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Shear bond strength and antibacterial effects of orthodontic composite containing TiO_2 nanoparticles

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SUMMARY Nanofillers can reduce enamel demineralization without compromising physical properties of the composite. The aim of this study was to evaluate shear bond strength (SBS) and antibacterial effects of an orthodontic composite after adding titanium oxide (TiO_2) nanoparticles.

Light cure orthodontic composite paste (Transbond XT) was blended with TiO_2 nanoparticles. A total of 30 extracted premolars were randomly allocated into two groups of 15. In order to bond brackets, Transbond XT adhesive and nanocomposite were used in each group, respectively. SBS of two groups were determined, and the adhesive remnant index (ARI) scores were assessed. A total of 45 composite discs specimen were prepared. Of the 45 discs, 30 discs were made from nanocomposite and tested for antibacterial properties immediately and 30 days after curing by direct contact test. The antibacterial properties of the remaining 15 discs that were made from the conventional composite were tested immediately after curing as control group. Student's *t*-test and chi-square tests were used to analyse the data with the significance level of 0.05.

No significant difference was found between SBS of conventional and nanocomposites, 24 hours after curing ($P = 0.58$). Chi-square test showed that ARI scores of two groups were not significantly different after debonding ($P = 0.69$). Comparison of antibacterial effects between conventional and nanocomposite demonstrated significant difference between two groups, with nanocomposites having a higher antibacterial activity ($P = 0.03$). Colony count revealed no significant difference in bacterial growth immediately and 30 days after curing in nanocomposite group.

Adding TiO_2 nanoparticles to orthodontic composite enhances its antibacterial effects without compromising the SBS.

Introduction

Decalcification of the enamel surface adjacent to fixed orthodontic appliances is an important and prevalent iatrogenic effect of orthodontic therapy (Derks *et al.*, 2004). Prevention of white spot lesions begins by implementing a good oral hygiene regimen including proper tooth brushing with a fluoridated dentifrice. For less compliant patients, an antimicrobial bonding system around the bracket base would be advantageous. Hu and Featherstone (2005) and Buren *et al.* (2008) found that Pro Seal, a filled light-cured sealant, reduced the incidence of demineralization without patient compliance. Wilson and Donly (2001) showed that a resin-modified glass–ionomer cement (Fuji Ortho LC) exhibit significant inhibition of adjacent demineralization compared with the nonfluoride-releasing adhesives. The antimicrobial efficacy of this material has been confirmed in other studies (Paschos *et al.*, 2009). Fluoride varnishes have also been shown to decrease enamel demineralization. Their application is extremely easy and almost promising in preventing white spot lesion (Schmit *et al.*, 2002; Bishara and Ostby, 2008; Farhadian *et al.*, 2008).

Amorphous calcium phosphate (ACP) composites have also been reported to reduce bacterial adherence and lesion formation around orthodontic brackets (Uysal *et al.*, 2010a; Chow *et al.*, 2011).

Decalcification occurs when specific bacteria are retained on the enamel surface for a long time and produce organic acids; therefore, if a bonding agent could prevent bacterial growth, it could inhibit demineralization adjacent to orthodontic brackets. Today, one of the most important advances in the dental material field is the application of nanotechnology to resin composites. Ahn *et al.* (2009) have shown that orthodontic adhesives containing silver nanofillers can help preventing enamel demineralization without compromising physical properties. Recently, it has been shown that nanocomposites and nanoionomers may be suitable for bonding because they fulfil the previously suggested shear bond strength (SBS) ranges for clinical acceptability (Uysal *et al*., 2010b). It has also been shown that *Streptococcus mutans* is sensitive to nanoparticles of silver, zinc oxide, and gold, which would allow achieving important clinical effects (Hernández-Sierra *et al*., 2008)

The aim of this study was to evaluate SBS and antibacterial effects of an orthodontic composite after adding titanium oxide (TiO_2) nanoparticles. The null hypotheses to be tested were that orthodontic composites containing TiO_2 nanoparticles have (1) statistically significant difference in SBS and (2) no statistically significant difference in antibacterial properties compared with conventional composites.

Materials and methods

Nanocomposite preparation

Light cure orthodontic composite paste (Transbond XT; 3M Unitek, Monrovia, California, USA) was blended with TiO_2 nanoparticles (standard P_{25}, dry nanopowder, mixed rutile/anatase phase, average primary particle size: 21 ± 5 nm; specific surface: 50 ± 10 m^2/g; purity: >99.5%) by a high-speed mixer (3500 revolutions per minute) in dark environment for 5 minutes. In a pilot study, it was found that mixing 1, 2, and 3% TiO_2 nanoparticles with composite had similar antibacterial effects; therefore, the new paste was made with the lowest concentration, by mixing 1% (w/w) TiO_2 nanoparticles. Scanning electron microscope (SEM) examination was performed on the new paste to check uniform distribution of the nanoparticle in the composite paste.

SBS test

A total of 30 extracted upper first premolars were randomly allocated into 2 groups of 15. Teeth with cracks or damaged enamel surface were excluded. After mounting the teeth in PVC tubes, they were cleaned for 5 seconds, with non-fluoride pumice slurry and low-speed hand piece, etched for 30 seconds with 37% phosphoric acid, rinsed for 15 seconds, and then dried with an oil-free air spray. In both groups, a thin layer of Transbond XT primer (3M Unitek) was applied on the enamel surface and cured for 10 seconds by a light curing unit (Astralis 7, Ivoclar, Vivadent, Schann, Lichtenstein). In the first group, Transbond XT adhesive (3M Unitek) was used to bond orthodontic stainless steel brackets (Dentaurum, Ispringen, Germany) to enamel surface. The brackets were positioned parallel to the long axis of the tooth at the buccal surface by a metal index and cured for 20 seconds (10 seconds from each mesial and distal side of bracket). In the second group, the bonding process was similar to the first group except that nanocomposite was employed for bonding. The teeth were later immersed in deionized water for 24 hours at 37°C. Bond strength was determined by a universal testing machine (Zwick, GMbH, Vlm, Germany) with 1 kN load cell at crosshead speed of 1 mm/minute. An occlusal-gingival load was applied to the bracket with a blade-end steel rod attached to the crosshead of the universal testing machine. This produced a shear force at the bracket–tooth interface. The load at bracket failure was recorded electronically in Newtons by a computer connected to the Zwick machine. The SBS values were calculated in megapascal by dividing the force by the area of the bracket base (MPa = N/mm^2). The maximum load necessary to debond or initiate bracket fracture was recorded in Newton and then converted into megapascal as a ratio of Newtons to the bracket surface area. After debonding, the teeth were examined by a stereomicroscope with ×10 magnification and the adhesive remnant index (ARI) score was assessed regarding the remnant resin material on the enamel surface, as defined by Artun and Bergland (1984).

0: no composite remained on the tooth surface; *1*: less than 50% of the composite remained on the tooth surface; *2*: more than 50% of the composite remained on the tooth surface; and *3*: the entire composite remained on the tooth surface, with a distinct impression of the bracket base.

Student's *t*-test and chi-square tests were used to compare SBS and ARI scores between two groups, respectively.

Disc preparation

A total of 45 composite discs specimen (6 mm diameter and 3 mm thickness) were prepared in plastic moulds. Of the 45 composite discs, 30 discs were made from nanocomposite and 15 discs were made from the conventional composite without nanoparticles. After the mould was filled with composite, it was covered with celluloid matrix strips and light cured for 20 seconds (Astralis 7 visible light curing unit; Ivoclar) from each side. Following setting, the specimen was removed from the mould.

Antibacterial effects

Streptococcus mutans is the main bacteria responsible for enamel demineralization (de Soet and de Graaff, 1998). Its bacterial suspension (ATCC 25175) was prepared in brain–heart infusion broth and placed in incubator for 1–2 hours in 37°C to reach logarithmic phase of bacterial growth.

The antibacterial properties of the experimental nanocomposites were tested immediately and 30 days after curing. The control group consisted composite discs made from conventional composites immediately after curing. In each group, 15 samples were placed in separate sterile microtubes and 180 µL of the standard bacterial suspension was added to them by scaled samplers. The microtubes were incubated at 37°C for 48 hours, and then 0.01 cc of the suspension from each microtube was cultured in blood agar Petri dishes. The Petri dishes were again kept in incubator at 37°C for 48 hours and the bacterial growth was evaluated by colony counting on each plate. The data were analysed by SPSS software (Statistical Package for Social Sciences, Version 11.0, Chicago, Illinois, USA), and then, the normal distribution of the data and equality of variances were confirmed by Kolmogorov–Smirnov and Levene test, respectively. Student's *t*-test was used to compare the antibacterial effect between groups. In all statistical tests, the significance level was considered 0.05.

Results

The results of *t*-test detected no significant difference between SBS of conventional and nanocomposites, 24 hours after curing ($P = 0.58$; Table 1).

Chi-square test showed that ARI score of conventional and nanocomposites were not significantly different after debonding ($P = 0.69$; Table 2).

In comparing antibacterial effect between conventional and nanocomposite discs, we found significant difference between two groups, with nanocomposites containing TiO_2 nanoparticles having a higher antibacterial activity ($P = 0.03$; Table 3).

Colony count revealed no significant difference in bacterial growth between experimental groups containing nanocomposite, immediately and 30 days after curing (Table 4). This means that antibacterial effects of composites containing TiO_2 nanoparticles did not differ significantly immediately or after 30 days of curing ($P = 0.85$).

Table 1 Comparison of shear bond strength (SBS) between conventional and nanocomposite 24 hours after bracket bonding.

Composite	SBS
Conventional	14.4 ± 1.2
Nanocomposite	14.3 ± 1.26
P-value	0.58

Table 2 Frequency of adhesive remnant index (ARI) scores between conventional and nanocomposite after bracket debonding.

	ARI score		
Composite	1	2	3
Conventional	2(13.3%)	5(33.3%)	8 (53.3%)
Nanocomposite	1(6.6%)	7(46.7%)	7 (46.7%)
Chi-square test	*P*-value = 0.69		

Table 3 Comparison of bacterial growth (colony count) between conventional and nanocomposite after curing.

Composite	Colony count
Conventional	69.1 ± 14.59
Nanocomposite	8.2 ± 3.95
P-value	0.03*

Table 4 Comparison of bacterial growth (colony count) between nanocomposites immediately and 30 days after curing.

Composite	Colony count
Nanocomposite immediately after curing	8.2 ± 3.59
Nanocomposite 30 days after curing	8.1 ± 3.31
P-value	0.85

Discussion

Patient co-operation in preserving good oral hygiene is always challenging during orthodontic treatment, therefore, many clinicians prefer methods that do not rely on patient compliance (Wilson and Donly, 2001; Hu and Featherstone, 2005; Paschos *et al.*, 2009; Chow *et al.,* 2011). Fluoride releasing agents are claimed to be advantageous in non-compliant patients for their sustained fluoride release, but the application usually occurs in the clinician's office only; therefore, there is a limitation on the frequency of exposures that the patient will receive (Farhadian *et al*., 2008).

This research revealed that orthodontic composites containing 1% TiO_2 nanoparticles (w/w) could significantly reduce bacterial growth compared with conventional composites, without compromising the SBS. There are a number of studies that verify the antibacterial activity of nanoparticles, for example, it has been shown that silver nanoparticles have antibacterial activity against multiresistant bacteria (Alt *et al*., 2004). Silver nanoparticles create a dark grey colour change in composite and are not suitable for dental application.

Ahn *et al.* (2009). found that an experimental composite adhesive containing silica nanofillers and silver nanoparticles can help prevent enamel demineralization around bracket surfaces without compromising physical properties. Incorporation of polyethylenimine nanoparticles in a resin composite had a long lasting antimicrobial effect against a wide range of bacteria with no measured effect on biocompatibility (Beyth *et al*., 2008). However, studies over a long term have not been carried out on the possible interactions with cells and tissues to determine the optimum therapeutic dose and to verify no long-term detrimental effects. Cheng *et al*. (2012) showed that composites containing ACP nanoparticles possessed good mechanical properties and potent antibacterial properties.

In this study, light cure orthodontic composite containing 1% (w/w) TiO_2 nanoparticles was quite effective in inhibiting bacterial growth, both in fresh and aged specimen. This result reveals that adding TiO_2 nanoparticle to composite could have long-term antibacterial effects. Similar results were obtained by Elsaka *et al*. (2011) after incorporating

TiO_2 nanoparticle to glass–ionomer powder. They showed that GI-containing 3% (w/w) TiO_2 nanoparticles is a promising restorative material with improved mechanical and antibacterial properties.

The SBS results revealed that there is no significant difference between nanocomposite and Transbond XT, which is contrary to Uysal *et al.* (2010b) findings. In our study, TiO_2 nanoparticles with particles size less than 50 nm were mixed with the composite paste by a high-speed mixer. This made a homogenous distribution of nanoparticles in composite structure and did not affect its consistency, whereas in Uysal *et al*. (2010b) study, the compact consistency of the paste compromised its SBS. Many studies confirm no change or improved physical properties after adding nanoparticles to composites. Zhang *et al*. (2011) believes that nanosilica content could improve flexural properties of composite resins, and Weir *et al*. (2012) have shown that combining nano-CaF_2 with glass particles yielded nanocomposites that exhibited long-term mechanical properties, which are promising for load-bearing and caries-inhibiting restorations.

Conclusion

Adding TiO_2 nanoparticles to orthodontic composite enhances its antibacterial effects without compromising the physical properties.

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