Original Article

Evaluation of Antimicrobial Efficiency, Shear Bond Strength, and Adhesive Remnant Index of TiO_2 Infiltrated Orthodontic Adhesive - An *In Vitro* Study

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Abstract

Background: Enamel demineralization is an unavoidable adverse effect encountered with bonding brackets in orthodontic therapy. Introducing nanoparticles into the composite adhesive paste can prevent enamel demineralization. Titanium dioxide (TiO_2) is known to exhibit direct antimicrobial efficiency. This study aimed to assess the antibacterial efficiency and shear bond strength (SBS) of an orthodontic bonding composite infiltrated with TiO_2 nanoparticles. **Materials and Methods:** This *in vitro* study evaluated the efficiency of TiO_2 nanoparticle–incorporated light-curing orthodontic composite paste (ENLIGHT, ORMCO). Twenty extracted premolars were randomly and equally allocated to the two study groups, N = 10. While a conventional composite was utilized for the bonding brackets in Group I, a TiO_{2-}incorporated composite was used in Group 2. The adhesive remnant index (ARI) scores given by Artun and Bergland *et al.* and SBS were determined. Furthermore, the antimicrobial efficiency was estimated using the minimum inhibitory concentration (MIC)/minimum bactericidal concentration (MBC) and agar well diffusion assay for six composite disc specimens. The results were statistically analyzed using the chi-square test and Student's t test, at $P < 0.05$. **Results:** After 24 h of curing, no statistical mean difference was observed between the two groups in terms of ARI or SBS scores ($P > 0.05$). However, there was a significant increase in the antimicrobial efficiency of Group II when compared with Group I ($P < 0.05$). **Conclusion:** TiO_2 nanoparticle–incorporated orthodontic composites improve the antimicrobial efficiency with no significant change in the SBS. The ARI scores indicate the presence of 50% remnant orthodontic composite on the tooth enamel surface post debonding.

Keywords: *Antibacterial, nanoadhesive, shear bond strength, titanium dioxide*

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Introduction

The increased propensity for enamel demineralization, which sustains the development of white spot lesions next to the bonded bracket, is a significant adverse effect of orthodontic therapy.[1] This could be avoided by adopting excellent oral hygiene maintenance practices, which include correct tooth brushing and the use of fluoride-containing/releasing toothpaste. As this process requires maximum patient cooperation, various alternatives with increased antimicrobial efficiency, such as incorporated orthodontic composites, can

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be used. In a study conducted previously, a decrease in enamel demineralization was observed with use of a light-cured sealant, ProSeal, for bonding.[2,3] Donly and Wilson (2001) reported that a modified glass–ionomer cement, Fuji Ortho LC, with a resin filler component, reduced the incidence of enamel demineralization when compared with non-fluoride-releasing composites.[4,5] Furthermore, it has been demonstrated that fluoride varnishes work well to prevent enamel demineralization.[6,7] Additionally, composites infiltrated with amorphous calcium phosphate (ACP) have been shown to avert or decrease the tendency of bacterial adherence, thus preventing enamel demineralization around brackets.[8,9]

When bacteria adhere to the tooth surface for prolonged periods, they form organic acids that result in demineralization of the enamel surface. Therefore, if orthodontic composites are incorporated with antimicrobial agents, bacterial growth could be curtailed, thereby preventing enamel demineralization. Various nanoparticles have been incorporated into orthodontic materials, which include silver nanoparticles coated on titanium orthodontic mini-implants,[10] zinc oxide nanoparticle–incorporated composites used as adhesives for orthodontic brackets,[11] chitosan nanoparticles admixed with silver nanoparticles used as fillers in orthodontic composites,[12] copper oxide nanoparticles used for antimicrobial efficiency in resin composites,[13] titanium oxide nanoparticles on titanium surfaces of dental implants,[14] gold nanoparticles coated onto orthodontic aligners to improve antimicrobial efficiency,[15] and carbonate hydroxyapatite nanocrystals incorporated into mouthwashes, toothpastes, and composite resins for their antimicrobial and anti-demineralizing effects.[16] Research on orthodontic materials has advanced significantly with the addition of nanoparticles to resin composites. Different nanoparticles, such as 1% gold, 1% silver, 1% chitosan, 1% zinc, and 1% titanium dioxide (TiO_2), synthesized using physical, chemical, and biological methods, have been widely used in orthodontics to improve antimicrobial efficiency.[17]

Nanoparticles are routinely synthesized via chemical methods, which cause environmental hazards, result in increased energy consumption, and impose different systemic problems. To overcome the abovementioned adverse effects, green synthesis can be used as a viable alternative. This process involves reducing agents derived from plant or microbial extracts to reduce the metal ions.[18] These extracts are obtained by soaking plants in water or ethanol under favorable environmental conditions and then mixing them with ion solutions (silver, gold, etc.). Green synthesis is cost-effective, alleviates environmental degradation, and is safe from the human health perspective.[18]

Nanoparticles are associated with enhanced antimicrobial properties owing to their inherent characteristic of improved antimicrobial efficiency. Ahn *et al.* (2009) reported that silver nanofiller–infiltrated orthodontic composites could prevent demineralization of enamel without influencing their physical characteristics.[19] Furthermore, recent studies have indicated that nanocomposites and nano-ionomers might be clinically acceptable for bonding as they meet the recommended shear bonding strength (SBS) criterion of 5.9–7.8 MPa given by Reynold *et al.* for clinical acceptance.[20,21] The sensitivity of *Streptococcus mutans* against nanoparticles, such as silver, zinc oxide and gold, has been established, which holds promise for achieving substantial clinical effects.[22]

When compared with other nanoparticles exhibiting increased antimicrobial efficiency, TiO_2 nanoparticles possess superior optical properties owing to their high reflective index, increased chemical stability, and good color stability when incorporated into composites.[23,24] It has been demonstrated that the infiltration of nanoparticles improves the mechanical characteristics of materials, particularly composites, including their diametral tensile strength, hardness, compressive strength, and SBS.[25] Furthermore, research has shown that TiO_2 nanoparticles have enhanced antimicrobial efficacy without having a discernible impact on the SBS, preventing demineralization of enamel and the development of white spot lesions.[26-29]

However, green synthesis has not been utilized in any of the previously conducted studies. As this method also augments the antimicrobial efficiency during the synthesis of TiO_2 nanoparticles, TiO_2 nanoparticles green-synthesized using *Eucalyptus globulus* were incorporated into orthodontic composites, and their properties were evaluated. The aim of this study was to evaluate the SBS and antibacterial activity of orthodontic composites containing TiO_2 nanoparticles. The null hypothesis was used to test (a) no significant difference in SBS and ARI scores and (b) no significant difference in the antimicrobial efficiency of TiO_2 nanoparticle–incorporated composites when compared to conventional composites. The study was reported as per CRIS Guidelines.[30]

Materials and Methods

Study design

The study was conducted in the Department of Pharmacology at Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, India. The SIMATS, Chennai Scientific Research Board gave its approval for conducting this study.

Nanocomposite preparation

The TiO_2 nanoparticle–incorporated orthodontic composite was prepared by the author, and its characteristics were confirmed in previous studies.[31,32] Briefly, 0.04 g of previously prepared TiO_2 nanoparticles that were green-synthesized using the reducing agent *E. globulus* was combined with 4 mL of dichloromethane in a beaker to produce 1% TiO_2 nanoparticle–infiltrated orthodontic composite in this study. Silver foil was used to completely seal the glass beaker, preventing any influx of white or natural light. The mixture was then manually stirred after addition of 4 g of orthodontic composite (Ormco Enlight light-cured composite, ORMCO). After that, the beaker was placed on an orbital shaker set to run for 24 h at 500 rpm to guarantee that the nanoparticles were evenly distributed throughout the composite matrix. To prevent the material from being exposed to light, the final product was retro-filled into a black syringe.

Sample size determination

The sample size was determined using G*Power software version 3.1 (Heinrich Heine University, Düsseldorf, Germany) and with reference to the study by Felemban *et al.*[29] The power was set at 95%, effect size was 1.536, and α error was 5%. The total sample size obtained was 14. However, a total of 20 samples were recruited for studying both antimicrobial efficiency and SBS. These samples were randomly categorized into two groups (n = 10/group). This research was a double-blinded study as the laboratory technician and the statistician who performed the statistical analysis were unaware of the sample category.

Tooth specimen

Twenty human maxillary first premolars that had just been extracted were collected and kept in a 0.1% thymol solution. Teeth with an intact buccal enamel surface were not treated previously, and those that did not have cracks or decay were selected. Using silicone molds, the samples were prepared by mounting the teeth in self-cure acrylic. The samples were then randomly numbered as G1 (n = 10) and G2 (n = 10), as represented in the CONSORT flow diagram [Figure 1]. The buccal or bonding surface of each tooth was prepared using pumice (non-fluoridated) and rubber cups.

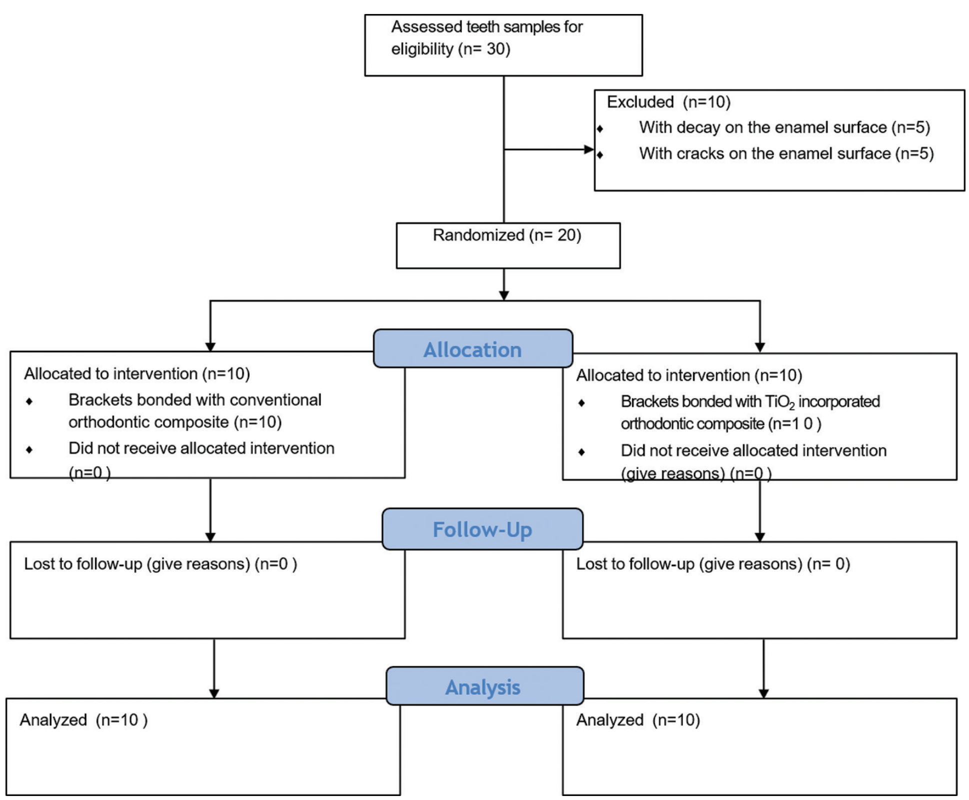

Figure 1: CONSORT flow diagram

Procedure

Using the two composites, metal brackets (3M Unitek) with a base area of 10.5 mm^2 on average were bonded. All samples were etched for 20 s using 37% orthophosphoric acid, rinsed with water, and air-dried. ORMCO primer was applied for 20 s, dried using mild airflow, and then cured using 450 nm blue light for 10 s.[29]

Group 1 consisted of samples where an orthodontic composite (ORMCO, Enlight Light Cure composite) was used to bond the brackets to the tooth surfaces. In Group 2, the orthodontic composite containing TiO_2 nanoparticles was used to bond the brackets to the tooth surface.

Variables evaluated

1. SBS
2. ARI
3. Antimicrobial efficiency

SBS

The universal testing machine (UTM, INSTRON Universal Testing Machine E-3000) was used to perform shear bond testing on all samples after they had been immersed in distilled water at 37°C for 24 h [Figure 2]. The upper member of the UTM comprised a knife-edge attachment. The prepared sample embedded in acrylic was stabilized using the lower member of the machine. The positions of both the upper and lower members were standardized to ensure uniform force application and debonding direction. Debonding forces were applied parallel to the tooth's long axis at the bracket–tooth interface in the occluso-gingival direction. Until debonding, the load was applied perpendicular to the tooth–adhesive–bracket interface with the crosshead speed held at 0.5 mm/min [Figure 3]. SBS in kg/cm^2 was determined using the following formula:

$$SBP=\frac{P}{(\pi \times r^2)},$$

where P represents the shear load applied by the UTM (kg) and r is the radius of the sample (mm). Finally, the results were multiplied by 0.09807 (constant) to obtain the SBS values in MPa.

ARI scores

After the SBS evaluation, the teeth's bonded surface was inspected under a 10× magnification by using a stereomicroscope (Leica Microsystems, Wetzlar, Germany). This examination was performed to

Figure 2: Sample preparation for shear bond strength evaluation

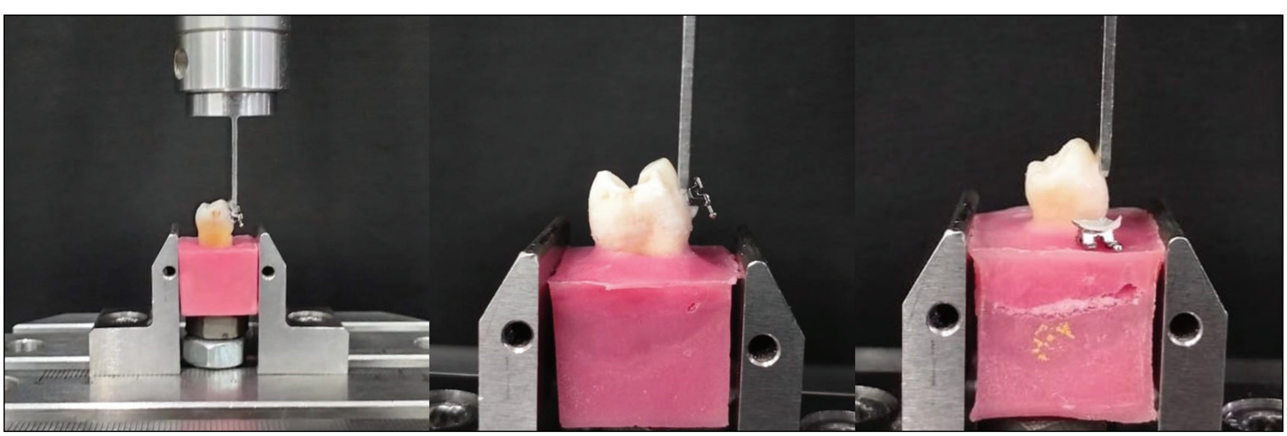

Figure 3: Shear bond strength evaluation using a universal testing machine

determine the ARI scores given by Artun and Bergland, 1984,[33] which employs the following grading scale:

0: 0% composite remaining on the enamel
1: ≤50% composite remaining on the enamel
2: >50% composite remaining on the enamel
3: 100% composite remaining on the enamel, with visible impression of the bracket mesh

Antimicrobial efficiency

Agar well diffusion method

The *in vitro* antimicrobial activity of the sample was assessed by combining the agar disc diffusion technique with the direct contact test, as advised by the Clinical and Laboratory Standards Institute (CDC, 2018).[34] Tests were conducted against a panel of clinically relevant pathogens, including *Staphylococcus aureus, Streptococcus mutans, and Lactobacillus* sp.

The Mueller–Hinton agar (MHA) was prepared and autoclaved for 15–20 min at 121°C to achieve sterilization. After that, sterile Petri dishes were filled with the sterile MHA medium, which was then left to solidify. Following solidification, swabs of *S. mutans*, *S. aureus*, and *Lactobacillus* sp. were prepared using sterile cotton buds. Powdered samples of TiO_2, 20 µg and 40 µg, measured using a laboratory weighing machine, were infused into the orthodontic composite. Along with antibiotic and control samples, these were loaded into wells in the solidified MHA medium. For 24 h, the plates were incubated at 37°C. Following the incubation period, the culture plates were examined, and a vernier caliper was used to measure the sizes of the zones of inhibition in millimeters.

Minimum inhibitory concentration (MIC)/minimum bactericidal concentration (MBC)

Enamel demineralization is primarily caused by *S. mutans*.[35] To encourage bacterial growth in the logarithmic phase, a bacterial suspension (ATCC 25175) was prepared from brain–heart infusion broth and cultured for 1 to 2 h at room temperature (37°C).

The antibacterial efficacy of the TiO_2-incorporated experimental orthodontic composite was investigated. Molds were used to create N = 10 conventional orthodontic composite discs (G1: control group) and N = 10 TiO_2 nanoparticle–incorporated orthodontic composite discs (G2: test group). Using calibrated samplers, 10 samples were added to sterile microtubes in each group, and 180 µL of standardized bacterial solution was added. After that, the microtubes were incubated at 37°C for 48 h. The suspension from each microtube was then grown in 0.01 cc on blood agar Petri dishes. The bacterial growth on each plate was measured using the colony counting method after these Petri dishes were incubated for 48 h at 37°C.

Statistical analysis

Data were collected and tabulated in Microsoft Excel before being imported into SPSS version 24.0 (Statistical Package for the Social Sciences Inc. IBM, NY, USA) for statistical analysis. The standard deviation and mean were used in the descriptive statistical analysis. The data's normal distribution was ascertained through Shapiro–Wilks test. The SBS and bacterial colony count were compared between the two groups using an independent *t* test for normally distributed data. To investigate the ARI values, the chi-squared test was applied. A significance threshold of $P < 0.05$ was utilized for the purpose of comparison.

Results

SBS

Table 1 provides a comparison of the average SBS between the two groups. Mean SBS values for Groups 1 and 2 were 15.15 MPa and 15.45 MPa, respectively. There was no statistically significant difference ($P > 0.099$) between the two groups. Compared to the conventional composite, the orthodontic composite containing TiO_2 had a higher SBS.

ARI

The results of the ARI scores are shown in Table 2. Between the two groups under analysis, there was no discernible difference in ARI scores ($P > 0.05$). Both groups' scores ranged from 2 (less than 50%) to 3 (all of the composite left).

Table 1: Comparison of mean shear bond strength in MPa between the two composite groups

Group	ARI Scores	N (%)	*df*	Chi square (Σ^2)	*P* value
G1: Conventional composite	2 (>50% of composite left)	4 (40%)	1	3.600	0.058
	3 (100% of composite left)	6 (60%)			
G2: TiO_2-incorporated composite	2 (>50% of composite left)	5 (50%)			
	1 (100% of composite left)	5 (50%)			

Antimicrobial efficiency of TiO_2-incorporated orthodontic composite

Agar well diffusion assay

The mean zone of inhibition (ZOI) exhibited an increase in the TiO_2-incorporated orthodontic composite having a volume of 20 mL and 40 mL when compared with the antibiotic and control groups. Both concentrations (20 mL and 40 mL) of the TiO_2-infiltrated orthodontic composite resulted in ZOI values equivalent to those achieved by ampicillin against *S. mutans, S. aureus,* and *Lactobacillus* sp. [Figures 4 and 5].

MIC/MBC

When comparing the antimicrobial efficiencies of conventional and TiO_2-incorporated orthodontic composite discs, a significant discrepancy was observed between the two groups. Notably, the TiO_2-incorporated orthodontic composite exhibited a higher antimicrobial efficiency.

Table 2: Comparison of adhesive remnant index scores between the two composite groups

Group	N	Mean ± SD	*P* value
G1: conventional composite	10	15.15 ± 0.57	0.099
G2: TiO_2-incorporated composite	10	15.45 ± 0.26	

Discussion

This study evaluated the SBS, ARI, and antibacterial efficiency of a new TiO_2-infiltrated orthodontic composite and compared it with those of the conventional composite [Table 3]. According to the findings of this investigation, the incorporation of TiO_2 nanoparticles into the composite had no statistically significant effect on the SBS of the material. The composite group containing the TiO_2 nanoparticles displayed a mean SBS of 15.45 ± 0.26 MPa, whereas the SBS of the conventional group was 15.15 ± 0.57 MPa. The SBS of the orthodontic composite must be within the clinically acceptable range for it to withstand the dynamic pressures applied during mastication and those exerted by orthodontic wires and attachments. An SBS of 5.9–7.9 MPa has been reported to be the acceptable clinical range.[24] However, higher values

Table 3: Comparison of bacterial growth (colony count) between conventional and titanium dioxide–incorporated orthodontic composites after curing

GROUPS	Mean ± SD	*P* value
G1: conventional composite	69.1 ± 14.59	0.030
G2: TiO_2-incorporated composite	8.2 ± 3.95	

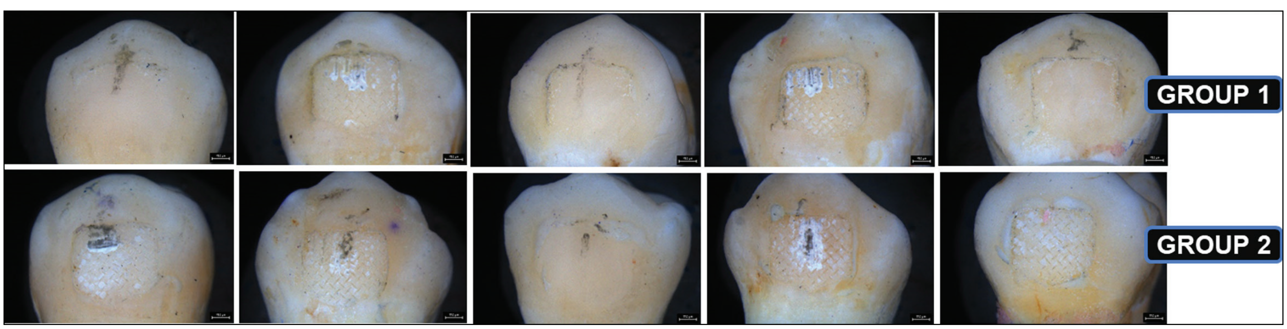

Figure 4: Stereomicroscope images of samples for ARI evaluation

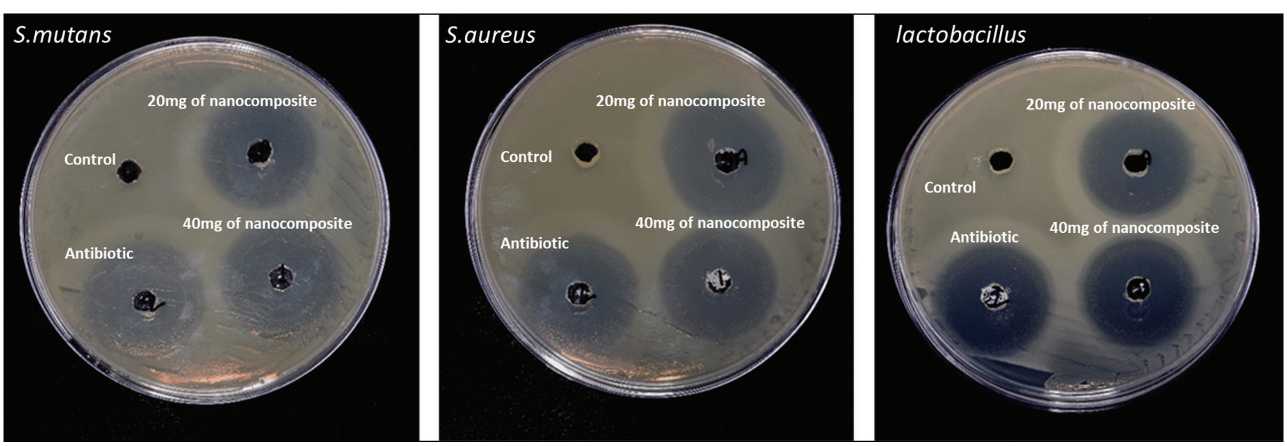

Figure 5: Agar well diffusion assay to evaluate the antimicrobial efficiency of TiO_2 nanoparticle–incorporated orthodontic composites against *Streptococcus mutans, Staphylococcus aureus*, and *Lactobacillus* sp.

closer to those of restorative materials might exert deleterious effects on the enamel surface as it might be damaged during debonding procedures.[26] These standard values are not consistent with laboratory-derived experimental values as the surrounding oral environment and constant dynamic forces acting within the oral cavity influence the bond strength.[36]

With reference to previous studies, 1% (w/w) TiO_2 nanoparticles were used as they have increased antimicrobial efficiency without causing any cytotoxic effects.[23,25,36] Furthermore, a study has recommended that SSB should be evaluated 24 h post-bonding to permit post-curing residual monomer polymerization, thereby ensuring the homogeneity and consistency of the bonded material.[36,37]

Similar to the results of the present study, Sodagar *et al.*[23] reported that 1% TiO_2 nanoparticle–incorporated orthodontic composites had higher SBS than 3% and 5% TiO_2 nanoparticle–incorporated orthodontic composites. In addition, Poosti *et al.* have established the absence of significant differences in the SBS values of orthodontic composites with and without TiO_2 nanoparticle infiltration.[26] Contrary to the present results, Felemban *et al.* observed that a homogenous mixture of zirconia and TiO_2 nanoparticles when incorporated into orthodontic composites improved the SBS.[29] Farzanegan *et al.* also failed to discern any difference in SBS values.[28] However, the small sample size used for the experimental analysis presents a key limitation.

On the contrary, Pourhajibagher *et al.* perceived a reduction in SBS after the incorporation of 1% TiO_2 nanoparticles.[38] Behnaz *et al.* noted that the SBS was increased by 20 units when conventional orthodontic composites were used.[27] Reddy *et al.* reported 30% reduction in the SBS of infiltrated composites in comparison with conventional composites.[39] These observations could be attributed to methodological errors during sample preparation, decreased sample size, and poor standardization protocols when compared with conventional techniques. Oral fluids and other contaminants have also been shown to influence the bond strength.

The adhesive–bracket interface and the adhesive–enamel interface after debonding are evaluated for quality of adhesion using the Adhesion Rating Index (ARI). A low ARI score is preferred because it reduces the possibility of enamel damage, whereas a high score suggests strong adherence, which could damage the surface enamel.[21,27] The ARI scores in this study showed 50% residual composite on the tooth surface, and they were similar among the study groups. There was no statistically significant difference. Prior research has shown that there is less than 50% of residual composite on enamel surfaces and that there is no discernible difference in ARI scores between conventional and TiO_2-incorporated composites.[25,27,36] Nonetheless, factors such as surface area of the bracket base, bracket base mesh design, position of the tooth intraorally, bonding protocol, and bracket material have a direct influence.

The primary purpose of developing this novel orthodontic adhesive is for its properties of improved antimicrobial efficacy. When the antimicrobial efficacies of the two composites were evaluated, 1% TiO_2–incorporated orthodontic composites significantly reduced *S. mutans* bacterial colony growth. This finding is in accordance with previously reported results.[26,38,40] Sodagar *et al.*, Poosti *et al.*, and Assery *et al.* have established the increased antimicrobial efficiency of the TiO_2-incorporated orthodontic composite.[23,25,26] Salehi *et al.* have proven the enhanced antimicrobial efficiency of N-doped TiO_2-coated brackets when applied for 3 months, which prevents enamel demineralization.[41,42] The antimicrobial efficiency of TiO_2 could be ascribed to its anti-composite property against oral microbes, especially *S. mutans*, and oxidative damage of the microbial cell wall via photocatalytic activity.[22,29,38] The findings of the present study have asserted that infiltration of the composite with TiO_2 nanoparticles considerably reduces bacterial growth without altering its mechanical property (SBS).

Although the results of this study were validated, caution in interpretation is warranted. The outcomes are limited to a specific composite that was modified and, hence, may not be available commercially. Moreover, SBS is a static test; intraorally, fatigue loads are commonly present, which could influence the properties of the modified composite. Moreover, the viscosity of the newly formed material was higher than that of the conventional composite. Hence, rheological properties should be evaluated. Further *in vivo* studies are needed, and the long-term antimicrobial efficiency of the composite must be assessed with a larger sample size.

Conclusion

According to the findings of this investigation, incorporating 1% TiO2 nanoparticles into orthodontic composites improves their antibacterial efficacy without significantly impacting the SBS. The ARI scores suggest that >50% of the adhesive remains on the tooth surface post-debonding.

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None

Conflict of interest

None

Author declaration

All three authors (HL, AKS, and SP) designed the study, prepared and collected the samples, obtained and analyzed the data, and checked the statistics after the analysis. This article's final version has been authorized by all of its writers.

Ethical policy and institutional review board statement

The SIMATS, Chennai Scientific Research Board gave its approval for conducting this study. SRB REFERENCE NO: SRB/SDC/ORTHO-2004/21/TH-051

Patient declaration of consent

The premolars that were extracted (therapeutic) for orthodontic purpose were taken in this study.

Data availability statement

The authors of the present study can be contacted.

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