wavelength for dental applications, has been primarily used for tooth bleaching procedures.<sup>[13,14]</sup> There is a lack of information in the literature regarding the effect of laser irradiation on the bond strength of adhesive systems when used in cavity-disinfecting procedures.<sup>[12]</sup>

The objective of this study was to compare the effects of different disinfectants; CHX gluconate, OG, OW, PRO and KTP laser on  $\mu$ TBS of composite resin to primary dentin. The tested null hypothesis was that the different

cavity-disinfectants do not affect  $\mu$ TBS of composite resin on dentin.

## Methodology

This study was approved by the Ethical Committee of the Cumhuriyet University permission Sep 09, 2012. Twelve primary molar teeth, extracted for orthodontic reasons, without caries were used in this study. The teeth were stored in distilled water and used within 1-month.

### Specimen preparation

One-third of the teeth (from coronal portion) were 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. A flat dentin surface was exposed, after grinding the occlusal enamel on a wet #180 grit SiC paper. The exposed dentin surfaces were further polished on wet #600-grit SiC paper for 60 s to standardize the smear layer. Primary teeth were divided into six groups, which are shown in Figure 1.

### Materials

Chlorhexidine, PRO, OW, OG and KTP laser were used for this purpose.

#### Chlorhexidine group

A 2% CHX gluconate (Klorhex, Drogosan, 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 Turkey, Zara/Sivas (Propolis, Sivas, Middle Anatolia, Turkey). Hand collected PRO was kept desiccated and in the dark until processing. PRO samples were ground with an ultra-centrifugal mill (Retsch, Haan, Germany), and 25 g powder was dissolved in 50 mL dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, USA) (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% PRO solution for 20 s. The cavity surfaces of the teeth were then dried with air for 10 s.

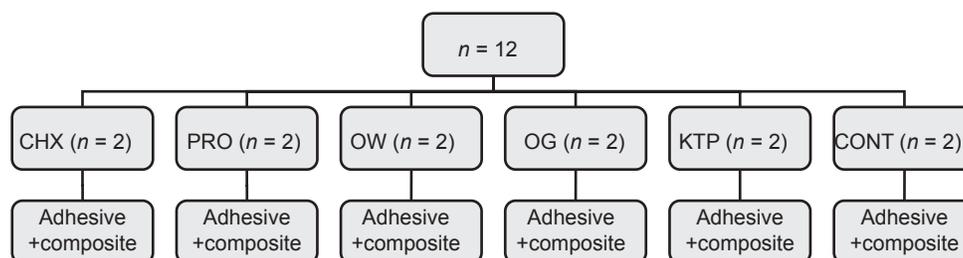


Figure 1: The schematic view of primary molar teeth

**Gaseous ozone group**

Gaseous ozone was applied with an ozone-generator KaVo HealOzone™ 2130C (KaVo Dental, Biberach, Germany) to the dentin for 30 s using a handpiece and silicone caps (for sealing).

**Ozonated water group**

The OW 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 probe, which was in the reaction tank connected to the generator. The digital indicator on the generator showed the ozone density of the distilled water in the reaction tank. The concentration of OW used for this study was between 3, 5 ppm and 4 ppm. The OW was used within 5 min after its preparation and applied to the dentin surface for 30 s.

**Potassium titanyl phosphate laser group**

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

**Control group**

Teeth in this group did not receive any treatment and served as control (CONT).

Following these procedures, adper prime and bond NT (Dentsply Detrey, Konstanz, Germany) was applied to the dentin for 20 s and light-cured with a LED curing light (Bluephase, Ivoclar Vivadent, Schaan, Liechtenstein) for 15 s and resin composite (Tetric N-Ceram, Ivoclar Vivadent, Schaan, Liechtenstein) was built up in 1 mm increments up to 4 mm.

After applying composite resin to dentine, 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 0.9 and 1 mm<sup>2</sup>. For each group, ten sticks were obtained. The sticks were stored in distilled water for 24 h. Then, the sticks were fixed to the universal testing machine with cyanoacrylate 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., West Sussex, UK) at a crosshead speed of 0.5 mm/min, and the  $\mu$ TBS was calculated and expressed in MPa.

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).  $\mu$ TBS data were analyzed using one-way ANOVA

and Tukey's *post-hoc* test. Assessments were made at a *P* level of *P* < 0.05.

**Table 1: The means, SDs, maximum and minimum values of  $\mu$ TBS**

| Groups | n  | Mean (MPa) $\pm$ SD | Minimum (MPa) | Maximum (MPa) |
|--------|----|---------------------|---------------|---------------|
| CONT   | 10 | 6.38 $\pm$ 2.47     | 3.30          | 9.89          |
| CHX    | 10 | 7.58 $\pm$ 3.18     | 4.69          | 15.14         |
| OW     | 10 | 11.12 $\pm$ 2.41    | 7.64          | 15.46         |
| OG     | 10 | 5.84 $\pm$ 2.62     | 2.30          | 10.57         |
| PRO    | 10 | 7.42 $\pm$ 2.28     | 4.12          | 9.79          |
| KTP    | 10 | 9.58 $\pm$ 2.92     | 5.63          | 14.41         |
| Total  | 60 | 7.98 $\pm$ 3.14     | 2.30          | 15.46         |

CHX=Chlorhexidine; CONT=Control; OW=Ozonated water; OG=Gaseous ozone; PRO=Propolis; KTP=Potassium titanyl phosphate; SDs=Standard deviations;  $\mu$ TBS=Microtensile bond strength

**Table 2: Multiple comparisons of the groups**

| Group | Significance |
|-------|--------------|
| CONT  |              |
| CHX   | 0.914        |
| OW    | 0.003*       |
| OG    | 0.997        |
| PRO   | 0.950        |
| KTP   | 0.096        |
| CHX   |              |
| CONT  | 0.914        |
| OW    | 0.049*       |
| OG    | 0.691        |
| PRO   | 1.00         |
| KTP   | 0.554        |
| OW    |              |
| CONT  | 0.003*       |
| CHX   | 0.049*       |
| OG    | 0.001*       |
| PRO   | 0.035*       |
| KTP   | 0.789        |
| OG    |              |
| CONT  | 0.997        |
| CHX   | 0.691        |
| OW    | 0.001*       |
| PRO   | 0.767        |
| KTP   | 0.031*       |
| PRO   |              |
| CONT  | 0.950        |
| CHX   | 1.00         |
| OW    | 0.035*       |
| OG    | 0.767        |
| KTP   | 0.471        |
| KTP   |              |
| CONT  | 0.096        |
| CHX   | 0.554        |
| OW    | 0.789        |
| OG    | 0.031*       |
| PRO   | 0.471        |

The mean difference is significant at the 0.05 level. CHX=Chlorhexidine; CONT=Control; OW=Ozonated water; OG=Gaseous ozone; PRO=Propolis; KTP=Potassium titanyl phosphate

## Results

The mean  $\mu$ TBS values and the differences between groups in primary teeth were showed in Tables 1 and 2. The range of  $\mu$ TBS values were OW > KTP > CHX > PRO > CONT > OG, respectively. OW showed significantly higher results than the other groups except KTP group. There were no significant differences between OW and KTP Groups. OW and KTP groups showed significantly higher  $\mu$ TBS values than OG ( $P < 0.05$ ).

## Discussion

According to the results of this study, the null hypothesis was rejected. The disinfectants have effects on  $\mu$ TBS. The use of cavity-disinfectants may reduce or completely remove the bacteria from tubules and this may reduce secondary caries, damage to pulp or failures of restorations and the dentists could avoid these problems with the use of cavity-disinfectant.

Chlorhexidine is a broad-spectrum disinfecting agent, which has been recommended for the irrigation of prepared cavities because of its disinfecting properties.<sup>[15]</sup> Many authors expected that CHX can improve dentin bond strength while exerting antibacterial effects but the results are controversial. Hiraishi *et al.*<sup>[11]</sup> reported that when CHX is applied to smear-covered dentine surfaces, it is more likely to bind to the loose apatite remnants within the smear layer than when it is applied to acid-etched dentin surfaces where phosphate groups are depleted due to etching and rinsing. Bonding of CHX to these loose, superficial apatites could have interfered with the functions of ED primer (Kuraray, Japan) monomers. Thus, further studies are required to clarify the property of CHX in the dentine matrix and its interaction with dental resin monomers. In this study,  $\mu$ TBS value of CHX was greater than PRO, CONT and OG, without a significant difference; whereas it was lower than KTP and OW. According to the results of this study, we are in agreement with other studies that the use of CHX in primary teeth, has no adverse effects on  $\mu$ TBS.<sup>[16-18]</sup>

Awawdeh *et al.*<sup>[19]</sup> found that PRO (30%) is very effective as an intracanal medication in rapidly eliminating *Enterococcus faecalis*. It has been stated that PRO possesses *in vivo* antimicrobial activity against *Streptococcus mutans* present in the oral cavity and might be used as an alternative measure to prevent dental caries. Arslan *et al.*<sup>[6]</sup> evaluated the effect of PRO as a cavity-disinfectant on microleakage of resin composites. They found that the PRO-treated group showed more microleakage than the CONT group when used with self-etch adhesive. However, they attributed this result to the mildly aggressive effect of self-etch adhesive on dentin. So when used with an etch and rinse adhesive, PRO had no effect on microleakage. In this study, there was

no difference between PRO group and CONT group when used with self-etch adhesive.

Evidence from *in vitro* studies using OG remains controversial; some authors found significant inactivation of *S. mutans* in a tooth cavity model.<sup>[8]</sup> The effect of ozone application on dental hard tissues prior to restoration has been poorly investigated. It is considered that the presence of oxygen and other oxidants after ozone application may delay or even inhibit the polymerization process and this may adversely affect the bond strength of dental adhesives. In a study, the influence of ozone on microleakage and penetration of nanoparticle fissure sealing resin and flowable composite was investigated. The results revealed that the treatment of the enamel with ozone after etching did not affect microleakage of either the flowable composite or the sealing resin.<sup>[20]</sup> In another study, ozone application did not negatively influence the leakage scores irrespective of the adhesive system used.<sup>[6]</sup> Magni *et al.*<sup>[4]</sup> reported that ozone treatment did not alter the mechanical properties of adhesive systems. In another study, application of two-step, self-etch adhesive to the ozonated dentin surfaces showed lower bond strength than the CONT group.<sup>[3]</sup> The different results among these studies may be due to the use of different types of adhesives, duration and doses of ozone applications, and variance of the ozone equipment.

Few reports on the use of KTP lasers have been published. KTP laser was used in the root canals for disinfection. In this study, KTP was used as a cavity-disinfectant and evaluated its effect on bond strength. Schoop *et al.*<sup>[21]</sup> 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.

There is not enough evidence about OW's effect on bond strength. In the present study, we found that the use of OW significantly increased  $\mu$ TBS values. OW was used in root canal therapies, but to the authors' knowledge this is the first study with primary teeth. The results with OW were significantly higher results than the other groups, and the reason of this result might be related with the effect of the oxygen. It was shown that OW was able to open the tubular structure by removing organic debris and the increased  $\mu$ TBS values may be affected due to opened tubules.<sup>[22]</sup>

Despite the limitations of the present study, such as the small number of sticks, which was hard to obtain from primary teeth and not waiting for long time in order to see the long-term effects of cavity-disinfectants on resin-dentin bond strength, the results are encouraging and add to those of other studies that have attempted to improve the long-term stability of resin-dentin bonds in the oral cavity.

## Conclusions

Within the limitations of this study, it could be concluded that OW and KTP laser might be used safely as cavity-disinfectants in primary teeth without compromising the bond strength of restorative materials. Further *in vivo* studies with a long-term followup are necessary to compare the effectiveness of OW and KTP laser as cavity-disinfectants.

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**How to cite this article:** Oznurhan F, Ozturk C, Ekci ES. Effects of different cavity-disinfectants and pot! assium titanyl phosphate laser on microtensile bond strength to primary dentin. *Niger J Clin Pract* 2015;18:400-4.

**Source of Support:** Nil, **Conflict of Interest:** None declared.