

Effect of ozone gas on the shear bond strength to enamel

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ABSTRACT

Ozone is an important disinfecting agent, however its influence on enamel adhesion has not yet been clarified. Objective: Evaluate the influence of ozone pretreatment on the shear strength of an etch-and-rinse and a self-etch system to enamel and analyze the respective failure modes. Material and Methods: Sixty sound bovine incisors were used. Specimens were randomly assigned to four experimental groups (n=15): Group G1 (Excite[®] with ozone) and group G3 (AdheSE[®] with ozone) were prepared with ozone gas from the HealOzone unit (Kavo[®]) for 20 s prior to adhesion, and groups G2 (Excite[®]) and G4 (AdheSE[®]) were used as control. Teeth were bisected and polished to simulate a smear layer just before the application of the adhesive systems. The adhesives were applied according to the manufacturer's instructions to a standardized 3 mm diameter surface, and a composite (Synergy D6, Coltene Whaledent) cylinder with 2 mm increments was build. Specimens were stored in 100% humidity for 24 h at 37°C and then subjected to a thermal cycling regimen of 500 cycles. Shear bond tests were performed with a Watanabe device in a universal testing machine at 5 mm/min. The failure mode was analyzed under scanning electron microscope. Means and standard deviation of shear bond strength (SBS) were calculated and difference between the groups was analyzed using ANOVA, Kolmogorov-Smirnov, Levene and Bonferroni. Chi-squared statistical tests were used to evaluate the failure modes. Results: Mean bond strength values and failure modes were as follows: G1- 26.85±6.18 MPa (33.3% of adhesive cohesive failure); G2 - 27.95±5.58 MPa (53.8% of adhesive failures between enamel and adhesive); G3 - 15.0±3.84 MPa (77.8% of adhesive failures between enamel and adhesive) and G4 - 13.1±3.68 MPa (36.4% of adhesive failures between enamel and adhesive). Conclusions: Shear bond strength values of both adhesives tested on enamel were not influenced by the previous application of ozone gas.

Key words: Shear strength. Dental bonding. Dental enamel. Ozone.

INTRODUCTION

Ozone is an important disinfecting agent due to its antibacterial action. Recent research revealed the bactericidal action of ozone against *S. mutans* and other bacteria commonly found in cervical carious lesions^{13,23}. In these studies, a 20 s HealOzone application resulted in a 99.9% reduction in microorganisms⁴.

Since it is not possible to confirm clinically that a dental cavity is bacteriologically aseptic, an

antibacterial treatment of the dental surface prior to cavity restoration has been advised^{6,22}. This can be achieved by ozone application to prevent secondary caries and thus, restoration failures³. However, studies of ozone influence on adhesion are very rare and few have been published⁷. Due to its strong oxidizing effect, ozone might have negative consequences on resin-tooth adhesion, since oxygen is a well-known polymerization inhibitor⁹. It has been demonstrated that oxygen and other oxidant agents (such as bleaching agents)

have a negative influence on dental adhesives bond strength²⁹.

Resin-enamel adhesion is one of the most significant advances in the history of dentistry and it is used in our days as a simple effective procedure when using an etch-and-rinse technique. However, the resin-dentin adhesion had to be improved through the years and new adhesive systems have been developed for that purpose^{11,20}. Self-etch systems based on acidic monomers that simultaneously condition and prime dental tissue, were developed to simplify and eliminate some of the clinical steps associated to wet dentin-bonding²⁰. With these systems, primer application dissolves the smear layer and incorporates it into the bonding process; as a result the tooth no longer requires rinsing, as it does with etch-and-rinse systems.

The first evaluations of these new adhesive systems have shown satisfactory bond strengths to dentin, while adhesion to enamel was less effective. One of the questions that arises with the use of self-etch adhesive systems is whether the acidic monomer is capable of promoting enamel demineralization and developing a reliable and durable bonding to this tissue¹⁸. In most studies¹² the highest mean bond strengths were obtained with etch-and-rinse adhesives to intact or roughened enamel. Also, shear bond strength (SBS) evaluation of brackets and etch-and-rinse systems exhibited fewer failures when compared to self-etch systems¹⁰.

Since bonding procedures have to take into account not only adhesion to dentin but also the proximity of cavity margins to the prepared or intact enamel, the effectiveness of these adhesive systems, in terms of bond strength to enamel, has to be ascertained. Compromised bond strength to enamel results in microleakage and subsequent failure of the restoration over a period of time²¹. This fact can be even more relevant if polymerization

of the adhesive is compromised by the use of a potential inhibitor like ozone.

In light of these developments, this study was undertaken to determine whether ozone gas pretreatment interferes with the SBS of an etch-and-rinse (Excite®) and a self-etch adhesive system (AdheSE®) to enamel.

MATERIAL AND METHODS

Sixty sound bovine incisors were extracted and kept in distilled water at 4°C, for no longer than a month. After this period, teeth were kept in a 0.5% chloramine solution for a week and sectioned in a microtome (Accuton-Struers, Ballerup, Denmark) to separate the crown from the root. The buccal surface was gradually polished with ascending grades of silicon-carbide sandpaper up to 320-grit (Carbimet Buehler-met, Buehler, Lake Bluff, IL, USA) to create a flat surface and simulate a smear layer. Polyester film (Mylar, Dupont Corp., DE, USA), with a 3 mm diameter hole was used to confine the adhesion area. Materials used in this study are listed in Figure 1 along with the manufacturers' compositions, batch numbers and code numbers.

Specimens were randomly assigned to 4 experimental groups (n=15), and composite resin cylinders were added to the tested surfaces after the application of the adhesive, according to the manufacturer's instructions. G1 (Excite® with ozone [Ivoclar Vivadent AG, Liechtenstein]) and G3 (AdheSE® with ozone [Ivoclar Vivadent AG, Liechtenstein]) were conditioned for 20 s with ozone gas from the HealOzone unit (Kavo®, Dental GmbH, Bismarckring, Germany - continuous stream of ozone of 615 cc/min with a concentration of 2,100 ppm, more or less 5%) using a 5 mm delivery cup (green) immediately before the adhesive procedures. G2 (Excite®) and G4 (AdheSE®) were used as controls. Specimens were then kept in distilled water for 24 h at 37°C to obtain the

Material	Function	Composition	Batch#
Excite® (Ivoclar Vivadent AG, Liechtenstein)	Adhesive system	Mixture of dimethacrylates, alcohol, phosphonic acid acrylate, HEMA, SiO ₂ , potassium fluoride, initiators and stabilizers	J01968
AdheSE® (Ivoclar Vivadent AG, Liechtenstein)	Adhesive system	Primer: Mixture of dimethacrylat, phosphonic acid acrylate, water, initiators and stabilizers; Bond: Bis-GMA, HEMA, GMDA,	J03385
Total Etch® (Ivoclar Vivadent AG, Liechtenstein)	Acid conditioner	37% phosphoric acid	J17398
Synergy D6 (Coltene whaledent GmbH + Co. KG Germany)	Restorative material	Methacrylates, silanized barium glass, hydrophobed amorphous silica	145721

Figure 1- Restorative and adhesive materials

maximum resin polymerization, before being thermally cycled (Aralab, mod 200E, Cascais, Portugal) for 500 cycles between 5°C and 55°C for 20 s in each bath and submitted to shear testing at a crosshead speed of 0.5 mm/min (Instron, Model 4502, Series H3307, Instron Ltd, Bucks, UK). The mode of failure was analyzed under a field emission scanning electron microscope (SEM; JEOL JSM 6301F/Oxford INCA Energy 350/Gatan Alto 2500, Tokyo, Japan). Specimens were dehydrated in ascending grades of ethanol and sputtered-coated with gold. Failures were classified as adhesive, cohesive (composite or enamel) or mixed. From the analysis of assumptions, The Kolmogorov-Smirnov test was used to check for normality of data among the groups. SBS means and standard deviations were calculated and the differences between the groups were analyzed by one-way ANOVA. The Levene test was used to determine homoscedasticity. The Bonferroni method was employed for multiple comparisons of results and Chi-squared statistical tests were used to evaluate the failure modes. A significance level of 5% was set for the statistical analyses.

RESULTS

SBS Mean values were as follows: Mean bond strength values and failure modes were as

follows: G1 - 26.85 ± 6.18 MPa (33.3% of adhesive cohesive failure); G2 - 27.95 ± 5.58 MPa (53.8% of adhesive failures between enamel and adhesive); G3 - 15.0 ± 3.84 MPa (77.8% of adhesive failures between enamel and adhesive) and G4 - 13.1 ± 3.68 MPa (36.4% of adhesive failures between enamel and adhesive).

No statistically significant differences were found between G1 and G2 ($p > 0.05$) or between G3 and G4 ($p > 0.05$). Both G1 and G2 showed significantly higher mean SBS values than those of G3 or G4 ($p < 0.05$).

As observed with SEM, the enamel surfaces conditioned by the acid from the etch-and-rinse adhesive system showed a more defined etching pattern than did the ones treated with the self-etch system (Figure 2).

Regarding the failure mode, the results are presented in Figure 3. Chi-squared statistical analyses were used to compare the type of fractures. The different values were not statistically significant. In this study, the most frequent type of fracture was adhesive between enamel and adhesive, which occurred 46.7% of the time. The less frequent type of fracture was adhesive between the adhesive and resin (8.9%).

Figures 4 to 6 show SEM micrographs of a typical adhesive fracture and a mixed fracture where the three layers appear in the same proportion.

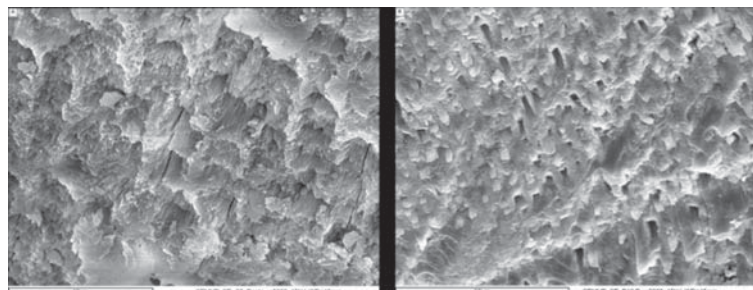


Figure 2- FE-SEM (scanning electron microscope) micrographs at 2,000x of enamel surfaces after application of: A: a total-etch adhesive system (Excite®) and B: a self-etch adhesive system (AdheSE®)

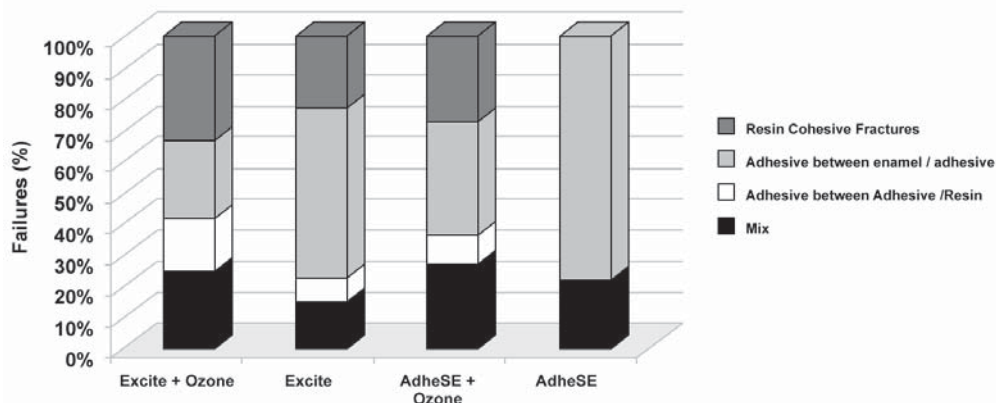


Figure 3- Different failure modes per group, in percentage

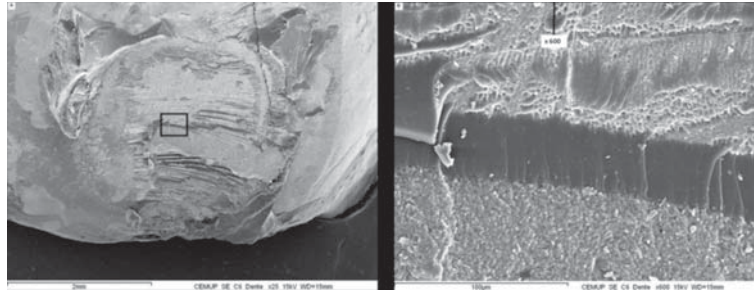


Figure 4- FE-SEM (scanning electron microscope) micrographs at 25x (A) and 600x (B) magnification. At 600x, three zones can be identified: the enamel on the top, on the middle there is the adhesive layer and at the bottom is the composite. This represents a typical adhesive failure mode, between adhesive and composite

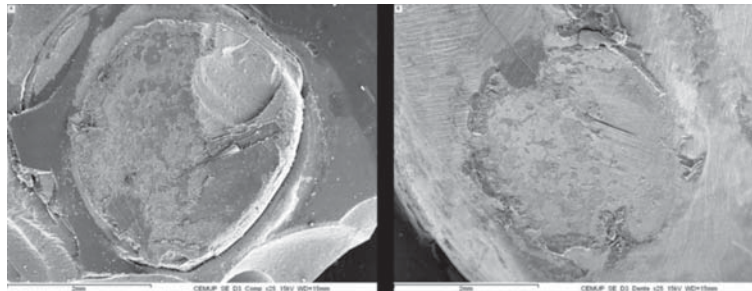


Figure 5- FE-SEM (scanning electron microscope) micrograph at 25x magnification of a mixed failure. Both composite (A) and enamel (B) surfaces show adhesive, composite and enamel layers in the same proportion

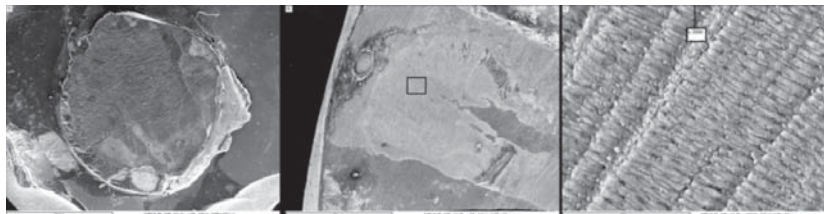


Figure 6- FE-SEM (scanning electron microscope) micrographs at 25x magnification, showing composite surface mostly covered by adhesive (A) and enamel surface free of adhesive (B) and at 2,000x magnification, showing enamel surface, with visible enamel prisms (C)

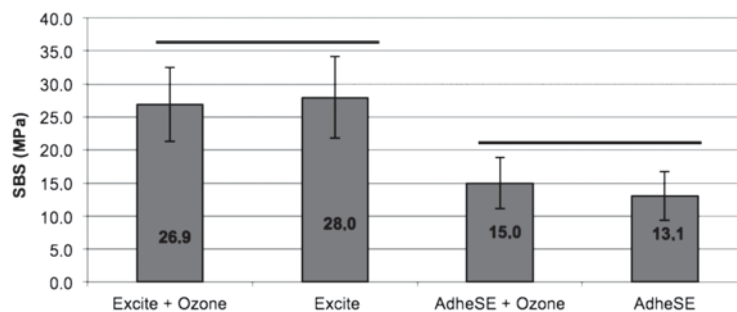


Figure 7- Mean shear bond strength (SBS) values (MPa±standard deviation). Groups under the same line show no significant differences

DISCUSSION

The application of ozone to enamel may be important for cavity disinfection in several situations, as for example, before fissure sealants

or orthodontic bracket adhesion. Recent studies^{2,16} led to substantial evidence that indicates *in vitro* application of ozone as a useful prophylactic antimicrobial treatment, prior to etching and placement of dental sealants and restorations. However, like bleaching systems and other

potential oxidants, ozone may cause inhibition of polymerization, leading to a decrease in conversion degree of the adhesive, which may in turn lead to a decrease in adhesion^{19,26}.

Two different bonding systems were tested in the present work, each one chosen to represent a distinct category of available materials: an etch-and-rinse (Excite®) and a self-etch (AdheSE®) adhesive system.

Etch-and-rinse adhesives are applied on a demineralized substrate, due to preliminary application of phosphoric acid followed by extensive rinsing with water. Therefore, we may speculate that the etching removes both superficial mineralized components and residual oxidants. On the other hand, with self-etch adhesive systems no rinsing occurs, and residual oxygen may be incorporated within the smear layer and smear plugs, making this adhesion more susceptible to oxygen.

Conversely, the result of this *in vitro* study showed no influence of ozone in 24 h SBS values of both tested adhesive systems. This fact could be related to the instability of ozone. Once delivered to the enamel surface, gaseous ozone is unable to maintain its chemical stability and decomposes completely, reacting instantaneously with the organic compounds¹⁴. Thus, we may speculate that only a residual amount of gaseous ozone is present on the dental substrate during bond resin application.

In the present study, ozone was applied to enamel for 20 s. According to the literature, after this period, ozone seems to eliminate 99.9% of bacteria present³, despite some more recent microbiological studies suggesting that gaseous ozone should be applied for a more sustained time²⁴. The very short time of ozone gas application when compared with the application time of other oxidants, like bleaching agents, could be another explanation for the lack of ozone effect on SBS values in the present study^{7,8}.

Another critical factor that could potentially affect the adhesion to an ozone-pretreated substrate is ozone concentration. In a recent study, the use of an ozone generator operating at a high concentration showed that ozone might impair the bond strength of an etch-and-rinse adhesive system to dentin²⁴.

Our findings also showed that SBS values were much superior for etch-and-rinse systems than for the self-etch systems, independent of ozone application.

Two-step self-etch adhesive systems, like AdheSE®, were developed to reduce application time and technical sensitivity of the adhesion process, since they may substitute wet bonding²⁸. However, our findings are consistent with other studies where AdheSE® SBS values in enamel were lower than values with the Excite® adhesive

system^{20,21}. The main reason for this fact might be the etch-and-rinse system's ability of deeper demineralization of enamel, exposing all the enamel prisms and leaving an adhesion surface superior to that in self-etch adhesive systems, as seen in the SEM images of the conditioned enamel surfaces (Figure 7).

However, Souza-Junior²⁷ (2012) found similar SBS values using self-etch adhesives with or without pre-etching the enamel surface, suggesting that a better and deeper demineralization might not mean higher SBS values. Other authors have shown that although demineralization patterns are not as accentuated with self-etch adhesive systems, they can reach highly satisfactory levels of bond strength²⁰.

Concerning the failure mode, in G1, 33.3% of the failures were attributed to cohesive failures in the adhesive, whereas in the other three groups the predominant failure mode was adhesive between the enamel and the bonding agent. AdheSE® yielded more adhesive failures between the enamel and the bonding agent. The use of this self-etch adhesive may be encouraged in the clinical adhesion of orthodontic brackets, since most failures are adhesive and when the bracket is removed, the enamel is not lost⁵. Even though adhesive failures between enamel and the bonding agent seem to occur when bond strength is low¹, there is no consensus in the literature concerning the meaning of the failure modes experienced after shear bond tests²⁰.

Laboratory testing of adhesive materials should always precede clinical application; however, adhesive testing must be performed in a standard manner to allow reliable comparisons¹⁵. Unfortunately, due to the innovation and constant introduction of new products, this does not always happen.

Owing to the difficulty in obtaining 60 sound human incisors, bovine enamel was used in the present study. Bovine enamel has been used in several other studies as a substitute model for human enamel and some authors have found no statistically significant differences in SBS when comparing bovine and human enamel^{17,30}.

In this study, ISO standards¹⁵ for adhesive testing were followed for the storage and disinfection of specimens, which contributed to minimizing changes on dental substrates likely to affect SBS values. According to the ISO standards, to obtain the most real oral conditions, the specimens should be thermally cycled for 500 cycles¹⁵. However, using an etch-and-rinse adhesive system, Paradella²⁰ (2007) observed similar SBS values despite the usage of ISO-standard thermal cycling. This may lead us to believe that the 500 thermal cycling cycles recommended by ISO¹⁵ and followed in the

present study may not have been enough to age the samples up to the point of yielding differences in bond strength. In fact, a recent review concluded that 10,000 cycles correspond approximately to an one-year lifetime *in vivo*, which would suggest that ISO thermal cycling standards are not sufficient to study long-term adhesive behavior²⁵. Research focusing on the long-term effects of ozone application in adhesive interfaces is recommended in further research. In addition, *in vivo* studies are recommended to better determine the disinfecting potential and oxidant ability of ozone gas.

CONCLUSION

SBS to enamel after a 24-h period was not influenced by pretreatment with ozone gas. It was also proven that the SBS mean values were higher for etch-and-rinse adhesive system than for the self-etch system, independent of ozone application. There was no evidence that the different groups presented specific failure modes.

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