Effect of titanium dioxide nanoparticle addition into orthodontic adhesive resin on enamel microhardness

A Andriani, Krisnawati* and M K Purwanegara

Department of Orthodontics, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia
*E-mail: krisnawati_et@yahoo.com

Abstract. White spots are an early sign of enamel demineralization, which may lead to development of dental caries. Enamel demineralization can be determined by examining the microhardness number of the enamel. Addition of antibacterial agents such as TiO$_2$ nanoparticles into the orthodontic adhesive (TiO$_2$ nanocomposite) is expected to prevent enamel demineralization. The objective of this study is to evaluate the effect of TiO$_2$ nanocomposites in maintaining enamel microhardness around orthodontic brackets. The bracket was bonded to the premolar using Transbond XT (group 1), 1% TiO$_2$ nanocomposites (group 2), and 2% TiO$_2$ nanocomposites (group 3). Group 4 was the control group, and it was not given any treatment prior to the microhardness test. The samples of groups 1, 2, and 3 were soaked in BHI solution containing Streptococcus mutans, and then stored in an incubator at 37°C for 30 days. Demineralizations were determined on cross-sectioned tooth 100µm and 200µm cervical to the bracket by the Vickers microhardness test. The microhardness values were significantly different between every group, with the highest value obtained for control group, followed by the 2% TiO$_2$ nanocomposite group, 1% TiO$_2$ nanocomposite group, and then the Transbond XT group. The results of this study reveal that 2% TiO$_2$ nanocomposites have the ability to maintain enamel microhardness around the orthodontic bracket.

1. Introduction

Enamel demineralization in the form of white spot lesions frequently occurs during and after orthodontic treatment. In a longitudinal study, Mitchell found than an overall prevalence of 18.5% of the tooth surface was affected [1]. This lesion can be clinically induced within a span of 4 weeks, which is typically within the time period between orthodontic appointments. In the highly cariogenic environment adjacent to orthodontic appliances, this lesion can progress rapidly and may produce carious cavitations. Thus, the prevention of white spot lesions is crucial to maintain the integrity of dentition during orthodontic treatment [2].

Conservative treatment to prevent and inhibit the development of white spot lesions include oral hygiene education to the patient, topical fluoridation, topical application of chlorhexidine, and the use of dentifrice containing fluoride or chlorhexidine. These treatments require the patient’s cooperation and could lead to gingiva and tooth discoloration. Therefore, scientists are investigating the effect of antibacterial agent incorporation into orthodontic adhesives to prevent white spot formation [2].

Titanium dioxide (TiO$_2$) nanoparticles exhibit better antibacterial properties compared to chlorhexidine. Bacteria are less likely to develop resistance against this material, so the use of TiO$_2$ nanoparticles has a good potential for preventing white spot formation [3,4]. The aim of this study is to investigate the effect of TiO$_2$ nanoparticle addition into orthodontic resin adhesive on the enamel microhardness.
2. Materials and Methods
This study was a laboratory experimental study and was carried out at the Oral Biology Laboratory Faculty of Dentistry Universitas Indonesia and Physical Metalurgy Laboratory Faculty of Engineering Universitas Indonesia. Light cure orthodontic composite paste (Transbond XT, 3M Unitek, USA) was mixed manually with TiO₂ nanoparticles (dry nano powder, particle size: 21 nm) by using a cement spatula and mixing pad in a dark environment. In this study, two concentrations, 1% (w/w) and 2% (w/w), of TiO₂ nanocomposites were employed. Scanning electron microscopy (SEM) analysis was performed on the nanocomposite to confirm the uniform distribution of the nanoparticles in the composite paste. A total of 40 healthy extracted upper first premolars were randomly allocated into 4 groups of 10. Teeth with cracks and damaged enamel surface were excluded. Brackets were bonded into the buccal surface of the teeth using Transbond XT (group I), 1% TiO₂ nanoparticles (group II), and 2% TiO₂ nanoparticles (group III) after being etched using 35% fosforic acid and smeared by Transbond XT primer. Then, all samples from groups I, II, and III were immersed in BHI solution containing Streptococcus mutans and placed in an incubator at 37 °C for 30 days. Group IV was the control group and not given any treatment prior to the microhardness test.

After 30 days, all samples were rinsed with aquadest and cleaned with an ultrasonic cleaner for 5 min. All samples were separated from their roots and were then sectioned buccopalataly by using a double-faced diamond bur to produce mesial and distal pieces. All the mesial pieces were embedded in individual acrylic resin blocks with the sectioned side facing up. The specimens were then polished using rubber and a low speed hand-piece under water spray. The microhardness test was carried out by using a Vickers testing machine. Indentations were made at two locations, which were 100 µm and 200 µm cervical from the bracket base. A load of 50 g was applied, and the indenter was dropped perpendicular to the polished enamel surface mounted on the acrylic block. The loading time for each block was 20 s. Five indentations of depths between 70 and 150 µm were made and measured. The indentations were measured at x400 magnification. The enamel microhardness values of the experimental group were analyzed by an independent t-test using Statistical Package for the Social Science 17.0 (SPSS 17.0) software.

3. Results and Discussion
3.1 Results
Table 1 shows the mean and standard deviations of each experiment group. Enamel microhardness values of group I (Transbond XT group) are 322.46 VHN and 322.34 VHN, for group II (1% TiO₂ nanoparticles group) are 326.20 VHN and 327.04 VHN, and for group III (2% TiO₂ nanoparticles group) are 345.30 VHN and 345.78 VHN. Enamel microhardness values of the control group are 356.76 VHN and 355.34 VHN. These values are considered normal enamel microhardness values.

| Group | Indentation Location | Mean   | SD     | Minimum | Maximum |
|-------|---------------------|--------|--------|---------|---------|
| I     | 100 µm              | 322.46 | 3.400  | 313     | 328     |
|       | 200 µm              | 322.34 | 4.556  | 312     | 330     |
| II    | 100 µm              | 326.20 | 3.692  | 320     | 336     |
|       | 200 µm              | 327.04 | 3.368  | 321     | 336     |
| III   | 100 µm              | 345.30 | 4.225  | 336     | 353     |
|       | 200 µm              | 345.78 | 4.409  | 336     | 355     |
| IV    | 100 µm              | 356.76 | 3.280  | 351     | 362     |
|       | 200 µm              | 355.34 | 3.396  | 351     | 363     |

Table 2 shows significant differences between the microhardness values for the experimental groups. The results indicate that the control group has the highest enamel microhardness value, followed by the
2% TiO2 nanocomposite and 1% TiO2 nanocomposite groups. The Transbond XT group exhibited the lowest enamel microhardness values.

Table 2. Independent t-test between experimental groups

| Location | Group                                | Mean Difference (VHN) | Sig. |
|----------|--------------------------------------|-----------------------|------|
| 100 µm   | Control vs. 1% TiO2 nanocomposite     | 30.56                 | 0.00 |
| 100 µm   | Control vs. 2% TiO2 nanocomposite     | 11.46                 | 0.00 |
| 100 µm   | Control vs. Transbond XT             | 3.3                   | 0.00 |
| 1% TiO2 nanocomposite vs. 2% TiO2 nanocomposite | -19.1                | 0.00 |
| 1% TiO2 nanocomposite vs. Transbond XT          | 3.74                  | 0.00 |
| 2% TiO2 nanocomposite vs. Transbond XT          | 22.84                 | 0.00 |
| 200 µm   | Control vs. 1% TiO2 nanocomposite     | 28.3                  | 0.00 |
| 200 µm   | Control vs. 2% TiO2 nanocomposite     | 9.56                  | 0.00 |
| 200 µm   | Control vs. Transbond XT             | 33                    | 0.00 |
| 1% TiO2 nanocomposite vs. 2% TiO2 nanocomposite | -18.74               | 0.00 |
| 1% TiO2 nanocomposite vs. Transbond XT          | 4.7                   | 0.00 |
| 2% TiO2 nanocomposite vs. Transbond XT          | 23.44                 | 0.00 |

Table 3 shows the independent t-test results for the experimental groups with respect to the indentation location. The test showed that the enamel microhardness values at 100 µm cervical from the bracket base are not significantly different compared to the enamel microhardness values at 200 µm cervical from bracket base.

Table 3. Independent t-test between indentation locations

| Group               | Indentation Location | Sig. |
|---------------------|----------------------|------|
| Transbond XT        | 100 µm vs. 200 µm    | 0.78 |
| 1% TiO2 nanocomposite | 100 µm vs. 200 µm  | 0.87 |
| 2% TiO2 nanocomposite | 100 µm vs. 200 µm  | 0.77 |
| Control             | 100 µm vs. 200 µm    | 0.65 |

3.2 Discussion

According to Craig and Peyton (1958), a 50 g load applied to the specimen represents optimum conditions. This procedure resulted in well-defined indentations with minimum fractures around the edges. A smaller load will present an unclear indentation that is difficult to measure accurately [5]. The difference in loading time was not significant for the enamel tested at the same test load. This suggests that an indentation time of 20 s is sufficient for permanent indentation on the tooth surface to take place [6].

The microhardness values of the control group are 356.76 VHN and 355.34 VHN. These values are in agreement with previously published data, which state that the microhardness values for sound enamel are between 250 and 360 VHN [7]. The addition of TiO2 nanoparticles into orthodontic adhesive resin aims to improve the antibacterial activity of the adhesive, thus preventing reduction in the enamel microhardness values. The microhardness values of the 2% TiO2 nanocomposite group (345.70 and 345.78 VHN) are significantly lower than those of the control group, but those values are still in the range of normal enamel microhardness values, according to Gutierrez and Reyes [6]. The microhardness
values of the 1% TiO2 nanocomposite group are 326.20 and 327.04 VHN. These values are significantly lower than the normal enamel microhardness values, but are higher than those of the Transbond XT group. A previous study conducted by Poosti (2013) showed that 1% TiO2 nanocomposites have better antibacterial activity when compared to Transbond XT. The results of this study are in accordance with those of the previous study, but the antibacterial activity of 1% TiO2 nanocomposites is not adequate to prevent reduction of the enamel microhardness.

Enamel microhardness values at 100 µm and 200 µm cervical from the bracket base are not significantly different, indicating that the antibacterial activity of the nanocomposite affected the surface not in direct contact with the nanocomposite. These results are in accordance with those from a previous study by Besinis [4], who stated that TiO2 nanoparticles have antibacterial ability on the surface distant from the nanoparticles.

The microhardness values of the Vickers hardness test are influenced by specimen preparation, diagonal length reading error, variation in chemical composition, age, and location in the tooth [8]. To minimize the effect of these factors in this study, operations such as specimen preparation and polishing were done by one person with the same tools, materials, and methods. The Vickers hardness test was also carried out by one operator who was certified to use the machine. The hardness test was carried out for the same location for every sample, which are 100 µm and 200 µm cervical from the bracket base. The chemical composition and the patient age were not controlled in this study.

4. Conclusion

Based on the results of this study, we can conclude that 2% TiO2 nanoparticles in orthodontic adhesive resin have the ability to increase the antibacterial effect of the adhesive when compared to Transbond XT. The 1% TiO2 nanocomposites have better antibacterial activity than Transbond XT, but their antibacterial activity is not adequate to prevent reduction of enamel microhardness.

References

[1] Willmot D 2008 White spot lesions after orthodontic treatment. Semin Orthod. 14 209–19.
[2] Bishara S E and Ostby A W 2008 White spot lesions: formation, prevention, and treatment. Semin. Orthod. 14 174–82.
[3] Borzabadi-Farahani A, Borzabadi E and Lynch E 2014 Nanoparticles in orthodontics, a review of antimicrobial and anti-caries applications. Acta Odontol. Scand. 72 413–7.
[4] Besinis A, De Peralta T and Handy R D 2014 The antibacterial effects of silver, titanium dioxide and silica dioxide nanoparticles compared to the dental disinfectant chlorhexidine on Streptococcus mutans using a suite of bioassays. Nanotoxicol. 8 1–16.
[5] Craig R and Peyton F 1958 The microhardness of enamel and dentin. J. Dent. Res. 37 661–668.
[6] Gutiérrez-salazar P and Reyes-gasga J 2003 Microhardness and chemical composition of human tooth. Mater. Res. 6 367–73.
[7] Poosti M, Ramazanzadeh B, Zebarjad M, Javadzadeh P, Naderinasab M and Shakeri M T 2013 Shear bond strength and antibacterial effects of orthodontic composite containing TiO2 nanoparticles. Eur. J. Orthod. 35 676–9.
[8] Chuenarrom C, Benjakul P and Daosodsai P 2009 Effect of indentation load and time on Knoop and Vickers microhardness tests for enamel and dentin. Mater. Res. 12 473-76.