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## Effects of Chlorhexidine Gluconate and Ozone on Bond Strength

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## **ORIGINAL ARTICLE**

# **Effects of Chlorhexidine Gluconate and Ozone on Bond Strength**

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## **ABSTRACT**

**Objective:** To examine the effects of chlorhexidine gluconate (Chx) and ozone on the bond strength of currently used restorative materials to dentin. **Methods:** Ninety third molar teeth were randomly divided into three groups of 30 each. Samples in Groups 1 and 2 were disinfected with Chx and ozone, respectively, whereas those in Group 3 were not disinfected (controls). Subsequently, the samples in all three groups were further divided into three subgroups of 10 teeth each and restored with Filtek Silorane (a), Gradia Direct (b), or Quixfil (c). Shear force was applied to the samples at 1 mm/min until breaking point. Fracture types were determined by examining the broken surfaces under a stereomicroscope. **Results:** No significant differences in bond strengths were noted between the Chx and control groups. However, the bond strengths in the ozone subgroups were found to be significantly lower than that of the control subgroups ( $p < 0.05$ ). Adhesive type fractures were observed in majority of the treatment groups. **Conclusion:** As Chx did not affect the shear bond strength of the restorative materials, it may be considered for use as a cavity disinfectant before restoration; conversely, ozone should be used with caution for cavity disinfection.

**Keywords:** bond strength, chlorhexidine gluconate, ozone

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## **INTRODUCTION**

One of the main problems in restorative treatment is the incomplete removal of infected dentin tissue following mechanical cleaning of the cavity, which is usually not enough for the elimination of microorganisms that may continue to remain on the walls of the cavity or the smear layer, or within the dentin tubules.<sup>1-3</sup> Hence, the use of cavity disinfectants or antibacterial adhesive systems is recommended to prevent the occurrence of postoperative sensitivity, secondary decay, and pulpal inflammation. Besides laser irradiation, the agents used for cavity disinfection include chlorhexidine gluconate (Chx), hydrogen peroxide, sodium hypochloride, and benzalkonium chloride.<sup>1-7</sup>

Chlorhexidine gluconate is a water-soluble compound with positive weighting that separates easily in physiological pH, and is widely used for disinfection. The antibacterial effect is exerted via the easy binding of the cationic molecules to the negative-

weighted microbial cell wall. Thus, adhesion of the bacteria to the external surface can be prevented by inhibiting the formation of calcium bridges between the bacteria and the external surfaces.<sup>8</sup> In addition to the antibacterial effect, another important aspect of the use of chlorhexidine is that it inhibits the matrix metalloproteinase (MMP) enzymes, which are responsible for the degradation of all extracellular matrix components. Previous research has shown the preventive effects of chlorhexidine against endogenic collagenase activity in the dentin.<sup>9-12</sup>

In recent years, ozone has been presented as an alternative antibacterial disinfectant. Owing to its strong oxidation property, ozone has been shown to fragment the cell membrane thereby releasing the intracellular components. It has been advocated for use in mucosal lesions, temporomandibular joint lesions, cavity and root canal disinfection, the treatment of periodontal pockets, implantology, pedodontology, and whitening procedures.<sup>7,13-17</sup> Furthermore, oxidizing substances such as ozone are reported to have a

negative effect on adhesion during restorative treatment and prevent the polymerization of monomers.<sup>18,19</sup>

Rapid developments in the types of restorative materials used have directed clinicians to the application of more conservative cavity preparations by protecting healthy dental tissue. However, despite these developments, complete success has not been achieved in adhesive bonding between dental hard tissue and resin.<sup>20</sup> The aim of this in vitro study was to examine the effects of Chx and ozone, when used as cavity disinfectants, on the bond strengths of current restorative materials (composite and silorane).

## METHODS

Third molars removed for surgical reasons were cleaned of remaining soft tissues and kept in distilled water at >4 °C until the experiment stage. Smooth dentin surfaces were obtained under water cooling (Plus, CE1023, size 19.0/1.6, diameter 1.58–1.61). To obtain a standard smear layer, the dentin surfaces were sanded with sandpaper (200, 400, or 600 grit; Boch, C355, Switzerland). The teeth were placed in cylindrical plastic molds (2 cm diameter, 3 cm height) with the roots inside and the dentin surface parallel to the occlusal plane. The samples were randomly separated into three groups (n = 30 in each) based on the agents used as follows: Group 1, Chx; Group 2, ozone; and Group 3, control. Each group was further separated into three subgroups (n = 10 each) as follows: a, agent + Silorane Adhesive + Filtek Silorane; b, agent + self-etch adhesive G-Bond + Gradia direct; and c, agent + acid + Prime&Bond NT + Quixfil.

In the Chx group, ceraxidin-C (IMICRYL, Konya, Turkey) was applied to the prepared dentin surfaces with a single-use applicator (902T micro applicators, Premium plus, UK). After 60 s, surplus disinfectant solution was removed with a dry cotton pellet. In the ozone group, prozone (W&H, Bürmoos, Austria) was applied to the prepared dentin surfaces from a distance of 1 mm using a Coro tip in 6 s cavity disinfectant mode. Silorane self-etch primer (3M ESPE, Germany) was applied for 15 s. It was spread using a light air spray and polymerized for 10 s with an LED light source (Light Emitting Diode-Elipar Freelight, 3M ESPE, Germany). Silorane bond (3M ESPE, Germany) was applied and a homogenous layer was formed using a light air spray; polymerization was applied for 10 s. Filtek Silorane (3M ESPE, USA) was applied as layers (2 mm thick) inside clear silicone molds (diameter, 3 mm; height, 4 mm), and each layer was polymerized for 20 s. In the case of Gradia direct, G-bond self-etch adhesive (GC, Tokyo, Japan) was applied without bond distribution; after a 5 s wait, the surface was dried under maximum air pressure for 5 s. Polymerization was performed for 10 s using an LED light source. Gradia Direct (GC, Tokyo, Japan) was applied to the dentin (2 mm-thick

layer) and each layer was polymerized for 20 s in clear silicone molds (diameter, 3 mm; height, 4 mm). In the Quixfil subgroups, the dentin surface was roughened for 15 s with Prime&Bond NT (Dentsply, Germany) and 37% phosphoric acid gel (Etch-37 TM, Bisco, Germany). The samples were washed with water for 5 s and dried with air spray. Prime&Bond NT was applied to the dentin surface for 20 s, dried under a low-pressure air spray for 5 s, and polymerized for 10 s. Quixfil (Dentsply, Germany) was applied to the dentin as layers (2 mm thick); each layer was polymerized for 20 s inside clear silicone molds (diameter, 3 mm; height, 4 mm).

All the prepared samples were incubated for 24 h at 37 °C (Nuve Incubator EN 50, Ankara, Turkey). Subsequently, force (1 mm/min) was applied to the samples in an Instron test device (Esetron, Turkey) until breaking point. The breaking point values were recorded in Newtons (N), and the megapixel (MPa) values were obtained by dividing the N values by the block base area of the resin (Mpa = N/mm<sup>2</sup>). Surface fracture was examined under a stereomicroscope (NIKON SM 800, Japan) at 40× magnification, and photographs were taken (Nikon Coolpix MDC lens, Japan). The fracture type was defined as adhesive, cohesive dentin, cohesive resin, or mixed type.

The study protocol was approved by the Dentistry Ethics Committee of Dicle University (File No: 2015-12) and was supported by the Scientific Research Project Co-ordination Unit of Dicle University (Project No: DIS. 15.009).

## Statistical analysis

Statistical analyses were performed using The data did not conform to normal distribution when assessed with the Kolmogorov–Smirnov test; therefore, the non-parametric Kruskal–Wallis variance analysis method was applied. Mann–Whitney U test with Bonferroni correction was used for paired comparisons between the groups.

## RESULTS

The bond strength values obtained in the study are shown in Table 1. Statistically significant differences in bond strength values were observed during paired comparisons in both Groups 1 and 2 ( $p < 0.05$ ). The lowest bond strength values were seen in Groups 1b and 2b. In the paired comparisons of groups with control, statistically significant differences in bond strength values were found between Group 3b and 3c ( $p < 0.05$ ). The bond strength value of Group 3b was found to be lowest of the three subgroups.

Furthermore, paired comparisons of the groups restored with silorane followed by ozone or chlorhexidine application demonstrated a statistically significant

**Table 1.** Median values of the groups in the shear force resistance tests

Groups	CHX (Mpa)	Ozone (Mpa)	Control (Mpa)
Silorane (Groups 1a, 2a, 3a)	11.01	7.48	14.47
Gradia Direct (Groups 1b, 2b, 3b)	8.07	4.87	11.94
Quixfil (Groups 1c, 2c, 3c)	17.18	10.09	20.95

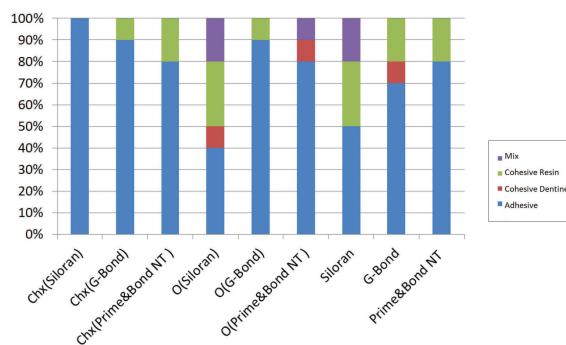
difference ( $p=0.0001$ ) in the bond strength values between Groups 2a and 3a. The bond strength value in Group 2a was found to be the lowest among the three subgroups. A statistically significant difference in the bond strength values between Group 2b and 3b was noted during paired comparisons of the groups restored with Gradia Direct ( $p=0.004$ ). The bond strength value in Group 2b (with ozone) was found to be lower than that of the control Group 3b. Bond strength values between Group 2c and 3c in the paired comparisons of the groups restored with Quixfil presented with a statistically significant difference ( $p < 0.05$ ). The bond strength value in Group 2c was found to be the lowest among the three subgroups.

The fracture types formed in the dentin samples during the shear force resistance test and the percentage distributions are shown in Figure 1. Adhesive fracture type was the most common in all groups.

## DISCUSSION

The clinical success of restorations depends on the continuity and quality of hybridization of dentin bonding, which provides the connection between resin material and dental tissues. The structural properties of dentin tissue, preparation of the surface, and differences in the application methods of bonding are factors that affect the clinical performance of the hybrid layer.<sup>5,21</sup>

The type bonding systems used and the stages at which they are applied are important factors to be considered when the effects of cavity disinfectants on dentin bonding are examined.<sup>22</sup> The application of disinfectants before or after acid use can affect the bonding. By making the surface resistant to roughening, the application disinfectants to the dentin surface can render acid treatment unnecessary.<sup>23</sup> In previous studies, it was shown that Chx had a bacteriostatic effect at a low concentration and to be an intracellular sedimentary bactericide at a high concentration.<sup>1,8,24</sup> It has been reported that 2% chlorhexidine shows an antibacterial effect against *Streptococcus mutans* (*S. mutans*).<sup>6,25</sup>



**Figure 1.** Bar graph illustrating the fracture types and percentages.

In the present study, the effect of 2% Chx on bonding was compared with that of ozone gas. While no statistically significant differences were noted within the chlorhexidine and control groups, the bond strength of Group 1c was found to be statistically significantly higher than that of Group 1b. No statistically significant difference in shear bond strength to the dentin was noted between the two-stage self-etch silorane adhesive systems after chlorhexidine application and the other two groups. In a study by Castro et al. using three different adhesive systems, the application of 2% Chx before and after acid was reported to have no effect on the micro-mechanical bonding of composite to dentin. Acid application increases the bonding tendency of chlorhexidine to the dental surface. Owing to its positive ionic weighting, chlorhexidine increases the surface energy of the dentin surface by binding to phosphate groups on the dental surface. Thus, application of chlorhexidine after acid application can increase the dentin wetting ability of the primer.<sup>23</sup>

The results of the current study showed no difference in dentin bonding strengths between the self-etch G-Bond and the two-stage self-etch silorane groups with chlorhexidine, and between both groups and the control group. A recent study investigating the antibacterial activities of 2% chlorhexidine, 6% hypochloride, and 0.01% urushiol against *S. mutans* reported that 2% chlorhexidine had no effect on bond strength.<sup>26</sup> Jang et al. reported that the application of 2% chlorhexidine had no negative effect on the micro bond strength of composites in single-stage self-etch adhesives to dentin (G-Bond, XenoV, Clearfil S<sup>3</sup> Bond).<sup>27</sup>

Carrilho et al. found that initial bond strengths, immediately after restoration, were not affected by the application of 2% chlorhexidine; however, after 6 months of immersion in synthetic saliva, a reduction in bond strength was noted in both the chlorhexidine and the control groups.<sup>9</sup> Nevertheless, when compared with the initial values, the reduction in the control group following storage in synthetic saliva was 43.5%, whereas that in the chlorhexidine group was found to be 23.4%. After 6 months, the unprotected collagen fibrils,

especially in the inferior part, were better protected in the area most susceptible to enzymatic destruction because they were exposed to chlorhexidine, which was then sealed within the fibrils by adhesive resins.<sup>9</sup> Manfro et al. showed similar bond strength values to dentin in milk teeth restored with Adper single bond 2 and Filtek Z250 and chlorhexidine at concentrations of 0.5% and 2% after acid-etching; both concentrations had no adverse effects on the bond strength to dentin in milk teeth.<sup>28</sup>

Insufficient diffusion of resin monomers in adhesive systems results in incomplete hybridization. Collagen fibrils left unprotected may be left defenseless against hydrolytic deterioration. Even in the absence of bacteria, it is thought that MMPs are activated and are responsible for the auto-degradation of collagen fibrils in the hybrid layer. The application of chlorhexidine after acid-etch and before adhesive systems is thought to slow the deterioration process in the adhesive interface by inhibiting MMP in addition to reducing bacterial counts.<sup>12,29</sup> Chaharom et al. used a cavity disinfectant containing 2% chlorhexidine before using the two-stage self-etch Clearfil SE Bond and the single-stage self-etch Clearfil S<sup>3</sup> Bond adhesive systems; no effect on the bond strengths to dentin was observed.<sup>29</sup>

One of the disadvantages of chlorhexidine application is the increased number of clinical application stages. In recent studies, the use of chlorhexidine mixed with an acid solution in primer or adhesive has been recommended in order to obtain antibacterial, antiproteolytic, re-wetting, and buffering properties at ideal concentrations, thereby simplifying the clinical application stages.<sup>10,30,31</sup> The study by Zhou et al. aimed to determine the ideal chlorhexidine concentration (0.05%, 0.1%, 0.5%, 1%) that could be used in the primer in the two-stage self-etch adhesive system (Clearfil SE Bond) and reported that the initial bonding values were not affected in any of the groups.<sup>31</sup> Sabatini evaluated the inclusion of chlorhexidine in the adhesive rather than topical application of the agent before using the adhesive.<sup>10</sup> However, no differences in initial and 6-month bond strength values were noted between the group with adhesives containing commercially added chlorhexidine and the control group (no chlorhexidine added). In a study by Arslan et al., which examined the bond strengths of cavity disinfectants in silorane-based resin composites (chlorhexidine, sodium hypochloride, propolis, ozone, Er, and Cr: YSGG laser), the disinfectant was applied before the silorane adhesive system.<sup>32</sup> The cavity disinfectant agents tested in the silorane-based resin composite (Filtek Silorane) were reported to have no effect on the bond strength. In the current study, the effect of application of 2% chlorhexidine before silorane self-etch primer and adhesive on shear bond strength was investigated, and chlorhexidine was found to have no statistically significant effect on bond strength when compared with the control groups. Contrary to the findings of the

current study, studies have shown that chlorhexidine increased or decreased bond strength values.<sup>11,33-36</sup> It is thought that if chlorhexidine is used before the self-etch adhesive systems and not removed from the smear layer before acid application, the debris that forms on the dentin surface and in the dentin tubules reduces bond strength. Due to the cationic property of chlorhexidine, it can bind to apatite phosphate groups on the smear layer or the dentin surface and have a negative effect on resin infiltration. Application after acid-etch may leave the surface wet; thus, moisture control after application is important.<sup>33,36</sup>

Despite the proven antibacterial effect of ozone gas on microorganisms, different views have been reported in studies evaluating its effect, as a disinfectant, on bond strength.<sup>3,6,11,8,32,37</sup> Polydorou et al. concluded that the application of ozone might be successful in the elimination of bacteria remaining under restorations, especially, *S. mutans* and *Lactobacillus casei*, two important bacteria responsible for tooth decay.<sup>38</sup> Johansson et al. evaluated the effect of ozone on karyogenic bacterial strains in the presence or absence of saliva and on saliva proteins. Following 60 s of ozone application, almost 100% bacteria were affected by the ozone gas.<sup>17</sup> Yetkiner et al. reported that the bactericidal effects of 6 and 12 s prozone application on *S. mutans* and *Lactobacilli* were not statistically significant, with the 6 s mode proving sufficient for decay prophylaxis.<sup>39</sup> In the current study, when the groups with etch-and-rinse adhesive system (Prime & Bond NT), self-etch adhesive system G-Bond, and self-etch two-stage silorane adhesive system were compared with the control groups, a significant decrease in shear bond strength to dentin was noted in the ozone group. Rodrigues et al. applied ozone before and after acid-etching in an adhesive system containing ethanol.<sup>18</sup> Subsequently, the samples were washed in a 10% sodium ascorbate solution and compared with a control group that had not undergone any procedures. The micro bond values to dentin in the ozone group (before and after acid-etch) were reduced when compared with the control group, and washing with 10% sodium ascorbate solution after ozone application did not make any difference. In addition, to observe whether phosphoric acid was affected by the application of ozone gas, the order of acid application was altered; interestingly, the application of phosphoric acid after ozone gas was found to reduce the micro bond strength.<sup>18</sup>

Ozone is an indeterminate molecule and rapidly decomposes to oxygen. If oxygen is present during polymerization of the adhesive, the process may be affected by the growing monomer chains that react with free radicals. Incomplete polymerization of adhesive systems may reduce the bond strength of the restorative material. Similarly, following dental whitening with hydrogen peroxide or carbamite peroxide, any remaining oxygen may decrease the bond strength.<sup>18</sup>

Schmidlin et al. examined the effect on bond strength to enamel and dentin following whitening with 35% hydrogen peroxide alone or in conjunction with ozone or a Healozone reductant.<sup>37</sup> No statistically significant differences in the bonding to enamel between the ozone application group (60 s of application; Healozone, KaVO, Brugg, Switzerland) and the control group (no application) were noted. In the enamel samples, the ozone only group showed better bonding values than the group treated with the whitening agent alone and the group in which the Healozone reductant was used. The use of a reductant fluid after the application of ozone reduced the bond strength. Hence, the results of that study showed that whitening had a negative effect on bond strength in enamel samples. In the dentin samples, the bonding values in the control group were seen to be higher than that of the groups where the Healozone reductant and hydrogen peroxide were used, statistical significance notwithstanding.<sup>37</sup>

The study by Pires et al. examined the effect of ozone on bond strength in the enamel; no statistically significant difference was reported in the groups applied with ozone (20 s) in the Excite and AdheSE adhesive systems when compared with the control group.<sup>40</sup> Moreover, the bond strength values in the Excite adhesive system samples (G1, Excite + ozone; G2, Excite) were found to be higher than that of the AdheSE adhesive system samples (G3, AdheSE + ozone; G4, AdheSE). After 24 h, ozone gas was found to have no effect on the bond strength to enamel.<sup>40</sup>

Arslan et al. researched the effects of disinfectants on bonding in silorane composites and reported that ozone did not affect bonding to dentin.<sup>32</sup> In another study, 2% chlorhexidine and ozone were used as disinfectants in the adhesive system (Prime&Bond NT) before the application of acid in milk teeth<sup>3</sup>. Statistically significant differences in bond strength were reported between the test groups; the chlorhexidine group showed lower bond strength values than the control and ozone groups. Furthermore, the application of ozone was reported to increase the bond strength.<sup>3</sup> Magni et al. examined the effect of Prime&Bond NT, Excite, Syntac/Heliobond, and silorane on the mechanical properties and reported that the application of 120 s of ozone gas before the bonding procedure did not constitute a risk.<sup>41</sup> Irrespective of the chemical composition and solvent content, ozone gas treatment did not impair the mechanical properties of these adhesives. The effect of ozone application before the bonding procedure on the characterization of the adhesive layer has not been clarified<sup>11</sup>. The findings of the above-mentioned studies demonstrate that different results can be obtained depending on the laboratory conditions, duration of ozone application, non-standardization of doses, type of dental tissue, and the contents of the adhesive system.

In the current study, we aimed to identify the fracture types that occurred during the shear bond tests in the experimental groups. In previous studies, cohesive fractures between the surfaces have been evaluated as high bond resistance, whereas adhesive fractures are considered as low bond resistant.<sup>42-46</sup> The observation of cohesive fracture types with high shear bond resistance in the control group in the present study supports the findings of previous studies. Adhesive fracture type was seen in the majority of samples as a breakage at the dentin and adhesive interface.

## CONCLUSION

The shear bond strength in the samples with chlorhexidine was not significantly different when compared with that of the control groups, suggesting that chlorhexidine could be used as an antibacterial agent following the two-stage silorane adhesive and self-etch adhesive systems and etch-and-rinse before restoration. As the shear bond strength in the samples applied with ozone was found to be significantly low when compared with the control groups, it should be used with caution; additional *in vitro/in vivo* studies determining the reasons for this decrease in shear bond strength are warranted.

## CONFLICT OF INTEREST

The authors declare that there were no conflicts of interest related to this study.

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