Original Article

The effect of nitrogen-doped titanium dioxide–modified stainless steel brackets on *Streptococcus mutans*: *A randomized clinical trial*

Avula Monica[a]; Sridevi Padmanabhan[b]

ABSTRACT

Objectives: To evaluate the effect of nitrogen (N)-doped titanium dioxide (TiO_2) coated stainless steel brackets activated with natural visible light and dental operating lights on *Streptococcus mutans* concentration in the plaque of orthodontic patients at 30 and 60 days.

Materials and Methods: A total of 30 patients were recruited for this split-mouth study; 60 upper lateral incisor brackets constituted the study sample. A total of 30 brackets (15 right and 15 left) were coated with N-doped TiO_2 using the (radio frequency) magnetron sputtering method. Plaque samples were collected at 30 days and 60 days after appliance placement. *S mutans* concentration was evaluated using real-time polymerase chain reaction.

Results: At both time intervals, the concentration of *S mutans* in the control group was greater than that in the study group ($P = .005$). In both the study and the control groups, the *S mutans* concentrations significantly increased from 30 to 60 days ($P = .005$).

Conclusions: N-doped TiO2, on exposure to natural visible light and dental operating light, was effective in reducing the plaque concentration of *S mutans* in orthodontic patients. The efficacy was better at 30 days than at 60 days after placing the orthodontic appliances. (*Angle Orthod.* 2022;92:396–401.)

KEY WORDS: Nanoparticles; Titanium dioxide; *Streptococcus mutans*

INTRODUCTION

The introduction of an orthodontic appliance affects the ecology of the oral microflora, causing increased levels of plaque accumulation attributed to the complex design, morphologic irregularities, and increased surface area of fixed orthodontic appliances. Hindrance to the self-cleansing ability creates a nidus for bacterial adhesion, thereby causing microbial imbalance and a fall in the pH.[1,2,3] *Streptococcus mutans* is one of the first organisms to colonize, with increased concentrations during active orthodontic treatment.[4] White spot lesions, which are more prevalent with orthodontic treatment, can occur even within 1 month of placement of fixed appliances.[1,5,6] Although various measures have been used to prevent them, dependence on patient compliance has led to alternate methods to control the microflora.[7,8]

Recent research has focused on the application of nanotechnology by incorporating nanoparticles into orthodontic materials such as composites, wires, and brackets.[9–11] The use of semiconductor materials such as titanium dioxide, zinc oxide, silver oxide, and others have provided an opportunity to enhance the antibacterial properties of orthodontic appliances. Titanium dioxide (TiO_2) has attracted considerable attention because of its low-cost, high-photo-catalytic activity; chemically stable properties; and resistance to photocorrosion.[12]

Coating of brackets and wires with TiO_2 is effective against microorganisms.[13,14] However, few studies have evaluated the effects in vivo, possibly because of a lack of information on their cytotoxic effects. A recent cell-line study found that the anatase phase of TiO_2 is less cytotoxic than the rutile phase while maintaining efficacy against *S mutans*.[15]

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Accepted: December 2021. Submitted: June 2021.
Published Online: January 24, 2022

Recent studies evaluating the influence of TiO_2 on orthodontic archwires in a clinical setting found that it reduced roughness of the wires and reduced bacterial adhesion in the initial phases.[16,17] However, the unique photocatalytic activity of TiO_2 is best harnessed by exposing it to light irradiation. Previous studies used ultraviolet (UV) irradiation to stimulate photocatalysis because TiO_2 has a wide-band gap of 3.2 eV, where its absorption edge occurs below 400 nm (UV region) and, hence, only a small fraction of solar spectrum is absorbed.[18] Although the use of UV-A light is effective, its use clinically is not advisable because of recognized health hazards.[19]

Research has tried to reduce the optical gap to visible light by doping with metal or nonmetal elements. Doping with metal ions reduced the overall activity of the photocatalyst, causing thermal instability.[20,21] Thus, nonmetal ion elements such as carbon, nitrogen (N), sulfur, phosphorus, and fluorine have replaced metal elements as they introduce localized oxygen states below the conduction band minimum, that is, 0.75–1.18 eV.[21] Nitrogen has gained popularity because of its increased optical properties.[22] It has been suggested by Asahi et al.[21] that visible light of less than 500 nm would be sufficient to activate N-doped TiO_2.

Although a recent in vitro study found that N-doped TiO_2-coated stainless steel brackets when exposed to visible light were effective against cariogenic bacteria,[23] no study has evaluated this in a clinical setting. Thus, this study was conceived to evaluate the effect of N-doped TiO_2-coated stainless brackets exposed to regular interior lighting and light used during orthodontic procedures on *S mutans*.

MATERIALS AND METHODS

The study was conducted at Department of Orthodontics, Faculty of Dental Sciences, Sri Ramachandra University, Chennai, India, and was approved by the institutional ethics committee (no. CSP/15/DEC/44/76). The design of the study was a one-center, parallel-group randomized clinical trial with a split-mouth design, where the patient's mouth was divided into two halves as right and left quadrants.

Based on the study of Baby et al.,[15] sample size was calculated with a two-paired mean with a 1:1 allocation ratio, assuming normality (power, 0.80; $\alpha = 0.05$). A total number of 30 participants were recruited for the study with a sample size of 30, where the upper lateral incisor brackets constituted the study sample.

Orthodontic patients in the age group of 15 to 25 years who fulfilled the inclusion criteria were recruited after obtaining informed consent. The study sample included 19 females with an age range of 16 to 23 years and 11 males with an age range of 16 to 29 years.

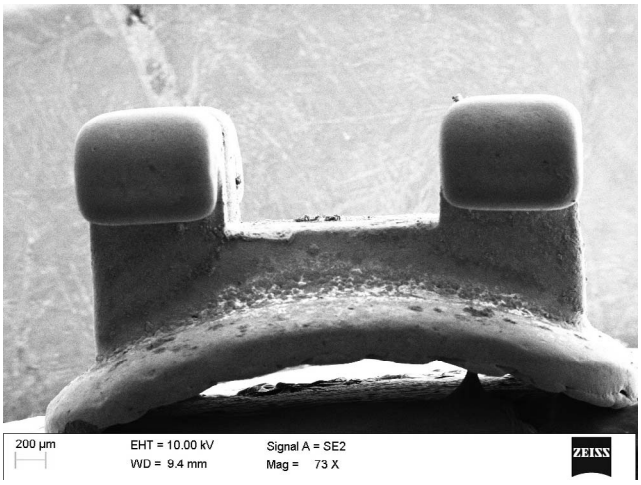

Figure 1. SEM image of TiO_2-coated bracket.

Patients with no medical history and a Simplified Oral Hygiene Index[24] score of 0–1.5 were included. All patients were treated as nonextraction with preadjusted edgewise appliances. Patients having teeth with hypoplastic areas, cracks, or irregularities of the enamel surface of maxillary lateral incisors or missing or deformed maxillary lateral incisors and taking antibiotic or anti-inflammatory medications were excluded.

Preparation of Brackets

A total of 60 upper lateral incisor brackets (MBT prescription, 0.022 × 0.028 inch; Mini Master Series, American Orthodontics, Sheboygan, WI) constituted the sample. Of these, 30 brackets (15 right and 15 left) underwent the N-doped TiO_2-coating procedures. These brackets were replaced in the original kit and allocated for the study group. The other 30 brackets served as control.

Surface coating of stainless steel brackets with N-doped TiO_2 was carried out by the RF (radio frequency) magnetron sputtering (Anelva Sputtering Unit Model SPF-332H; Canon Anelva Corp, Kawasaki, Japan) method. The stainless steel brackets were used as substrate, and the target material was TiO_2. High-purity argon and N at a ratio of 30:1 acted as the ambient gas under controlled pressure and temperature.[19,21] Pre-sputtering was performed for 10 minutes to remove pollutants from the target surface. The coated brackets were then cooled at room temperature and annealed in an N atmosphere at 450°C in a muffle furnace (Sastha Scientific Agencies, Bangalore, India). After annealing, the coated brackets were analyzed under a scanning electron microscope (Zeiss, Oberkochen, Germany) (SEM; Figure 1). A thickness in the range of 50–80 nm of TiO_2 thin film was coated on the brackets (Figure 2). X-ray diffraction meter (XRD) analysis was done to

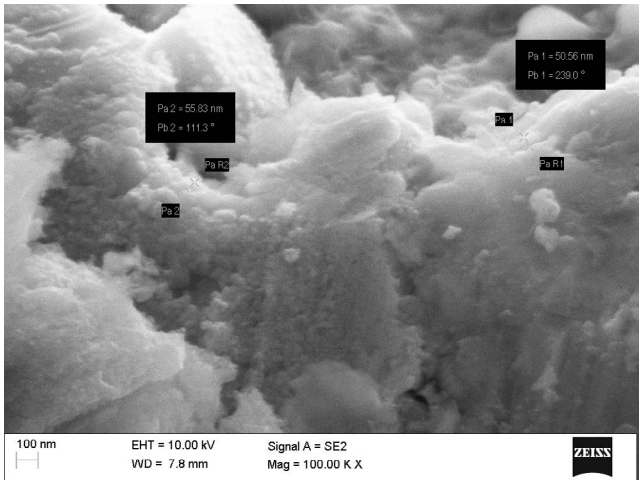

Figure 2. SEM image showing thickness of TiO_2 coating.

confirm whether the TiO_2 existed in the anatase phase (Figure 3).

Randomization

The randomization was done by using the web site randomization.com (http://www.randomization.com), and an allocation sequence was generated. Block randomization was done to distribute equal numbers of participants in both groups.

Allocation Concealment

Operator 1 generated the sequence and maintained the allocation mechanism. During enrollment, operator 1 would allocate to operator 2 for bonding. The alphabets A were allocated to right side and alphabet B to the left side. If coated brackets were bonded to the allocated side, the contralateral side served as the control and vice versa.

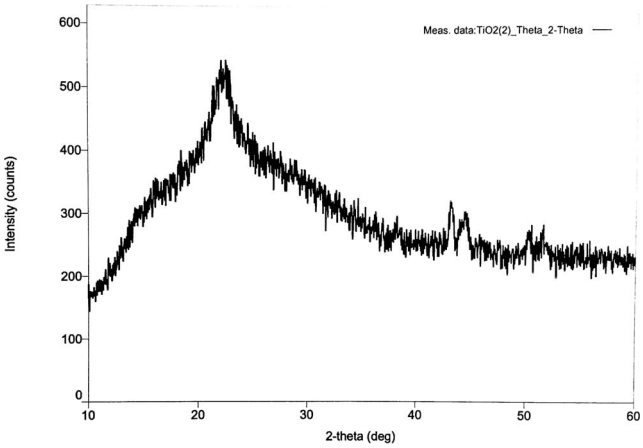

Figure 3. XRD image.

Blinding

The operator and participants were blinded until allocation. Further blinding was not possible as there was a color difference between the coated and uncoated brackets. The bonding procedure followed routine prophylaxis: etching with 37% phosphoric acid gel (D Tech, Pune, India), rinsing, and drying. Brackets were bonded using Transbond XT (3M Unitek, Monrovia, CA) and cured using a quartz tungsten halogen light unit (QLH75 Dentsply, Dentsply Sirona, Charlotte, NC) for 40 seconds. Patients were advised to use Colgate Total dentifrice and brush twice daily and not to use any chewing gum, fluoridated mouthwash or antibiotics during the period of the study, which was for 60 days.

Follow-Up

Plaque was collected from the area surrounding the maxillary lateral incisors using the four-pass technique[25] (Figure 4) at the following two time intervals: 30 days (T1) and 60 days (T2) after bonding and appliance placement. The samples collected were placed into individual 2-mL microcentrifuge tubes containing phosphate buffer saline and were coded and sealed for transport to the Central Research Facility, Sri Ramachandra Institute of Higher Education and Research, Chennai, India, for DNA isolation.

The Ultrapure Genomic DNA Spin Miniprep Kit (Medox Biotech India Pvt. Ltd, Chennai, India) was used, and the quality of the DNA obtained was measured using a nano drop technique. The samples were then processed for real-time polymerase chain reaction (PCR) analysis, which was done using the fast 7900HT machine (Applied Biosystems, Waltham, MA) using SYBR Green assay for the relative quantification of bacteria. The oligonucleotide primers specific for the *S mutans* used were Sm F5 5^{o}-AGC CAT GCG CAA TCA ACA GGT T and Sm R4 5^{o}-CGC AAC GCG AAC ATC TTG ATC AG.

The PCR values were obtained in the form of a graph that was interpreted using the RQ (relative

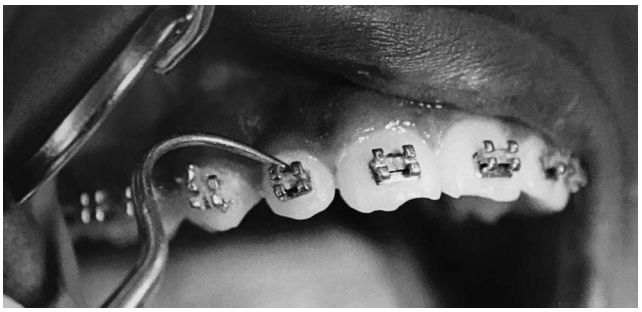

Figure 4. Clinical photo of N-doped TiO_2-coated bracket.

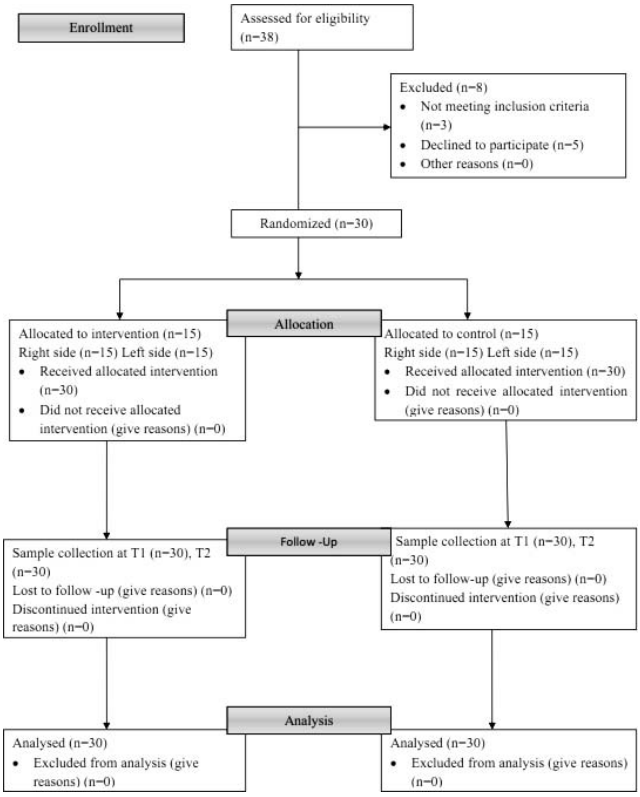

Figure 5. Consolidated Standards of Reporting Trials flowchart.

quantification) Manager software by Applied Biosystems.

Statistical Analysis

The collected data were analyzed with IBM (Armonk, N.Y.) SPSS statistics software version 23.0. To describe the data, descriptive statistics, means, and standard deviations were used. To find significant differences between the groups, an independent Student's *t*-test was used for intergroup comparisons. To find significant differences between the bivariate samples in the paired groups, a paired-sample *t*-test was used. A probability value of .05 was considered significant.

There were no dropouts in the study, and the participants in the study did not show any allergic reactions and did not complain about the color of the brackets (Figure 5).

RESULTS

At both T1 and T2, the average cycle threshold value for the control group was less than that of the study group, indicating that the concentration of *S mutans* in the control group was greater than in the study group. The difference was statistically significant (Tables 1 and 2).

Table 1. Intergroup Comparison of Mean Real-Time PCR Cycle Threshold Values at T1

Group	N	Mean	Standard Deviation	Standard Error Mean	*P* Value
Control	30	34.71	2.03081	0.37077	.005*
Study	30	38.54	1.81382	0.33116	

* $P < .05$.

In both the study and the control groups, the *S mutans* concentrations increased significantly from T1 to T2 (Table 3).

DISCUSSION

Advances in nanotechnology have motivated researchers to alter the biofilm by incorporating nanoparticles in orthodontic materials or by coating them.[26] Nanoparticles have a greater surface-to-volume ratio (per unit mass), interacting more closely with microbial membranes and providing considerably larger surface area for antimicrobial activity. They are, therefore, able to modify the oral biofilm.[27] Of these, during the past decade, TiO_2 has gained popularity because of the photocatalytic activity it exhibits when exposed to UV-A light, which is crucial for its antibacterial activity. The hydroxyl radicals and superoxide ions produced are extremely reactive when in contact with organic molecules that damage the cell wall initially, and later, after partial decomposition of the cell membrane, these ions reach the cytoplasm membrane and cause cell death.[28]

In an attempt to overcome the problem of UV light, researchers have done extensive work on doping TiO_2 with metal or nonmetal elements to extend its light absorption to visible light. Previous studies on nonmetal elements have shown that N-doped TiO_2 was most effective in narrowing the band gap and had greater optical properties as a result of occupied localized states in the conduction band and oxygen-deficient sites in grain boundaries.[20–22,29,30] Therefore, in this study, N-doping was done so that the photocatalytic activity of TiO_2 under visible light could be studied.

TiO_2 exists in three phases, namely, anatase, rutile, and brookite. Because the anatase phase has more photocatalytic activity and no, or minimal, cytoxic effects compared with rutile,[15] the brackets in this study were coated with the anatase TiO_2. No other

Table 2. Intergroup Comparison of Mean Real-Time PCR Cycle Threshold Values at T2

Group	N	Mean	Standard Deviation	Standard Error Mean	*P* Value
Control	30	31.89	2.15130	0.39277	.005*
Study	30	36.84	2.12276	0.38756	

* $P < .05$.

Table 3. Intragroup Comparison of Real-Time PCR Cycle Threshold Mean Values Between T1 and T2

Group	T1	T2	Mean Difference (T1−T2)	Standard Deviation	Standard Error Mean	Lower	Upper	*P* Value
Control	34.71	31.89	2.81	2.99091	0.54606	1.69951	3.93316	.005*
Study	38.54	36.84	1.69	2.18393	0.39873	0.88084	2.51183	.005*

* $P < .05$.

study evaluated the efficacy of N-doped TiO_2 against *S mutans* in orthodontic patients. Because N-doping supposedly exposes the photocatalytic activity to a wider spectrum, this property was studied only on exposure to normal interior light and light exposure during routine orthodontic procedures such as bonding. Asahi et al.[21] demonstrated that the photocatalytic activity of N-doped TiO_2 was more effective than TiO_2 when exposed to visible light but not UV light.

Because studies have shown that mature plaque can be seen 3 weeks after placement of fixed appliances,[31] plaque samples were evaluated at T1 and T2 after appliance placement. *S mutans* concentration was quantified using real-time PCR, which has high sensitivity, specificity, and accurate means for the quantitative detection of oral pathogens.[32] At both T1 and T2, the study group showed significantly less concentrations of *S mutans* compared with the control group. The significant reduction in *S mutans* concentrations at T1 must be considered clinically significant because there was 100% genomic expression of *S mutans* in the control group and only 57% in study group, which reflected negligible concentrations in 43% of the samples.

At T2, the concentrations of *S mutans* increased in both the control and study groups when compared with T1, although the control group showed significantly more concentrations of *S mutans*. There was 100% genomic expression of *S mutans* in the control group and 87% in the study group. The increases in *S mutans* concentrations from T1 to T2 were statistically significant in both groups.

The results of this study were consistent with those of Cao et al.,[19] who also showed reductions in microorganisms on N-doped TiO_2–surface modified stainless steel brackets. However, their evaluation was done under in vitro conditions on exposure to visible light (lamp, 100 W*2) for 24 hours. In addition, they did not evaluate subsequent efficacy. Salehi et al.[23] exposed brackets to 60 minutes of visible light and found that the brackets showed significant antimicrobial activity during a period of 90 days in an in vitro environment.

In contrast, the present study evaluated antibacterial efficacy under clinical conditions during a period of 60 days, depending mostly on interior lighting and exposure to visible light during routine appliance placement and follow-up. Although the coated brackets performed better than the control brackets at both time intervals, it appeared that the antibacterial effect of N-doped TiO_2 reduced from T1 to T2. It has been suggested that the intensity of photocatalysis was directly proportional to the intensity of light.[33] Kuznetsov and Serpone stated that photoactivation of TiO_2 depended on constant activation with either some form of heat or light.[34]

A previous study evaluating the integrity of the TiO_2 coating on orthodontic archwires showed that, although the TiO_2 coating initially contributed to reduced roughness, it exhibited delamination and deterioration after 1 month.[16] The integrity of the TiO_2 coating is also a factor to be considered for long-term efficacy.

This study evaluated the antimicrobial efficiency of N-doped TiO_2 for only 60 days. Long-term assessment during the complete duration of orthodontic treatment is necessary to establish this as an accepted clinical protocol and to further develop light-activation protocols of TiO_2 for orthodontic practice.

CONCLUSIONS

- N-doped TiO_2-coated stainless steel brackets showed reduced concentrations of *S mutans* when compared with uncoated stainless steel brackets at T1 and T2. This difference was statistically significant.
- Although the concentrations of *S mutans* increased in both groups, coated brackets showed significantly less bacterial concentration when compared with uncoated brackets.
- N-doped TiO_2 activated with visible light shows promise in future applications for dentistry and orthodontics, and further research is needed to explore this and devise suitable protocols for photoactivation intraorally.

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