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ORIGINAL ARTICLE

Effect of different disinfectant methods on the initial microtensile bond strength of a self-etch adhesive to dentin

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Abstract The aim of this in vitro study was to evaluate the effect of different disinfection methods on the initial microtensile bond strength of a two-step, self-etch adhesive to dentin. Twenty mandibular molars were sectioned parallel to the occlusal plane to expose the mid-coronal dentin. All of the teeth were divided into four groups ($n=5$ per group): (1) in group OZ, the dentin surfaces were exposed to ozone gas from the Ozonytron X delivery system (OzonyTron X-Bioozonix, Munich, Germany), (2) in group ND, the dentin surfaces were irradiated with an Nd:YAG laser (Pulsmaster 600 IQ, American Dental Technologies, U.S.), (3) in group CHX, the dentin surfaces were treated with a 2% chlorhexidine solution, and (4) in the control group, no treatment was applied. In all of the groups, the teeth were restored with Clearfil SE Bond (Kuraray, Tokyo, Japan) and Clearfil Majesty Posterior (Kuraray, Tokyo, Japan), according to the manufacturer's instructions. The teeth were sectioned perpendicular to the bonded surface (surface area of approximately 1 mm^2). Thus, six to seven specimens were obtained from each tooth, and a total of 34 specimens were analyzed in each group. The specimens were attached to the microtensile test machine (Micro Tensile Tester, T-61010 K, Bisco, U.S.). The data was analyzed using the one-way analysis of variance (ANOVA) and Tukey test ($p<0.05$). Fracture modes of each specimen were determined using a stereomicroscope (SZ-PT Olympus, Tokyo, Japan) and a scanning electron microscope (SEM). The lowest bond strength occurred in the OZ group. Significant differences were determined only between group OZ and the other groups (group ND, group CHX, and control group) ($p<0.05$). In conclusion, although ozone decreased the microtensile bond strength of the self-etch adhesive system to dentin, the Nd:YAG laser and 2% chlorhexidine did not change the microtensile bond strength so in context of the present study it would appear that the Nd:YAG laser and 2% chlorhexidine may be used as pre-restorative sterilization procedures on the dentin prior to the application of a two-step, self-etch adhesive.

Keywords Ozone · Nd:YAG laser · Microtensile · Bond strength · Chlorhexidine

Introduction

The primary way that dentists treat the clinical signs of infection caused by caries is through the surgical removal of the diseased parts of the tooth structure and obturation of the area with an inert filling material [1]. 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, which may induce not only secondary caries but may also damage the pulp [2].

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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 [3]. Investigators found that fermentative organisms remained viable under non-antiseptic restorations for as long as 139 days [4]. To remove all the bacteria from the cavity preparation and to reduce the potential for residual caries, an antibacterial solution has been suggested in addition to the physical removal of carious dentin for the disinfection of dentinal cavities [5, 6]. Chlorhexidine contains chlorhexidine gluconate that binds to the amino acids in the dentin and continues to kill bacteria for several hours, making it a good antibacterial agent [7]. In addition to CHX, the Nd:YAG laser and, more recently, gaseous ozone have been considered an alternative treatment to disinfect the dentinal cavities. It has been reported that ozone has a strong oxidizing power with a reliable microbicidal effect [8]. Previous studies suggested that ozone treatment kills microorganisms via a mechanism involving the rupture of their membranes [9, 10]. However, some researchers have stated that chemical disinfection methods have an inhibitory effect on the bond strength of adhesive techniques [11]. In this study, a two-step, self-etch adhesive was used to determine the effect of disinfection methods on bond strength. Recently, self-etch adhesives have become popular because they lessen the clinical application time and significantly reduce technique sensitivity [4]. Among self-etch adhesives, Clearfil SE bond produced excellent results for in vitro and in vivo studies and therefore can be considered the gold standard [4, 12, 13].

Thus, the aim of this study was to evaluate the effect of different disinfection methods on the initial micro-tensile bond strength of a two-step, self-etch adhesive to dentin. The null hypothesis is defined as "no difference among the disinfectant methods on the initial micro-tensile bond strength of two-step, self-etch adhesive to dentin."

Materials and methods

Twenty extracted mandibular third molars were used in this study. The teeth were cleaned with a toothbrush and water and then stored in sterile saline. The experimental setup is schematically presented in Fig. 1. A diamond bur with a water coolant was used to section the teeth parallel to the occlusal plane to expose the mid-coronal dentin. To create a standardized smear layer, each of the dentin surfaces was polished along the cut surface with the same series of wet silicon carbide disks (#600, #800, and #1000) and rinsed under water for 60 s. Then all of the teeth were randomly divided into four groups. In group OZ, the dentin surfaces were exposed to ozone gas using the Ozonytron X delivery system (OzonyTron X-Biozonix, Munich, Germany) with a GI probe for 60 s, at 1 mm from the cavity surface. In group ND, the dentin surfaces were irradiated with an Nd:YAG laser (Pulsmaster 600 IQ, American Dental Technologies, US) with 120 mJ/20 Hz for 20 s with a fiber-optic tip scanning the cavity surface. In group CHX, the dentin surfaces were treated with 2% chlorhexidine gluconate for 40 s. In the control group, no treatment was applied on the dentin surfaces. In all of the groups, the teeth were restored with a two-step, self-etch adhesive (Clearfil SE Bond, Kuraray, Tokyo, Japan) and a hybrid composite resin (Clearfil Majesty Posterior, Kuraray), according to the manufacturer's instructions. The samples were stored at 37°C for 24 h at 100% humidity and the teeth were sectioned perpendicular to the bonded surface using a low-speed diamond saw (Mecatome T201A, Pressi, France) to form a surface area of approximately 1 mm^2. Thus, six to seven specimens (four times 7 and once 6) were obtained from each tooth and in total 34 specimens were analyzed in each group. The final width and thickness of the bonded surfaces was measured with a digital caliper (Tresna, Guangxi Province, China). The specimens were then attached to the microtensile test machine (Micro Tensile Tester, T-61010 K, Bisco, US) with a cyanoacrylate adhesive (Zapit, Dental Ventures of America, Corona, CA, USA) and subjected to tensile stress at a crosshead speed of 1 mm/min. Bond strengths were calculated by dividing the failure load by the cross-sectional area of each specimen. In the current study, the test samples were prepared by only one person. The data was analyzed using the ANOVA and Tukey test ($p<0.05$). After the microtensile bond test, the fracture modes of each specimen were determined using a stereomicroscope (SZ-PT Olympus, Tokyo, Japan) at a magnification of×40 by one single operator. Fracture modes were classified into four categories: adhesive failure at the resin/dentin interface, cohesive failure in dentin, cohesive failure in composite and mixed adhesive failure at the resin/dentin interface, which included cohesive failure of the neighboring substrates. From each subgroup, five pairs of fractured composite-dentin specimens were prepared for observation under an SEM (JSM-840 A JOEL-Technique Co. Ltd, Tokyo, Japan) observation. To evaluate the debonding surface and fracture mode, both the dentin and composite sides of the fractured specimens were mounted on the slubs, gold sputter-coated, and examined under an SEM.

Results

The microtensile bond strength of each group and statistical comparisons are shown in Table 1. The lowest bond strength was determined in group OZ (12.09±5.49). There was a significant difference between group OZ and other groups (group ND, group CHX, and the control group) ($p<$

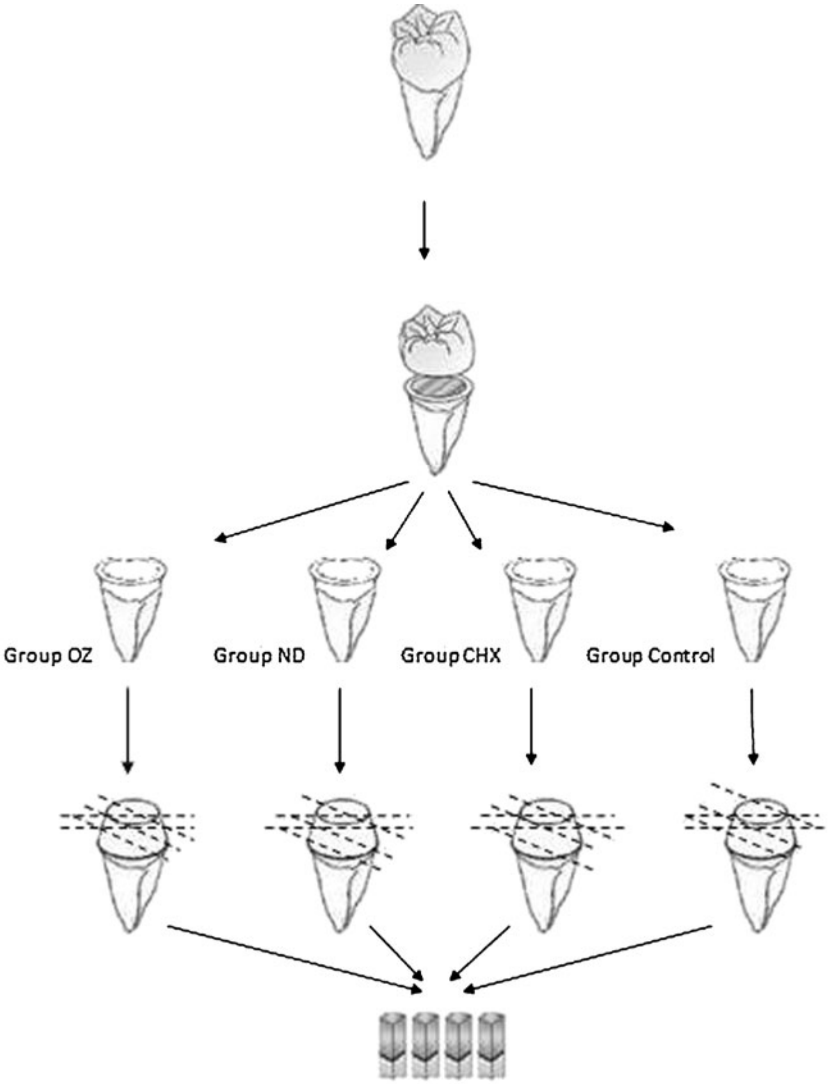

Fig. 1 Schematic representation of the study design

0.05). The mean micro-tensile bond strength values of group ND, group CHX, and the control group were 16.64±4.98, 19.06±6.19, and 17.30±7.88, respectively. There was not a significant difference between group ND, group CHX, and the control group ($p > 0.05$). Fracture modes of the groups are shown in Table 2. In all of the groups, most of the specimens showed an adhesive failure. Most of the adhesive failures were seen in the control group ($n = 32$). The number of adhesive failures in groups OZ, ND, and CHX were 30, 30, and 26, respectively. SEM illustrations of the fracture modes of the groups are in Figs. 2, 3, 4, and 5.

Discussion

Elimination of pathogenic bacteria from carious lesions cannot usually accomplished by mechanical removal [14]. Secondary caries have been reported as one of the most common reasons for a restoration's failure and may be related to the presence of residual bacteria under the restorations [15–17]. However, some researchers analyzed the effect of leaving residual caries and removing the soft dentin in the treatment groups [18, 19]. Ribeiro et al. reported that the clinical performance of the restorations on deciduous teeth was not adversely affected by the incomplete caries removal after a year [18]. Similarly, Kidd

Table 1 Mean values and standard deviation (SD) of microtensile bond strength ($n = 34$)

Groups	Mean (SD)
Group OZ	12.09 (5.49) A
Group ND	16.64 (4.98) B
Group CHX	19.06 (6.19) B
Control group	17.30 (7.88) B

Different cases represent the significant difference between the groups ($p < 0.05$)

Table 2 Distribution of pattern of failures (n=34)

Groups	Adhesive	Dentin cohesive	Composite cohesive	Mixed
Group OZ	30	-	4	-
Group ND	30	2	1	1
Group CHX	26	2	3	3
Control group	32	1	1	-

recommended the removal of partial caries in the deep caries to reduce the risk of carious exposure [19]. In addition, Gultz et al. stated that the problems associated with microleakage can be magnified by incomplete sterilization of the cavity as a consequence of a failure to mechanically remove infected tooth structures [20]. Martin et al. determined that the presence of bacteria in dentin and proximity to the pulp have clearly been associated with pulpal inflammation [2]. To reduce the potential for residual caries, an antibacterial solution or technologies like laser and ozone may be alternative treatment approaches for disinfecting the dentin after the cavity is prepared [5].

Ozone is a naturally occurring compound of three oxygen atoms. There are many advantages of using ozone as a potent oxidizing agent in food and other industries. Ozone is potentially useful for decreasing the microbial load and the level of toxic organic compounds [21]. Ozone, in its gaseous or aqueous phase, has been shown to be a powerful and reliable antimicrobial agent against bacteria, fungi, protozoa, and viruses [21, 22]. Baysan et al. determined that there was a significant reduction in *S. mutans* and *S. sobrinus* in ozone-treated samples [23]. In another study, Baysan and Lynch found that ozone treatment significantly reduced the number of microorganisms and re-mineralized most of the root carious lesions [24]. Therefore, the application of ozone on dental hard tissues prior to restoration has recently been proposed for disinfecting the cavity surfaces [23, 24]. However, the effect of an ozone application on dental hard tissues prior to restoration has been poorly investigated, and there is no definitive information about the bond strength of adhesives in ozonated dentin surfaces. In the present study, ozone gas, an Nd:YAG laser, and 2% chlorhexidine were compared as cavity disinfectants prior to the application of a two-step, self-etching dentin bonding agent for determining the effects on initial bond strengths.

It was reported that ozone did not negatively influence the micro-leakage and penetration of a sealant and did not impair the shear bond strength of dental adhesives to bovine dentin and enamel [25, 26]. Magni et al. reported that ozone treatment did not damage the mechanical properties of adhesive systems [6]. Cadenaro et al. reported that the use of ozone gas to disinfect the cavity before placing a restoration had no influence on immediate enamel and dentin bond strength [27]. Similar to these results, Gurgan et al. stated that pretreatments with ozone did not impair the two-step, self-etch adhesive Clearfil SE to coronal dentin [28]. However, in the current study, the application of two-step, self-etch adhesive to the ozonated dentin surfaces showed lower bond strength than in the control group. The different results among studies may be due to the use of different types of adhesives, time periods of ozone applications, and doses of the various ozone equipment. Glantz reported that the ozone treatment

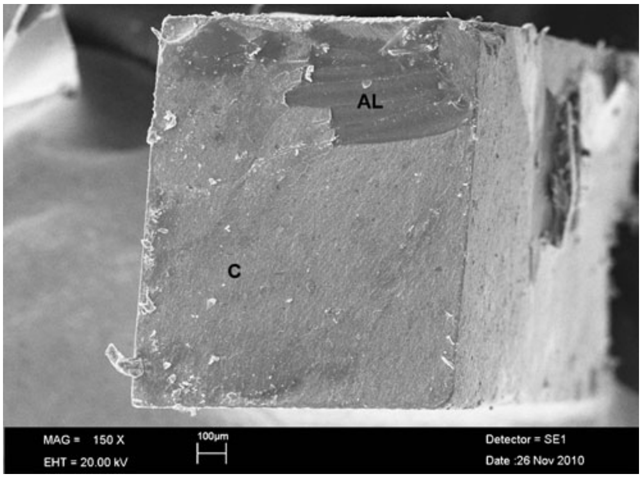

Fig. 2 SEM observation (×150) of fracture surface of group OZ. Mixed failure. *AL* adhesive layer, *C* composite

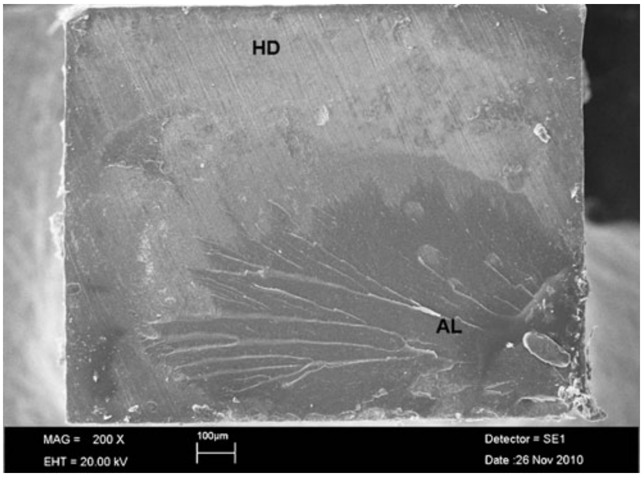

Fig. 3 SEM observation (×200) of the fracture surface of group ND. Mixed failure. *HD* hybridized dentin, *AL* adhesive layer

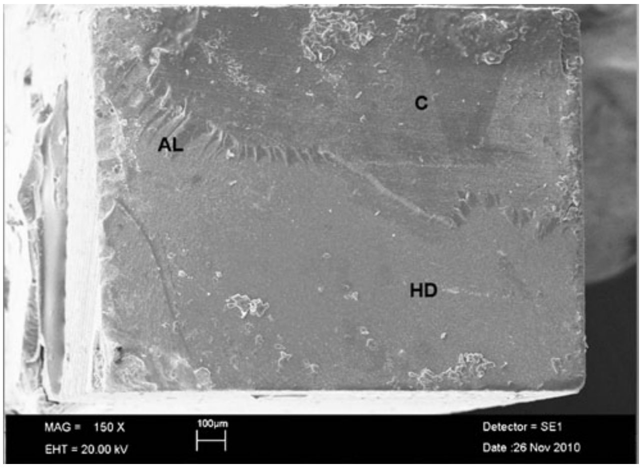

Fig. 4 SEM observation (×150) of the fracture surface of group CHX. Mixed failure. *HD* hybridized dentin, *AL* adhesive layer, *C* composite

reduced the wettability of the dentin surfaces [29]. Ozone is a strong oxidizing agent that can react with almost every organic material. In the case of a dentin surface, the possibility exists that the treatment altered organic surface constituents, such as collagen [30]. This changed surface may have negatively altered the bond strength of the self-etch adhesive.

Among all available antimicrobials in dentistry, chlorhexidine is still the most frequently used agent to reduce plaque, control caries, as it has a strong antibacterial effect on oral bacteria, notably *S. mutans* [31]. The use of chlorhexidine-containing products as a cavity disinfectant after tooth preparation could help to reduce the potential for residual caries and post-operative sensitivity [5]. However, some researchers have reported that the positive benefits would be negated if the solution decreased the composite resin bond strength to dentin. SEM examination by Meiers and Kresin showed that cavity disinfectants applied to dentin surfaces were resistant to acidic conditioning [5]. This acid-resistant layer might inhibit the impregnation of the adhesive resin to the dentin surface. Gürgan et al. determined that a cavity disinfectant of 2% chlorhexidine decreased the shear bond strength of the total-etch adhesive to dentin in the center of the buccal surface [11]. However, other studies showed that the application of chlorhexidine did not have a negative effect on shear bond strength [32–34]. Similarly, in the current study, no significant difference was determined between CHX and the control group. Moreover, the mean microtensile bond strength values of CHX are a bit higher than the control group. In other words, the results of the current study show that 2% chlorhexidine as a cavity disinfectant did not negatively affect the bond strength of two-step, self-etch adhesive Clearfil SE.

Nd:YAG lasers can be used in many clinical procedures, such as caries ablation, reduction of bacterial contamination, and treatment of root canals, reduction of dentinal hypersensitivity, remineralization of incipient dental caries, and pits and fissure sealing [35–38]. In addition, previous studies found that the Nd:YAG laser altered and modified the dentin and enamel surfaces [39, 40]. When examining SEM photographs of dentin irradiated by an Nd:YAG laser, melting and resolidification of dentin was observed [41]. Researchers reported that surface modification with a laser may increase the tissues' microhardness or decrease their permeability [39, 40]. Ferreira et al. determined that an Nd:YAG laser negatively influenced the bond strength of adhesive systems to dentin [42]. Paranhos et al. determined that an Nd:YAG laser decreased the bond strength of two different adhesive systems to dentin [43]. Rolla et al. reported that the application of an Nd:YAG laser to dentin provided an increase in the bond strength values of one-step self-etch adhesives [44]. However, Gürgan et al. stated that an Nd:YAG laser did not impair the bond strength of adhesive systems to coronal dentin [28]. In the current study, an Nd:YAG laser (120 mJ/20 Hz) was used as a cavity disinfectant because of its bactericidal activity. This laser application did not influence the bond strength of Clearfil SE Bond and did not seem to alter the dentin surfaces in this application mode and time, which may account for the reduction of the bond strength of adhesives. In the content of the present study, it would appear that an Nd:YAG laser and 2% chlorhexidine may be used as pre-restorative sterilization procedures on the dentine, prior to the application of a two-step, self-etching dentine bonding agent, without decreasing the bond strength to dentine.

In the current study, the effect of different disinfectant methods on initial (24-hour) bond strength of two-step, self-etch adhesive was determined, but the long-term effectiveness of these groups was not studied. To assess long-term effectiveness, it is crucial to first determine the initial bond

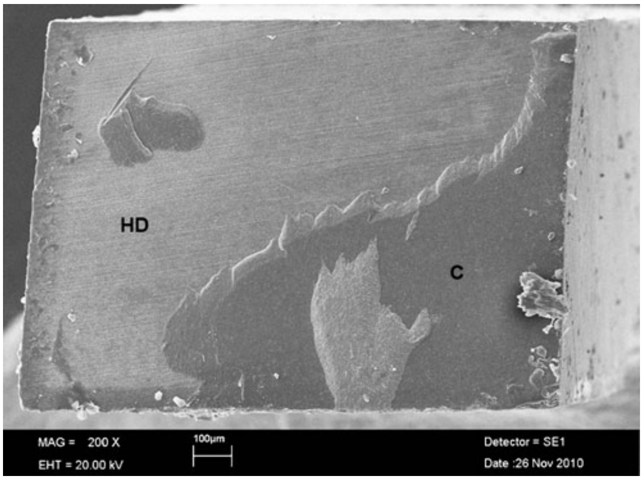

Fig. 5 SEM observation (×200) of fracture surface of control group. Mixed failure. *HD* hybridized dentin, *C* composite

effectiveness of the adhesives. These initial bond strength results can then serve as baseline data. The long-term bond strength of these groups may be studied in other research. The low number of teeth (five teeth per group) and the obtainment of six to seven specimens from each tooth may be another limitation of the current study. In addition to that, all of the specimens were selected from the central part of the tooth to eliminate substrate regional variability. Van Landuyt et al. used only the four central specimens from each tooth in their microtensile bond strength study, to eliminate substrate regional variability [45]. Another limitation of the current study is that only one type of adhesive system (self-etch) was used. Another type of adhesive system, like total-etch, could be tested and the effect of these disinfectant methods on bond strength could be studied. However, in many previous studies, the two-step, self-etch adhesives were defined as the gold standard and their in vitro and in vivo effectiveness approach to total-etch adhesive systems was reported [4, 12, 13].

Within the limitations of this in vitro study, the null hypothesis was rejected because for cavity disinfection:

1) An Nd:YAG laser and 2% chlorhexidine did not change the microtensile bond strength of two-step, self-etch adhesive system to dentin.
2) Ozone decreased the microtensile bond strength of two-step, self-etch adhesive to dentin.

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