Evaluation of the Preventive Effect of Composites Containing Silver and TiO₂ Nanoparticles on Demineralization around Orthodontic Brackets

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**Abstract**

**Aim:** The aim of this study was to investigate the antidemineralization effect of composites containing silver and titanium dioxide (TiO₂) nanoparticles used for bonding brackets to tooth specimens.

**Materials and methods:** A total of 75 freshly extracted teeth were etched and primed and then randomly assigned to three adhesive groups: (1) conventional orthodontic adhesive, (2) conventional adhesive mixed with TiO₂ nanoparticles, and (3) conventional adhesive mixed with silver nanoparticles. In each group, brackets were bonded with the pertinent adhesive. Teeth were painted with varnish on all surfaces except a 2-mm rim around brackets. Specimens were subjected to a cariogenic process in a circulating microbial model inoculated with Streptococcus mutans and Lactobacillus casei for 12 days and subsequently sectioned for cross-sectional microhardness testing. In each specimen, enamel microhardness was determined in three locations: 25–30 μm and 1.5 mm away from the bracket and under the varnish-protected enamel.

**Results:** Enamel hardness was higher at 25–30 μm away from brackets in both the experimental groups (p value < 0.05), and the nanoparticles acted similarly in this location (p value = 0.992). At 1.5 mm away from the brackets, there was no difference between experimental and control groups (p value > 0.05); the effect of TiO₂ attenuated in this location while silver remained as potent.

**Conclusion:** Both nanoparticles resulted in decreased demineralization at 25–30 μm from the bracket but farther away the effect of TiO₂ was diminished.

**Clinical significance:** According to the results of this study, composites containing silver and TiO₂ nanoparticles can be suggested as anti-demineralization adhesives in case their biocompatibility is proved.

**Keywords:** Adhesives, Antibacterial adhesive system, Caries prevention, Nanotechnology, Streptococcus mutans.

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**Introduction**

The endeavor toward providing the patient with an esthetic smile will not be possible if at the debond appointment it is revealed that the teeth are affected by the unsightly “white spot lesions (WSLs).” With an incidence rate of 2–96%,¹ these lesions may develop as early as 4 weeks from the beginning of the treatment.²

Compliance with the meticulous hygiene measures required during treatment has been proven to be as low as 13%, particularly among the target population of orthodontics, i.e., the adolescents.³ Therefore, various carries preventive methods, such as incorporation of antimicrobial nanoparticles into orthodontic adhesives,⁴–⁸ have been investigated to reduce the need for patient compliance.

Ahn et al.⁴ for instance evaluated the anti-Streptococcal effect of adhesives containing silver nanoparticles through bacteriological assays on disks of silver-containing adhesive. Their results demonstrated slower bacterial growth in the presence of silver-containing adhesives.

Poosti et al.⁵ and Elsaka et al.⁶ used similar methodologies for assessment of antibacterial activity of nanoparticles and demonstrated enhanced streptococcal growth inhibition of composite and glass ionomer disks containing titanium dioxide (TiO₂) nanoparticles. This is while other studies declared that TiO₂ nanoparticles are among the least effective nanoparticles with regard to bacterial growth inhibition,⁷ particularly in comparison to silver.⁸

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**Conflict of interest:** None

Although several studies⁴–⁸ evaluated the antimicrobial properties of these nanoparticles, no study has yet investigated their “antidemineralization” effect “around” bracket bases bonded to teeth in a microbial cariogenic environment. It may be proposed that under these conditions, either direct contact of bacteria with nanoparticles exposed to the environment could be limited or the quantity of toxic products that the small amount of composite releases could be scarce.
Therefore, the aim of this study was to investigate the effectiveness of composites containing silver and TiO2 nanoparticles, which are used as bracket adhesives in the prevention of WSLs that develop adjacent to brackets.

**Materials and Methods**

**Ethical Approval**

All procedures performed in this study were in accordance with the ethical standards of the institutional research committee. The study was approved by the institutional review board 1396-01-03-14697.

**Tooth Preparation and Group Allocation**

A total of 75 extracted premolars were collected and stored in deionized water. The criteria for tooth selection included intact enamel with no prior chemical treatment, no cracks, caries, or WSLs. The remaining soft tissue, calculus, and bone were removed. Roots were also removed 2 mm apical to cementoenamel junction. All teeth were cleaned with fluoride- and oil-free pumice and rinsed and dried with oil- and moisture-free compressed air.

To standardize the area exposed to etching and bonding procedures, the enamel surfaces were protected by a self-adhesive tape with a cutout window the size of the bracket base during all adhesive procedures.11 All teeth were etched for 30 seconds with 37% phosphoric acid gel (3M Unitek, Monrovia, California), rinsed for 20 seconds, and air dried thoroughly. An unfilled light-cured bonding agent (Transbond XT, 3M Unitek, Monrovia, California, USA) was then applied in a thin, uniform layer.

Teeth were then randomly allocated to three groups:

**Group I (Control)**

A conventional light-cure orthodontic paste (Transbond XT, 3M Unitek, Monrovia, California, USA) was used as bracket adhesive.

**Group II (Titanium Dioxide Group)**

A light-cure orthodontic paste (Transbond XT) was homogeneously blended with TiO2 nanoparticles (standard P25, dry nanopowder, mixed rutile/anatase phase, average primary particle size: 20 nm; specific surface: 50 ± 10 m²/g; purity: 99.5+%, US Research Nanomaterials Inc., USA) by a high-speed mixer (3500 rpm) (SpeedMixer™ DAC 150.1 FVZ, Germany) in a dark environment for 5 minutes. One study5 demonstrated similar anti-bacterial effects for 1%, 2%, and 3% TiO2 nanoparticles; therefore, the new bonding agent in this study was made with 1% (w/w) TiO2 nanoparticles to reduce the potential biologic side effects. To confirm uniform distribution of the nanoparticles, SEM/EDX examination was performed on a cured sample of the new adhesive.5

The obtained paste was used as bracket adhesive in this group.

**Group III (Nanosilver Group)**

A light-cure orthodontic paste (Transbond XT) was loaded with 0.5% (w/w)12 nanosilver particles (Ag, 99.99%, 20 nm, metal basis, Us Research Nanomaterials, Inc., USA) through homogeneously mixing the two materials with the high-speed mixer. Uniform distribution of the nanoparticle was checked on a cured sample of the new paste.

The obtained paste was used as bracket adhesive in this group.

In all groups, metal brackets (Victory Series; 3M Unitek, Monrovia, California, USA) were bonded to the buccal surface of the teeth with their previously specified adhesive. Each bracket was bonded by an experienced operator (N.B.) using the same amount of adhesive paste placed on the bracket mesh and with the same amount of firm pressure applied with a dental probe to minimize the thickness of the resin film. The adhesive tape and the excess adhesive were carefully removed by the probe, followed by light curing for 20 seconds from the mesial and distal ends.

Elastomeric rings were placed in order to induce plaque retention.

Teeth were then painted with a thin coat of acid-resistant varnish and allowed to set overnight. The varnish was painted on all surfaces leaving a 2-mm rim of exposed sound enamel surrounding the bracket.13 This was done to standardize the area exposed to the cariogenic process and to protect the varnished surfaces from demineralization so that each tooth could be considered its own control.

Lingual surface of each tooth was then affixed to one end of a Plexiglas rod. All specimens in each group were secured in their caries-forming vessels by gluing the ends of their Plexiglas rods to a round Plexiglas base (Fig. 1). The three vessels were then sterilized at a very low pressure, continuous flow of hydrogen peroxide vapor, and low-temperature gas plasma (STERRAD NX System, Johnson and Johnson Medical Ltd. UK).

**Microbial Cariogenic Environment**

Based on Fontana’s microbial cariogenic model,14 a mixture of overnight cultures of Streptococcus mutans (ATCC #35668) and Lactobacillus casei (ATCC #39392) in dextrose-free trypticase soy broth, supplemented with 5% sucrose (TSBS), at 37°C was prepared and used as the inoculum for the three groups. Tooth specimens were exposed for 12 days to circulating cycles of TSBS (30 minutes each, 3 times per day) and a mineral wash solution for the rest of the day in a 37°C incubator in a dark environment. Waste was constantly removed from the vessels.

To evaluate the cariogenic potency of the experimental environment, a pilot study was performed. Five teeth that met our inclusion criteria were subjected to the cariogenic process and sectioned for microhardness testing. Five other compatible teeth were stored in deionized water for 12 days and sectioned. Inspection of WSLs on the former verified the cariogenic potency of the environment. Average cross-sectional microhardness values were compared using the Student t test; a p value of 0.000 and the fact that hardness of teeth subjected to the cariogenic process was...
within the amounts previously reported for carious enamel further confirmed the cariogenic potential of this model.

**Evaluation of Enamel Lesions**

At the end of the process in each group, teeth were visually examined for evidence of demineralization before sectioning. The status of the exposed rim of enamel was categorized when applicable to “sound enamel”, “initial stage caries”, “moderate stage caries,” and “extensive stage caries” based on the International Caries Detection and Assessment System (ICDAS). Teeth were then embedded in molds of epoxy resin to prevent fracturing during sectioning (Fig. 2).

Transverse sections of the teeth were made buccolingually at the bracket level with a microtome (Mecatome T180, PRESI SA, France). All samples were then ground with silicon carbide paper P1200 and finally polished with a diamond spray (1 μm; Buehler). Cross-sectional microhardness was then measured with an automatic micro Vickers hardness tester (MHV-1000Z/V3.0, Shanghai Shangcai Testermachine Co., Ltd., China) in three different locations: on enamel in proximity (25–30 μm) to the bracket (location 1); at 1.5 mm from the bracket (location 2); and on the varnish protected enamel (protected enamel). (Fig. 3) During the measurement, the test load was continuously increased from 0 to 20 mN, and the indentation depth of the Vickers diamond was measured. Universal hardness was calculated based on the indentation depth and the maximum test load. In all three locations, microhardness measurements were performed at 25, 35, 45, 55, and 65 μm below the outer enamel surface (Fig. 3). For calibration of data based on each tooth’s original hardness, the data were converted into values of percentage hardness. Since the enamel was unaffected by the cariogenic process in the varnish-protected area, the mean of the values obtained in this location was used as the value (100 % hardness) of sound enamel for each tooth. All measurements were given in relation to that tooth-specific value, and index of enamel hardness was defined for each location as follows:  

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\text{Index of location } 1(2) = \frac{\text{location } 1(2) \text{ enamel microhardness} \times 100}{\text{reference enamel microhardness}}
\]

**Statistical Analysis**

Statistical analysis was done using the Statistical Package for Social Sciences (Version 22.0, SPSS Inc., Chicago, Illinois, USA). Descriptive analysis was performed for calculated hardness indices of enamel in all groups for both locations. The normal distribution of the data was tested with Kolmogorov-Smirnov normality test. Difference in hardness index values between the 3 groups were analyzed using two-way analysis of variance (ANOVA). A Tukey post hoc test compared the individual groups for statistically significant differences in each location. Averages of indices in each location were compared using the Student t-test. The level of significance for all tests was set at \(p < 0.05\).

**Results**

The hardness profile in the three groups in each location is summarized in Table 1 and Figure 4. Interaction between the groups and locations significantly affected the hardness index. \((p \text{ value } = 0.001)\) (Table 2).

Teeth in both the experimental groups had statistically higher hardness indices in comparison to the control group in location 1 \((p \text{ value } < 0.05)\). In this location, differences in hardness between TiO2 and silver groups were not significant \((p \text{ value } = 0.992)\). In location 2, there was no significant difference between the experimental groups and the control teeth \((p \text{ value } > 0.05)\). In this location, however, significantly higher hardness indices were calculated for teeth in the silver group in comparison to those in the TiO2 group \((p \text{ value } = 0.001)\).
Differences in average hardness index between the two locations were significant in the control and the TiO$_2$ groups, with the control being significantly harder in “location 2” than in “location 1” ($p$ value = 0.00) and TiO$_2$ group significantly harder in “location 1” than in “location 2” ($p$ value = 0.003). In the silver group, however, both locations had similar hardness indices ($p$ value = 0.527).

Visual inspection of the teeth in the two experimental groups revealed no alterations in the exposed enamel when the teeth were wet. After thorough drying (5 seconds), however, visual changes in enamel in the two groups were disclosed. This was determined to be compatible with the initial stage caries development (ICDAS code 1). In the control group, distinct visual changes in enamel could be inspected both in wet and in dry surfaces, classified as initial stage caries development (ICDAS code 2), but no enamel breakdown or dark shadows from the dentin were inspected in any of the teeth (Fig. 5).

**Discussion**

Thus far, several studies have confirmed the antibacterial potential of composites containing silver and TiO$_2$ nanoparticles through various microbiological analyzes, and it was deduced from these results that incorporation of antibacterial nanoparticles into orthodontic adhesives would prevent enamel demineralization.

It appeared to us, however, that the antibacterial activity of nanoparticle-containing adhesive disks in cultural media may not guarantee their antiplaque effect when used as actual adhesives in an actual cariogenic environment. In such environment, the amount of particle release or direct bacterial contact with the particles would be limited; hence, this study was designed to investigate the significance of these antibacterial activities in prevention of demineralization.

Cross-sectional microhardness test was implemented since its value as an alternative to Transverse Microradiography has been verified for evaluation of enamel lesions.17

After 12 days in a cariogenic environment, teeth in all our groups revealed visual signs of WSL development. At 25 to 30 μm to brackets bonded with adhesives containing nanoparticles, enamel remained significantly harder in comparison to control. It could be concluded that despite the small area of exposed adhesive beneath the bracket, bacterial activity was still attenuated due to the antibacterial effect of both nanoparticles. This is in accordance with the results of Ahn et al.4 who demonstrated the antibacterial effect of silver nanoparticles incorporated in composite disks. Poosti et al.5 and Elsaka et al.6 also incorporated TiO$_2$ in composite and Glass Ionomer disks, respectively, and both confirmed the anti-Streptococcal effect of this particle.

In none of our groups, however, enamel remained completely intact in this location.

At 1.5 mm from the bracket base, however, enamel hardness in the experimental groups was no longer significantly different from the control. In this location too, teeth in none of the groups remained completely intact. This lack of difference between the control and the experimental groups in this location may be due to the fact that in the control group, cariogenic activity was significantly attenuated farther away from the bracket base resulting in harder enamel with similar hardness index values to that in the experimental groups. This attenuation in cariogenic activity is to be expected, since the roughened adhesive surrounding the bracket and the bracket material itself provide a suitable environment for accumulation of cariogenic bacteria in proximity to the bracket18 and 1.5 mm away from the bracket base the effect of these plaque retaining areas is lessened.

At location 1, TiO$_2$ and silver acted similarly well in reducing the cariogenic activity. This may contradict the results of Besinis’s study10 who declared that TiO$_2$ nanoparticles had limited or no effect against *Streptococcus mutans*. The difference between the results of Besinis et al. and ours may be related to different environmental conditions under which the two studies were conducted.
proved that antibacterial potency of nanoparticles is influenced by various factors including environmental conditions.\textsuperscript{19}

Vargas-Reus et al.\textsuperscript{9} also declared that TiO\textsubscript{2} was significantly less potent an antimicrobial agent than silver. They, however, conducted their experiment on Gram-negative periodontal bacteria, and their results may only be applicable to these microorganisms, while the cariogenic bacteria in our study were both gram positive. Gram-negative bacteria are said to be more susceptible to silver nanoparticles due to their cell wall structure;\textsuperscript{20} gram-positive bacteria that do not have this structure may be similarly affected by both nanoparticles.

Contrary to the results of some studies that attribute the antibacterial effect of silver to direct electrostatic contact with bacterial membrane,\textsuperscript{4,10} in the setting of our study, silver nanoparticles probably acted through Ag\textsuperscript{+} ions they release,\textsuperscript{21,23} and it is probable that silver could not exert a higher antibacterial activity than TiO\textsubscript{2} in proximity to the brackets, since Ag\textsuperscript{+} ions were not maximally concentrated in that location, explaining the similar performance of Ag and TiO\textsubscript{2} in location 1. This is while TiO\textsubscript{2} as a stable metal oxide is said to exert its antibacterial effect through direct contact with the organisms;\textsuperscript{24} therefore, this nanoparticle’s maximum activity was in proximity to the bracket. This speculation is confirmed by the fact that the antibacterial activity of TiO\textsubscript{2} deteriorated 1.5 mm away from the bracket while silver remained as potent, resulting in statistically significant differences between the two groups in location 2.

An important property of TiO\textsubscript{2} is its photocatalytic activity that makes it a strong oxidizing agent in presence of light and water. Besides direct contact with bacteria, this is one of the most important antibacterial mechanisms attributed to TiO\textsubscript{2}.\textsuperscript{25} If activated by UV light, this nanoparticle reacts with water to produce reactive oxygen species (ROS). These ROS will in turn instigate secondary bacterial membrane damage, hinder protein function, cause DNA destruction, and result in excess radical production.\textsuperscript{19} In the application of TiO\textsubscript{2} nanoparticles as antibacterial agents incorporated in the adhesive in our study, it is probable that because of being hidden beneath metal brackets in the dark experimental environment, these nanoparticles were deprived of the influential effects of UV light. In the oral cavity, the probability exists that UV light catalyzes the formation of ROS, and the antibacterial activity of TiO\textsubscript{2} nanoparticles may be enhanced especially for brackets bonded to anterior teeth in patients with adequate incisal exposure at rest. It would be possible then that TiO\textsubscript{2} exerts its effect farther from the adhesive through dissolution of ROS in saliva.

In the TiO\textsubscript{2} group, the enamel at location 2 had significantly less hardness probably because the stable TiO\textsubscript{2} nanoparticles could not be released from the adhesive to have direct contact with the bacteria in this location. On the other hand, silver nanoparticles acted similarly well in both locations, probably since dissolved ions from these particles could exert protective effects both adjacent and far from the bracket base.

The finding that no tooth remained completely intact may be related to the small amount of adhesive needed for bonding brackets, which could have reduced the available amount of nanoparticles, resulting in inadequate bacterial growth inhibition to totally prevent enamel demineralization. If that is the case, incorporation of higher amounts of nanoparticle would be necessary which could provoke concerns about cytotoxicity of such excessive amounts.\textsuperscript{26} In addition, it is possible that high dosages of these nanoparticles adversely affect the optical properties of enamel after debonding the brackets.\textsuperscript{12}

Although increased microhardness values were shown with nanoparticles in comparison with control, none of the nanoparticles could provide an absolute prevention against WSLs, it is suggested that TiO\textsubscript{2} and silver nanoparticles be applied in combination with other noncompliance preventive agents.

Although cross-sectional microhardness test is as valid as transverse microradiography for evaluation of enamel lesions,\textsuperscript{17} employing other methods for assessment of mineral loss could have increased the internal validity of our study.

The cariogenic model used in this study, although dynamic, is far from an accurate simulation of oral cavity and its distinctive features. Therefore, investigations in human models during longer periods are recommended, provided that cytotoxicity of these particles is ruled out.

**Conclusion**

Both nanoparticles resulted in decreased demineralization at 25 to 30 μm from the bracket, but farther away the effect of TiO\textsubscript{2} was diminished. Future studies evaluating the effect of these nanoparticles in the context of clinical trials will add immensely to the results of our study.

**Clinical Significance**

According to the results of this study, composites containing silver and TiO\textsubscript{2} nanoparticles can be suggested as anti-demineralization adhesives in case their biocompatibility is proved.

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