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Effects of Orthodontic Bonding Containing TiO_2 and ZnO Nanoparticles on Prevention of White Spot Lesions: an *In Vitro* Study

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Abstract: Demineralization is a common problem following orthodontic treatments. Today using antibacterial nanoparticles in preventing white spot lesions is being discussed. Given that ZnO and TiO_2 nanoparticles have direct antibacterial known properties, this study aims to evaluate these nanoparticles' antibacterial effects in orthodontic bondings' composition on preventing white spot lesions. In this *in vitro* experimental study, 43 sound human premolar teeth were divided into five groups according to the adhesive utilized for bracket bonding: None group consisting of 12 teeth bonded with Transbond XT, TiO_2 Group consisting of 12 teeth with Transbond XT and TiO_2 nanoparticles, ZnO group consisting of 12 teeth with Transbond XT and ZnO nanoparticles, a positive control group consisting of 5 teeth without brackets and negative control groups consisting of 5 teeth in a sterile medium. All teeth were stored in a medium consisting of 1cc brain heart infusion (BHI) + sucrose 1%+ 0.5 McFarland *Streptococcus mutans* bacteria for 28 days. The medium was replaced every 48 hours. All the samples were examined every week for 4 weeks using DIAGNOdent and photography to detect white spots. The results of this study revealed that adding TiO_2 and ZnO nanoparticles to Transbond XT bonding caused a decrease in enamel lesions occurrence and incidence of white spots (p value= 0.00). The results did not reveal significant differences between TiO_2 and ZnO groups. Novel bonding agents containing TiO_2 and ZnO nanoparticles represent promising candidates in combating enamel white spot lesions.

Keywords: decalcification, nanoparticles, bonding, orthodontics

1. Introduction

White spot lesions (WSLs) are one of the most common side effects following orthodontic treatment. WSLs cause great aesthetic concern for orthodontists and patients. The roughness of orthodontic adhesives surfaces predisposes to bacterial accumulation and thereby WSLs formation [1]. WSLs cause great aesthetic concern for orthodontists and patients. These lesions are often irreversible and, if not treated promptly, ongoing demineralization can promote frank cavitated lesions that require invasive treatments [2]. Maintaining adequate oral

hygiene is difficult in orthodontic patients; therefore, it is a matter of great clinical importance to implement effective antibacterial agents to prevent new WSLs formation or halt the progression of any preexisting demineralization. In recent years, many caries-preventing agents have been proposed in the literature to overcome tooth surface demineralization following orthodontic treatments. They include but are not limited to the following: fluoride-containing agents, bioactive glass [3-5], Proseal, Octaflouropenthyl methacrylate, amorphous calcium phosphate and iodide amorphous calcium phosphate [6-8], silver nanoparticles [9], triazine, niobium pentoxide phosphate, etc. [10-18]. Incorporating these agents into the conventional orthodontic bonding agents suffers from some drawbacks: compromising mechanical characteristics and limitation of active component release, discoloration and esthetic challenges, and diminishing mechanical bond strength [19, 20].

These limitations may lead to less than ideal clinical performance. Because of the drawbacks of the materials mentioned above, none of the current caries preventing agents is recommended as the gold standard. Among the proposed materials, nanoparticles' application has gained the spotlight in recent years thanks to their remarkable antimicrobial and suitable physical properties due to their small size and increased surface area [21-26]. However, the antimicrobial properties and the new nano-adhesives' safety must be ensured before any clinical application [11, 20]. Therefore the mentioned issues are the motivation for this study, which sought to determine whether orthodontic adhesives containing antibacterial nanoparticles such as TiO_2 and ZnO are beneficial in preventing WSLs formation *in vitro* situations.

2. Materials and Methods

2.1. Specimens selection and preparation.

This *in vitro* experimental study was approved by the Ethics Committee of Shahid Beheshti University of medical sciences, dental faculty. Forty-three sound human premolars freshly extracted for orthodontic purposes were included in the study. All the samples were sound premolars extracted for orthodontic purposes gathered from different dental clinics in Tehran, Iran. According to the criteria determined for the study goals, specimens with sound buccal enamel, no pretreatment with chemical materials (e.g., H_2O_2), no signs of surface cracks, and free of caries and previous white spot lesions were chosen. All samples were stored in 0.9% normal saline immediately after extraction. The specimens were kept in deionized water at 37ºC for 24 hours. Then the teeth were cleaned and polished using coarse, oil-free pumice and rubber prophylactic cups for 10 seconds. Then the specimens were rinsed with water and kept in the incubator for 24 hours. Anatase TiO_2 nanoparticles in 2 wt. % concentration was added to Transbond XT (3M Unitek) bonding agent in a dark room after being weighed by a digital scale and mixed by a stirrer to produce a homogenous blend. To confirm the nanoparticles' homogenous distribution in the composite resin, SEM-EDX (Scanning electron microscopy with an energy dispersive X-ray analytical system) analysis was performed.

Etchant (Transbond XT etching gel, containing 35% phosphoric acid from 3M/Unitek) was rubbed to the cleaned buccal surface of the tooth for 15 seconds, rinsed for 10 seconds, and thoroughly evaporated using an air-water syringe for 20 seconds. The teeth surfaces were completely dried with oil- and moisture-free air blow to obtain the chalky white appearance of enamel.

2.2. Groups tested.

The specimens were randomly divided into five groups as follows: Group one (Transbond XT plus TiO_2): Twelve specimens were included in this group. Transbond XT primer (3M Unitek, Monrovia, CA, USA) was rubbed as a tiny layer on the etched enamel, spread on the surface by gentle air spray from a 15 cm distance, and cured for 10 seconds using a light-curing unit (Astralis 7, Ivoclar, Vivadent, Schann, Lichtenstein). Transbond XT composite (3M Unitek) was applied on a bracket base. Identical bicuspid stainless steel miniature mesh twin bracket brackets (Generous Roth, GAC, NY, USA) were used in the study. The brackets were placed on the middle third of the buccal enamel surface. Brackets were placed on the tooth with a constant force by one operator. An explorer applied adequate pressure to the slot to adapt the bracket to the tooth surface to simulate the clinical conditions. A hand instrument performed the excessive adhesive removal, and the bracket was cured for 10 seconds from the distal, 10 seconds from the mesial, 10 seconds from the gingival, and 10 seconds from the occlusal with a light intensity of 1000 mW/cm^2. Also, the light-curing unit was calibrated by a radiometer every 10 minutes to ensure the equal intensity of light for all samples. Finally, the samples were transferred to the numbered sterilized test tubes. Group two (Transbond XT plus ZnO): In another group, twelve specimens were included, and the same bonding procedure as the first group was performed except that Transbond XT orthodontic adhesive containing ZnO was utilized for bracket bonding. The samples were transferred to numbered sterilized test tubes. Group three (Transbond XT):

In the third group, twelve specimens were included, and the same bonding procedure as the first group was performed except that Transbond XT orthodontic adhesive without any nanoparticles was utilized for bracket bonding. The samples were transferred to numbered sterilized test tubes. Group four (Positive control): Five specimens were considered as a positive control. The teeth without any brackets were transferred to the numbered sterilized test tubes without any preparation for bracket bonding. Group five (Negative control): Five specimens were considered as a negative control. The teeth without any brackets were transferred to the numbered sterilized test tubes.

2.3. Microorganism.

Lyophilized culture of *Streptococcus mutans* (ATCC 25175) was used. For this study, *S. mutans* was purchased from the Industrial Fungi and Bacteria Collection Center (Tehran, Iran).

2.4. Experimental procedure.

The sterile teeth were numbered and placed in the test tubes. 1 mL of BHI was added to each tube containing the teeth. The tubes were incubated for 24 hours to ensure sterile conditions. Then 50 µL of the bacterial suspension was added to BHI in the numbered tubes (1 to 4) except tube 5, the negative control group (Figure 1).

The vortex mixer (Boekel, 270100 Tap Dancer-Vortex Mixer, Feasterville, PA) was used to ensure a homogeneous distribution of bacteria and culture in all tubes. Then the tubes were incubated at 37ºC in an anaerobic atmosphere for 48 hours [27, 28]. Then, every 48 hours, all the tubes were stirred using the vortex mixer, and the culture medium was evacuated.

Figure 1. Sterile tubes containing the experimental teeth and the culture medium.

The linear culture was made from two tubes randomly to rule out any possible contamination and ensure the pure *Streptococcus mutans* culture. Then 10 ml of fresh medium was added to each tube, stirred with a vortex mixer, and incubated at 37ºC in an anaerobic atmosphere. This procedure was continued for 28 days.

2.5. DIAGNOdent measurements and photography examination.

The sample surfaces were cleansed using sterile gauze pads. The baseline WSLs (before any bonding procedure) were scored using two methods: Quantitatively measured using DIAGNOdent device (Kavo, Biberach, Germany), which was calibrated according to the manufacturer's instructions before every use. The same examiner repeated all measurements 3 times in randomized order to eliminate the operator effect for each lesion and was calibrated before the study using DIAGNOdent in line with the manufacturer's instructions.

The second method applied for white spot lesion detection was the digital photography examination with the International Caries Detection and Assessment System II (ICDASII) index [29]. The stability, accuracy, and reliability of colorimetric measurements were evaluated as described previously [30, 31]. Briefly within a lightproof box and under standardized conditions (aperture of f45, shutter speed at 1/60 s, and image ISO sensitivity 200), digital photographs were obtained using a fixed SLR camera (Nikon D7000; Nikon, Tokyo, Japan). Images were taken with a macro lens (Nikon 105 mm, 1:2.8; Nikon) and a ring flash (Sigma Em-140 DG, Nikon). All the pre and post photographs were taken at a distance of 1.5 cm at the same condition. Every one week, after medium evacuation, the WSLs were again recorded using photography and the DIAGNOdent device at the exact same condition. The changes in the amount of the values and scores in each teeth specific area were recorded. The evaluation was performed at the following instants of times: T1: The first day, before initiation of the laboratory procedure, T2: At the end of the first week, T3: At the end of the second week, T4: At the end of the third week, T5: at the end of the fourth week. All the pre and post photographs were taken at a distance of 1.5 cm at the same condition. Every one week, after medium evacuation, the WSLs were again recorded using photography and the DIAGNOdent device at the same condition. Then the changes in the amount of the values and scores in each teeth specific area were recorded.

2.6. Statistical analysis.

SPSS software version 21.0 (SPSS Inc., Chicago, IL, USA) was utilized for statistical analysis. The data were analyzed using the Repeated Measures ANOVA test. Two by two comparison of groups was performed using Bonferroni correction. A level of 0.05 was chosen.

3. Results and Discussion

3.1. DIAGNOdent measurements.

The following results were recorded in five groups of one (containing TiO_2), two (containing ZnO), three (without any nanoparticles), four (positive control), and five (negative control) in five-time points of T1: The first day, before initiation of the laboratory procedure, T2: At the end of the first week, T3: At the end of the second week, T4: At the end of the third week, T5: at the end of the fourth week. In the 28 days of the experiment period, the mean DIAGNOdent measurements showed ascending values in all groups, and the highest number recorded belonged to group 3 without any nanoparticles (Figure 2).

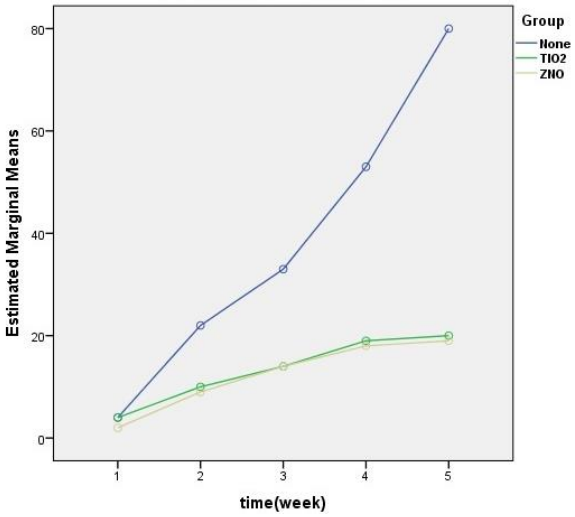

Figure 2. The estimated marginal means of DIAGNOdent (numbers) in different time points (T1-T5).

Groups 1 and 2 showed no statistical difference in the 28 days of the experiment time regarding the mean values of DIAGNOdent measurements. There was no statistically significant difference between groups 1, 2, and 3 at T1 (first day) in terms of DIAGNOdent mean values ($p<0.05$). The difference of the mean values of DIAGNOdent in the experimental groups was statistically significant at T5 (at the end of the fourth week) ($p>0.05$). More detailed information is demonstrated in Figure 3.

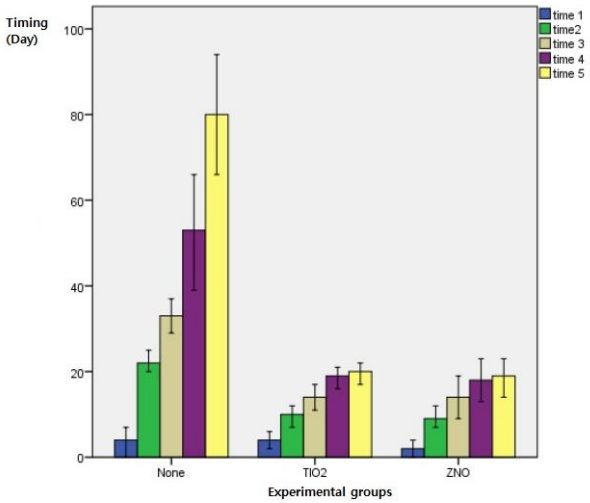

Figure 3. The mean differences of DIAGNOdent values in various experimental groups in different time points (T1-T5).

No statistical difference was observed between groups 1 (containing TiO2) and 2 (containing ZnO) regarding the mean values of DIAGNOdent ($p=1.00$). The results of this

study revealed that adding TiO2 and ZnO nanoparticles to Transbond XT bonding caused a decrease in enamel lesions occurrence and incidence of white spots (p value= 0.00).

3.2. Photographic records.

Comparison of the photos taken before and after the study operation revealed evident WSLs in the group without nanoparticles (group three) and the positive control group (group four). In groups one and two containing nanoparticles, no evident WSLs were observed, especially in the teeth samples' buccogingival area.

3.3. Discussion.

This *in vitro* study was designed to examine the potential of orthodontic adhesives containing TiO_2 and ZnO nanoparticles to prevent enamel demineralization lesions around orthodontic brackets by means of the combined use of digital photography and DIAGNOdent measurements. Considering the short duration of time during which WSLs can develop and become irreversible, adopting a suitable preventive method is considerably significant, as modern dentistry is focused on a preventive approach instead of invasive restorations of carious defects. One method that is independent of patient cooperation is the addition of anti-demineralization biocompatible materials to the bonding system. Several materials have been introduced to combat WSLs formation during fixed orthodontic treatment, but no one is recommended to prevent enamel demineralization. Bonding agents containing fluoride have been used as antibacterial orthodontic adhesives, but their bracket retention force is relatively low compared with that of conventional resin bonding. Moreover, research on commercially available fluoride-releasing adhesives has revealed that they do not demonstrate a significant advantage in their anti-demineralization effects compared with products that do not release fluoride. The use of other antibacterial agents such as GIC and CPP- ACP considerably reduced the lesion depth and caused an average reduction in the lesion volume. But they did not show a thorough reduction in the prevalence of WSLs since they are only capable of reducing WSLs up to a certain extent [30, 32, 33]. A recent long-term clinical split-mouth investigation revealed that a monomer-containing primer called Clearfil Protect Bond as an antibacterial agent has no efficacy in reducing enamel demineralization over the full course of orthodontic treatment. Although, this material contains antibacterial properties such as long-term releasing fluoride and 12-methacryloyloxydodecylpyridinium bromide (MDPB). To overcome these drawbacks, nanoparticles have attracted much attention in dentistry in recent years as antimicrobial agents. They have evolved over the past decade with several distinct advantages, including a high surface-to-volume ratio and the possibility to control the size that leads to the absence of agglomeration, thereby offering better properties. All these factors provide closer contact with microorganisms and an improved ability to anchor them [33, 34].

The result of the current study revealed no WSLs around brackets bonded with adhesives containing ZnO and TiO_2 nanoparticles which is in line with some previous studies demonstrating antibacterial effect for nanoparticles such as TiO_2 incorporated in orthodontic adhesives without compromising mechanical strength [36]. This result contrasts with the previous study that reported ZnO-containing nanoparticles are ineffective for biofilm inhibition at periods up to one month. The authors attributed this to the ZnO nanoparticles' insolubility, which prevents the diffusion of an adequate amount of Zn^{2+} to the surrounding environment to impart an observable antibiotic effect [35]. A study designed to evaluate the antibacterial effect

of ZnO nanoparticles concluded that the incorporation of 2 to 5 wt. % of ZnO may endow antibacterial effect to bonding agents without jeopardizing their physicochemical properties. This result is in line with our study, which applied 2 wt. % of nanoparticles incorporated into the bonding agent [35].

It is proposed that WSLs can become visible around fixed appliances within one month of bracket placement. So the current study was designed for one month to evaluate the antibacterial effects of the tested nanoparticles. It is recently reported that ion-release from orthodontic adhesives containing ions decreases in 2–3 months; whereby, the orthodontic treatment period is long, and it lasts for at least one year [37-39]. Therefore, future studies with a longer follow-up period are recommended to evaluate the proposed bonding material antibacterial capacity's time extent. In this study, DIAGNOdent is used to detect WSLs. Considering the acceptable sensitivity and specificity, it seems that DIAGNOdent is an appropriate modality for caries detection as a complementary method besides other methods. It is proposed that its use alone does not provide sufficient diagnostic information [40, 41]. Therefore we used the combination of DIAGNOdent and photograph for the detection of WSLs in the current research.

Several studies have evaluated WSLs by using photographic techniques [14, 42]. The quantification of white spot lesions around orthodontic brackets by means of image analysis of digitally photographed teeth is a reproducible and accurate method. Photographs are advantageous for evaluating WSLs because of evaluating the images under magnification and detecting tiny WSLs that may be overlooked during visual inspection [34]. However, it is hard to obtain consistency in lighting, reflection, and angulation. The camera might record details differently to the naked eye due to the tendency to overestimate the incidence of opacities, partly caused by the reflection of the flash from the tooth surface. The literature has proposed different methods to overcome extraneous light problems, including slanting the camera slightly or filtering out the flash using cross-polarizing filters [31, 43, 44]. In the current study, we did our best to standardize the light using a ring flash with cross-polarization filters and fixing the lens's focal length distance at 50 cm. In the current study, we used sound human premolar teeth and bonded brackets on their buccal surfaces. This enabled a simulation of the surface characteristics on which the biofilm attaches. Since the experimental setup was performed *ex vivo*, we were able to control the variables and determine the effectiveness of orthodontic adhesives incorporated with TiO_2 and ZnO on the tested bacteria used in the study. Although this *in vitro* study shows positive results of adhesives containing nanoparticles, further *in vivo* investigation should be performed.

In this research, the antibacterial test was conducted only on *S.mutans* because it is considered the first agent involved in bacterial colonization and biofilm growth. *S. mutans* has shown potential abilities such as the production of extracellular polysaccharides from the sucrose hydrolysis, which, in turn, simplifies the adherence of other bacterial species onto the enamel and contributes to the organization of the biofilm [45]. These were the rationale behind using a single *S.mutans* biofilm in the present study. Therefore, there is scope for future work to employ diverse bacterial species rather than *S. mutans* to investigate the preventive effects of nanoparticles on the formation of WSLs. It is also recommended to conduct future studies to investigate the nanoparticles' possible interaction added to orthodontic adhesives with cells and tissues to determine the optimal therapeutic dose and verify no long-term detrimental effects.

4. Conclusions

Nanoparticles have shown several advantages in the field of dentistry. Orthodontic adhesives, including nanoparticles, have demonstrated outstanding features compared to conventional adhesives without nanoparticles. It could be concluded that bonding agents containing TiO2 and ZnO nanoparticles have a preventive effect on white spot lesions formation in the 1-month evaluation period within this study's limitations. A longer observation period with an enlarged sample size is recommended to confirm whether the antibacterial effect is maintained.

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Conflicts of Interest

The authors declare no conflict of interest.

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