

# The Effects of Prophylactic Ozone Pretreatment of Enamel on Shear Bond Strength of Orthodontic Brackets Bonded with Total or Self-Etch Adhesive Systems

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## ABSTRACT

**Objectives:** The aim of this in vitro study is to determine (1) shear bond strength (SBS) of brackets bonded with self-etch and total-etch adhesive after ozone treatment (2) bond failure interface using a modified Adhesive Remnant Index (ARI).

**Methods:** 52 premolars were randomly assigned into four groups (n=13) and received the following treatments: Group 1: 30 s Ozone (Biozonix, Ozonytron, Vehos Medikal, Ankara, Turkey) application + Transbond Plus Self-Etching Primer (SEP) (3M) + Transbond XT (3M), Group 2: Transbond Plus SEP + Transbond XT, Group 3: 30 s Ozone application + 37% orthophosphoric acid + Transbond XT Primer (3M) + Transbond XT, Group 4: 37% orthophosphoric acid + Transbond XT Primer + Transbond XT. All samples were stored in deionised water at 37°C for 24 hours. Shear debonding test was performed by applying a vertical force to the base of the bracket at a cross-head speed of 1 mm/min.

**Results:** The mean SBS results were Group 1: 10.48 MPa; Group 2: 8.89 MPa; Group 3: 9.41 MPa; Group 4: 9.82 MPa. One-Way Variance Test revealed that the difference between the groups was not statistically significant (P=0.267). Debonded brackets were examined by an optical microscope at X16 magnification to determine the bond failure interface using a modified ARI. The results were (mean) Group 1: 2.38; Group 2: 1.31; Group 3: 3.00; Group 4: 1.92. Multiple comparisons showed that Groups 1 and 2, 2 and 3, 3 and 4 were statistically different (P=0.014, P<.001 and P=0.025).

**Conclusions:** Ozone treatment prior to bracket bonding does not affect the shear bond strength. (Eur J Dent 2010;4:367-373)

**Key words:** Ozone; Shear bond strength; Self-etch adhesive; Orthodontics.

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## INTRODUCTION

The prevention of enamel decalcification during orthodontic therapy is an important issue in orthodontics. The two methods that have been proposed to date to prevent white spot formation include 1) professional hygiene instruction;<sup>1</sup> and 2) enamel surface modification by means of fluoride agents,<sup>2</sup> chlorhexidine,<sup>3</sup> sealants<sup>4</sup> or Nd:YAG laser.<sup>5</sup> In the field of conservative dentistry, ozone, a natural oxidant consisting of three oxygen atoms that is also known as triatomic oxygen or trioxygen, has gained popularity of late as a means of preventing dental caries.<sup>6,7</sup> It has been suggested that ozone application on cavitated and noncavitated carious lesions may reverse, arrest, or slow the progression of lesions.

Recently, a number of studies have explored the effects of ozone on dental caries treatment and the reduction of oral microorganisms.<sup>7-11</sup> Ozone treatment is reported to inactivate microorganisms that cause tooth decay and allow for re-mineralization of the tooth structures, thus providing an alternative to "drilling and filling" in certain cases.<sup>7-9</sup> The application of ozone gas for a period of 10 seconds was found to be capable of reducing the number of *Streptococcus mutans* and *Streptococcus sobrinus* in vitro.<sup>10</sup> In addition, ozone treatment has been demonstrated to produce significant remineralization in fissure caries of permanent teeth regardless of lesion type or location.<sup>11</sup>

A recent review<sup>12</sup> of dental literature reported conflicting evidence for the in vitro application of ozone in endodontics, and as yet, insufficient evidence exists on the beneficial effects of ozone application during oral and maxillofacial surgery and upon oral diseases and implant therapy. On the other hand, the same review<sup>12</sup> concluded that substantial evidence indicates the in vitro application of ozone as a useful prophylactic antimicrobial treatment prior to etching and the placement of dental sealants and restorations, one with no negative interactions with the physical properties of enamel and adhesive restorations.

In orthodontics, clinicians tend to seek out an effective antimicrobial agent to apply prior to bracket bonding in order to prevent dental caries during multibracket treatment. Despite the current lack of substantiated evidence for the benefits of its application, ozone may prove a viable al-

ternative to conventional prophylactic treatments in the near future. In light of various findings in conservative dentistry,<sup>13</sup> it seems appropriate to speculate that pretreating the enamel with ozone might result in a reduction of white spot lesion formation in orthodontics. Not surprisingly, ozone is currently attracting attention in this field.<sup>14,15</sup>

Although ozone appears to be an integral part of noninvasive therapy of dental caries, specifically as a disinfectant prior to placing a direct restoration,<sup>7-13</sup> a dearth of knowledge of its effect on the bond strength of dental materials and orthodontic brackets exists. The present study aimed to evaluate the effect of ozone pretreatment on the shear-bond strength of conventional orthodontic brackets bonded to human enamel with either an acid-etch or a self-etch adhesive system. The null hypothesis proposed that ozone gas application may negatively affect the shear bond values of the adhesives tested due to oxygen inhibition.

## MATERIALS AND METHODS

Fifty-two caries-free and intact human premolars extracted for orthodontic purposes were collected and stored in distilled water. Teeth were cleaned and polished with pumice and rubber cups for 10 seconds. Tooth selection criteria included the absence of any visible irregularities or cracks of the enamel surface under a magnification of 4X. The teeth were randomly assigned to 1 of 4 groups (n=13) and received the following surface preparation and adhesive application treatments, according to the directions of the manufacturer.

Group 1: 30 s Ozone (Biozonix, Ozonytron, Vehos Medikal, Eryaman, Ankara) application + Transbond Plus Self-Etching Primer (SEP) (3M) + Transbond XT (3M).

Group 2: Transbond Plus SEP + Transbond XT.

Group 3: 30 s Ozone application + 37% orthophosphoric acid + 15 s rinse + Transbond XT Primer (3M) + Transbond XT.

Group 4: 37% orthophosphoric acid + 15 s rinse + Transbond XT Primer + Transbond XT.

Gemini brackets (3M, Unitek) with a base area of 9.61 mm<sup>2</sup> were bonded to human premolars. A halogen light-curing unit (Hilux, Benlioglu, Istanbul, Turkey) was used to cure the resin for 20 seconds from both the mesial and distal sides. The adequacy of the unit's irradiance was confirmed with a radiometer prior to photo polymerization.

Specimens were stored in distilled water for 4 weeks at 37°C, after which thermal cycling in de-ionized water was performed at 5±2°C to 55±2°C for 500 cycles, with a dwell time of 30 s and a transfer time of 10 s. Thereafter, the roots were removed by means of a low-speed diamond saw under coolant water, and the crowns were embedded in acrylic placed in phenolic rings, with a mounting jig used to align the labial surface of each tooth so that it was perpendicular to the bottom of the mold. Samples were next mounted in the jig attached to the universal testing device (Model 4204, Instron, Canton, Mass) for shear testing. The specimens were stressed in an occlusogingival direction with a crosshead speed of 1 mm/min, as in previous studies.<sup>16,17</sup> The force required to dislodge the bracket was recorded in Newtons and converted to Megapascals via the following equation:

$$\text{shear force (MPa)} = \text{debonding force (N)} / [w \times l] \text{ (mm}^2\text{)}$$

where  $w$  = the width of the bracket base,  $l$  = the height of the bracket base, and 1 MPa = 1 N/mm<sup>2</sup>. After debonding, the teeth and the brackets were examined under a stereomicroscope at a magnification of 10X for any remaining adhesive, in accordance with the modified adhesive remnant index (ARI).<sup>16</sup> ARI scores range from 5 to 1 as follows: 5 = no adherence of the composite on the enamel, 4 = less than 10% of the composite remaining on the enamel, 3 = more than 10% but less than 90% of the composite remaining on the enamel, 2 = more than 90% of the composite remaining on the enamel, and 1 = all of the composite remaining on the enamel, with the impression of the bracket base.

Statistical analysis of the data was performed utilizing SPSS 11.5 (SPSS Inc., Chicago, IL, USA). One-way variance test (ANOVA) was used to evaluate the differences in shear bond values between the groups, while Kruskal Wallis test was carried out so as to analyze the differences in ARI scores between the groups. Further comparisons of the ARI scores were performed via a multiple comparison test. The level of significance was set at  $P=0.05$ .

## RESULTS

The descriptive statistics for shear bond strength (SBS) values are presented in Table 1; results from the ARI are shown in Figure 1. In the overall evaluation, specimens pretreated with

ozonytron demonstrated higher SBS values. The lowest SBS values were obtained in Group 2, in which a self-etch adhesive system was used without Biozonix Ozonytron pretreatment. ANOVA test failed to reveal a statistically significant difference in SBS values between the groups ( $P=0.267$ ). Meanwhile, the Kruskal-Wallis test revealed significant differences between the groups in terms of ARI scores. Multiple comparisons revealed Groups 1 and 2; 2 and 3; 3 and 4 to be statistically significant ( $P=0.014$ ). When the ARI scores of all of the groups are evaluated, specimens pretreated with Ozonytron are observed to demonstrate higher ARI scores. The lowest ARI scores were observed in Group 2.

## DISCUSSION

In medicine, the use of ozone therapy has been investigated as a treatment of ocular diseases, acute and chronic infections, ischemic diseases, orthopedic diseases, as well as dermatological, pulmonary, renal, hematological and neurodegenerative diseases.<sup>18</sup>

In dentistry, since its introduction by Dr. E. A. Fisch,<sup>18</sup> ozonated water has been used to promote haemostasis, enhance the local oxygen supply, and inhibit bacterial proliferation in dental surgery.<sup>10,12</sup> Presently, its use in conservative dentistry is gaining in popularity. Theoretically, ozone can reduce the bacterial count in active carious lesions; therefore, it may temporarily arrest the progression of caries, resulting in the prevention or delay of the need for tooth restorations.<sup>19-21</sup> By oxidizing the biomolecules featured in dental diseases, ozone has a severely disruptive effect on cariogenic bacteria, resulting in the elimination of acidogenic bacteria. The strongest naturally occurring acid, produced by acidogenic bacteria during cariogenesis, is pyruvic acid. Ozone can decarboxylate this acid to acetic acid.<sup>20</sup> It has been demonstrated that the remineralization of incipient carious lesions can be encouraged when the production of acetic acid, or other high pKa acids found in resting plaque, buffers plaque fluid.<sup>22</sup>

An average of two out of three teeth bonded with either of the bonding material are reported to have been affected by some form of enamel opacity after orthodontic treatment, diffuse opacity being the most common type identified.<sup>23,24</sup> O'Reilly and Featherstone,<sup>23</sup> as well as Øgaard,<sup>24</sup>

have shown visible white lesions to be able to develop within 4 weeks. Although enamel-bracket interface is the most susceptible area for white spot lesion formation, two recent studies<sup>25,26</sup> have demonstrated that microleakage can invade beneath the bracket area. Thus, enamel prophylaxis is of great importance in orthodontics. In a recent study, Kronenberg et al<sup>15</sup> evaluated the

preventive effect of ozone on the development of white spot lesions during multibracket appliance therapy. The authors tested the HealOzone device and compared its effect with that of the Cervitec and Fluor Protector combination. The patients received either treatment monthly in a split mouth-design study with an average observation period of 26 months. As a result, the authors reported that the Cervitec and Fluor Protector combination proved significantly more effective than ozone application in preventing white spot lesions. This result is not surprising, as ozone, when used alone, is an antibacterial agent, rather than a remineralization tool. The result of the study might have differed had HealOzone been used with the remineralization kit. Clearly, further studies are needed in order to assess the effect of ozone pretreatment in orthodontics.

Ozonytron, which is approved by MDA and TUV, differs from HealOzone in the manner in which ozone gas is produced as well as in the concentration of ozone gas. The device produces an electromagnetic field so as to transform oxygen into ozone. The gas is then transmitted to the tissue via plasma probes (Figure 1). There are 6 different plasma probes for different application sites. The

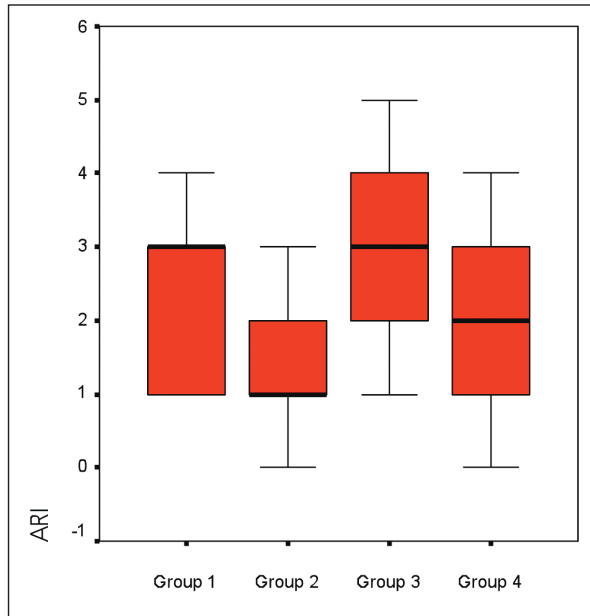


Figure 1. ARI scores of all groups.



Figure 2. Application of Ozonytron.

Table 1. Descriptive statistics of SBS values for all groups.

	Mean	Std. Deviation	Std. Error
Group 1	10.48	2.05	0.57
Group 2	8.89	2.13	0.59
Group 3	9.41	2.19	0.61
Group 4	9.82	1.91	0.53

maximum concentration that can be generated by Ozonytron is 300.00 ppm. For caries treatment, the producer recommends using the device with a minimum concentration of 100.00 ppm.

On the other hand, due to its strong oxidizing effect, ozone might have negative effects on resin-tooth adhesion related to the oxygen inhibition of polymerization.<sup>27</sup> To date, few studies have tested the effect of ozone pretreatment on the bond strength of dental materials. A recent study in the field of conservative dentistry demonstrated that the shear bond strength of bovine enamel and dentine to resin composite was not impaired after the exposure of dental structures to ozone when an acid-etch adhesive (etch&rinse) was used.<sup>28</sup> Similarly, Campbell et al<sup>29</sup> reported that a 25 s application of ozone did not impair the bond strength of the resin composite either to enamel or to dentin. However, contrary to these findings, studies conducted with glass ionomer materials report that ozone application may negatively affect the bonding of glass ionomer to human dentin.<sup>30</sup> So far, in dental literature, only one in vitro study<sup>14</sup> has evaluated the effect of ozone gas on shear bond strength of orthodontic brackets bonded to extracted human premolars. That study, however, was conducted with pre-coated APC brackets bonded with an acid-etch adhesive system. Although these brackets provide a reduction in the number of bonding procedures and improve tolerance to humidity, they are expensive and are less commonly used than conventional brackets. Thus, in the present study, conventional brackets were used.

In the present study, no significant difference in SBS values was observed among the groups. Although not significant, an increase in SBS values was observed when the self-etch system was used, following pretreatment with ozone. Previously, Endo et al<sup>31</sup> evaluated the bond strengths on enamel and dentin with a self-etch adhesive (iBond), with or without oxygen-inhibited surface layer, and reported that oxygen inhibition had no adverse effect on enamel bond strengths. This may be attributed to the different and complex chemical compositions of the self-etch adhesives.

For the ozone+acid-etch+Transbond XT (Group 3) group, the findings of the present study are in accordance with the studies in which no reduction of bond strength values was observed. As far

as the authors know, this is the first study to have tested the bond strength of a self-etch adhesive system after ozone application; thus, no comparison can be made in the self-etch group (Group 1).

Although no significant difference was found in SBS values, when ARI scores were examined, it was observed that specimens not pretreated with Biozonix demonstrated lower ARI scores. In the overall evaluation, the lowest SBS values and the lowest ARI scores were observed in Group 2. Accordingly, it can be interpreted that surface etching helps to counter the oxidizing effect of the ozone; however, the use of a self-etch adhesive system without ozone pretreatment revealed adhesion failure. ARI scores showed that the predominant failure mode for Group 2 was cohesive failure, and most of the resin remained on tooth surface. On the other hand, the ARI is an ordinal scale, which has its limitations, especially when the numbers do not represent linear gradations from one score to the next. Perhaps a more accurate adhesive retention index could have been developed in which 3D area calculation could be conducted precisely. More importantly, as Hildebrand et al<sup>32</sup> demonstrated, ARI scores do not always correlate with SBS values, and in vitro studies do not represent clinical situations precisely.

## CONCLUSIONS

Although the results should be confirmed clinically, the following conclusions were drawn within the limitations of this study:

- Enamel pretreatment with ozone did not affect the SBS of tested adhesive systems used for bracket bonding. SBS values of the ozone pretreated specimens were somewhat higher.
- The lowest SBS values and lowest ARI scores were obtained when a self-etch adhesive was used alone.
- In general, the ARI scores of the specimens without ozone pretreatment were lower. In clinical situations, ozone pretreatment prior to bracket bonding may result in less residual adhesives after debonding.

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