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Original Article

Evaluation of Photocatalytic Titanium Oxide Surface Modified Stainless Steel and Nickel-Titanium Orthodontic Wires for its Antiadherent and Antibacterial Properties - An *In Vitro* Study

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Abstract

Background: The purpose of this study was to assess the photocatalytic titanium oxide surface modified stainless steel and nickel–titanium (NiTi) wires for its antiadherent and antibacterial properties against *Streptococcus mutans*.

Materials and Methods: The study was preceded on 80 specimens of stainless steel and NiTi wires which were divided into four groups each having two subgroups. Groups containing uncoated wires acted as control groups for their respective experimental groups containing coated wires. Surface modification of wires was performed with TiO_2 sol which was further heated at 400°C. For assessment of antiadherent and antibacterial properties of wires, microbiological test against *S. mutans* was performed.

Result: Orthodontic wires coated with TiO_2 showed antiadherent effect against *S. mutans* as compared to uncoated wires. The groups containing surface modified wires showed a statistically significant decrease in the survival rate of *S. mutans* expressed in the colony-forming unit when compared to the groups containing uncoated wires.

Conclusion: Surface modification of the orthodontic wires can be used to prevent accumulation of dental plaque, hence, the development of periodontal problems as well as dental caries during fixed orthodontic treatment.

Key words: Antiadherent properties, Antibacterial properties, Nickel-titanium wires, Stainless steel wires, *Streptococcus mutans*, TiO_2

INTRODUCTION

Fixed orthodontic appliances are the most common habitat for dental caries and periodontal diseases. Although there are many reasons which may cause dental caries and periodontal diseases such as physical, chemical, and biological characteristics of dental plaque, the oral environment with fixed appliances provides ideal conditions for colonization of microorganisms as a result of their inherent morphological irregularities.[1]

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The incidence of enamel demineralization and periodontal diseases after fixed orthodontic treatment can involve up to 50% of the patients.[2]

Among different components of fixed orthodontic appliances, wires are present throughout the course of orthodontic therapy and hence pay a crucial part in causing demineralization of enamel surface.

The wire bracket interface provides unique conditions that hinder proper access to the tooth surface for cleaning.

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Streptococcus mutans and *Lactobacillus* are the microorganisms that have been identified as the main pathogen in periodontal diseases and dental caries, and their presence increases the risk of decalcification.[3]

To combat these ill effects resulting from fixed orthodontic therapy, it is essential to give proper oral hygiene instructions as well as to maintain supervision during the course of the treatment. This subsequently poses to be a challenge for the orthodontist.

To reduce the growth of oral microorganisms during fixed orthodontic treatment, surface modification of the appliances used in fixed orthodontics can be initiated, which will subsequently prevent enamel decalcification and dental caries.

The photocatalytic degradation of organic compounds in water treatment process and decomposition of air pollutants have been studied for the several decades.[4]

Many kinds of nano-photocatalytic coating have been reported such as TiO_2, ZnO, Ag, and $SrTiO_3$, in which TiO_2 has been reported as the most active.[5]

TiO_2 is a white-colored inorganic substance that is thermally stable, non-flammable, poorly soluble, chemically inert, and not classified as hazardous according to the United Nations Globally Harmonized System of classification and labeling of chemical.[4]

TiO_2 has been used for industrial as well as consumer goods including paints, adhesive paper, coatings, and water treatment agents. Thorough research on sterilization mechanism of photocatalytic coating is made and has proved that the cell wall is wrecked first, which lead to the change of osmotic pressure, then the intercellular structures are destroyed subsequently degrade the bacterial.[6]

Many studies have also proved the antiadherent property of TiO_2. The photocatalytic TiO_2 has strong oxidation and superhydrophilicity.[7]

Therefore, using this photocatalytic activity of TiO_2 may provide effective results in preventing bacterial adhesion and growth around fixed orthodontic appliances.

Among all the pathogens in the oral cavity, *S. mutans* and *Lactobacillus* have been identified as the main pathogens for dental caries and periodontal diseases.[8-10]

Thus, this study was framed to evaluate the antiadherent and antibacterial properties of surface modified photocatalytic TiO_2, stainless steel and nickel–titanium (NiTi) against *S. mutans*.

MATERIALS AND METHODS

The study was done on 80 specimens of 0.016″ stainless steel and NiTi wires of 4 cm length. The specimens were divided into four groups and further divided into two subgroups of each group have a control and experimental group, in which the control group consisted of uncoated wires and the experimental group consisted of photocatalytic TiO_2 coated wires. Group 1 and 3 consisted of stainless steel wires and Group 2 and 4 consisted of NiTi wires (Table 1). All the groups were then assessed for antiadherent and antibacterial properties.

Bacterial Strains

S. mutans was used for the antiadherent and antibacterial tests. *S. mutans* was inoculated overnight in 5 ml of brain heart infusion (BHI) for adhesion test, 10% of the broth after 24 h of inoculation was transferred to 10 ml of BHI broth which contained 10% sucrose, which was further incubated for 24 h.

Coating of Stainless Steel and NiTi Wires with Photocatalytic TiO_2

Coating of stainless steel and NiTi wires with photocatalytic TiO_2 was done by sol-gel method.[5] In this method, 0.40 g of titanium isopropoxide was dehydrolized in deionized aqueous solution. As a result, titanium hydroxide as a precipitate was achieved, which was separated by decantation process. The precipitate was further washed thoroughly with water until the alcohol generated during the hydrolysis was completely removed. The precipitate was then dissolved in 10 ml of hydrogen peroxide, which gave the yellowish orange solution of titanium peroxo complex. Thus, the TiO_2 sol was prepared (Figure 1).

The wires were cleaned, air dried, then dipped in solution for 5 min, and then were pulled out with motor-operated equipment in uniform motion. Wires were then dried at room temperature. Further, the coated wires were heated for 5 h under electric furnace at 400°C (Figure 2).

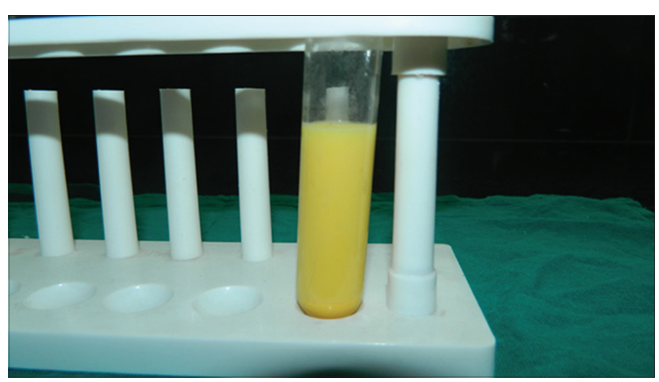

Figure 1: Prepared TiO_2 sol

Table 1: Groups of coated and uncoated wires used for antiadherent and antibacterial activities

Group 1	
Group 1a	Control group – Consisted of 10 uncoated stainless steel wires that was used for evaluation of bacterial adhesion to orthodontic wires
Group 1b	Experimental group – Consisted of 10 surface modified stainless steel wires with photocatalytic TiO_2 thin film which was used for evaluation of bacterial adhesion to the orthodontic wires
Group 2	
Group 2a	Control group – Consisted of 10 uncoated NiTi wires that used for the bacterial adhesion
Group 2b	Experimental group – Consisted of 10 surface modified NiTi wires with photocatalytic TiO_2 thin film which was used for bacterial adhesion
Group 3	
Group 3a	Control group – Consisted of 10 uncoated stainless steel wires used for evaluation of antibacterial property
Group 3b	Experimental group – Consisted of 10 surface modified stainless steel wires with photocatalytic TiO_2 thin film which was used for evaluation of antibacterial property to the orthodontic wires
Group 4	
Group 4a	Control group – Consisted of 10 uncoated NiTi wires that was used for the antibacterial property
Group 4b	Experimental group – Consisted of 10 surface modified NiTi wires with photocatalytic TiO_2 thin film which was used for antibacterial property

NiTi: Nickel titanium

Assessment of Bacterial Adhesion to Orthodontic Wires

Before adhesion test, adventitious macroscopic contaminations of the wire were removed by ultrasonicating in 2-propanol for 5 min and were dried in a desiccator. After that, the wires were sterilized in an autoclave and were pre-weighed on analytical balance and were stored in container having 10 ml of BHI broth (Figure 3). An overnight cultured *S. mutans* growth was inoculated to final concentration of 10% and was incubated for 24 h at 37°C under illumination of ultraviolet (UV)-A black light inside the incubator (Figure 4). Wires were carefully removed and then immersed in 10% formaldehyde solution for 30 min to immobilize the cells. The wires were then weighed under analytical balance for bacterial adherence.

Assessment of Antibacterial Properties of Orthodontic Wires

The antibacterial properties of orthodontic wires were demonstrated against *S. mutans*. The culture media used were BHI agar, and the disc diffusion method was carried out. BHI agar plates were brought at room temperature, and the inoculum preparation was done using a swab to transfer the colonies onto the plates. Turbidity was adjusted visually with broth to equal that of 0.5 McFarland turbidity standard. The suspension was standardized with a photometric device. After that, the inoculation of other plates was done in 15 min by adjusting the inoculum to McFarland turbidity standards; a sterile cotton swab was dipped against the walls of the tube above the liquid to remove the excess inoculum. After that, the wires were placed in the plate, and the plates were illuminated with a UV-A black light inside the laminar air flow chamber with an intensity of 1.0 MW/cm^2 for 60 min (Figure 5). Antibacterial activity was described as the survival rate for colony-forming units (CFUs) for *S. mutans*.

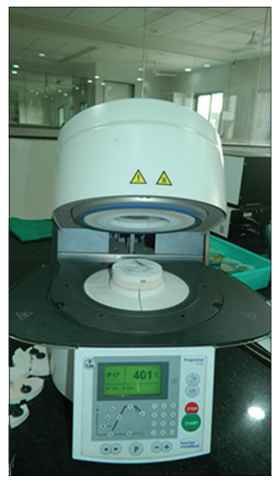

Figure 2: Wires kept in furnace at 400°C

Figure 3: Analytical balance used for weighing wires

Statistical Analysis

The results were evaluated by SPSS 12.0 K software (SPSS Inc., Chicago, IL, USA). A paired *t*-test was used for the bacterial adhesion test, and Kruskal–Wallis *H*-test was applied for the antibacterial activity tests.

RESULTS

Adhesion of *S. mutans* on the Surface of Orthodontic Wires

From the data shown in Table 2, it is observed that there was a significant increase in the weight of the uncoated wires of Group 1b and 2b and coated wires of Group 1a and 2a showed less change in weight than the uncoated wires and hence, showing the antiadherent property of coated wires.

Antibacterial Activity of Surface Modified Orthodontic Wires on *S. mutans*

The survival rate of bacterial cells was calculated in terms of CFU as shown in Table 3, Group 3a and 4a having uncoated wires showed a significant increase in bacterial colony when compared to Group 3b and 4b which contained the surface modified wires showing a statistically significant decrease in bacterial colony.

Figure 4: Wires kept in incubator

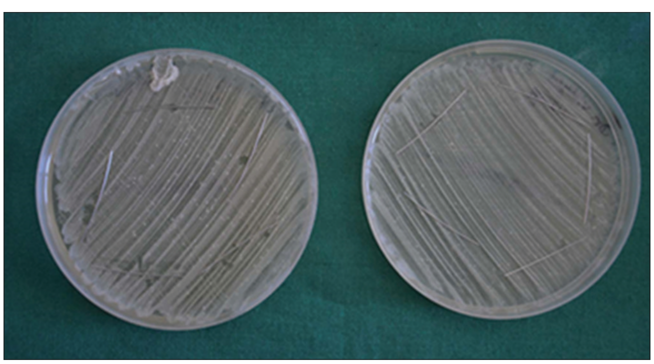

Figure 5: Colonies of *Streptococcus mutans* on agar plate. On right side control group and on left experimental group

DISCUSSION

The present study was undertaken to modify the surface of stainless steel and NiTi wires with photocatalytic TiO_2 and to assess their antiadherent and antibacterial properties against *S. mutans*. It is possible that a sufficient amount of reactive oxygen species such as OH was released from the TiO_2 coated wires as a result of UV-A light, whereas reduced adhesion of *S. mutans* to the TiO_2 coated wires might be a result of decomposition of surface organic molecules of *S. mutans* such as M protein. This phenomenon might further cause the cell walls of bacteria to become more fragile.[11]

The photocatalytic activity of illuminated TiO_2 has been actively investigated in adverse areas such as water treatment processes, air cleaning agents, and antibacterial agents. TiO_2 might be sufficient to degrade the microbial cell components which are mainly composed of organic compounds.[12]

Among the various infectious organisms in the oral cavity, *S. mutans* is one of the most closely investigated organisms in dentistry to cause enamel decalcification and periodontal diseases.[8-10]

The main problem faced by orthodontists during fixed orthodontic treatment is the development of dental plaque, which is initiated by the adhesion of *S. mutans* to the tooth surface on the fixed orthodontic appliance.[13-15]

Several studies have been conducted for the antibacterial properties of TiO_2.

Zhang *et al.* in their study concluded that because of TiO_2, there is disintegration of bacterial cell wall.[16] Jacobi *et al.* also proved that organic matters in the cell can be decomposed completely by photocatalysis of TiO_2.[17] Caballero composited the TiO_2 photocatalyst into acrylic paint, and this coating could kill *Escherichia coli*.[18]

Various studies have also been conducted for the antiadherent property of TiO_2. Shi *et al.* excogitated a

Table 2: Comparison of initial, final and change in weight of different coated and uncoated wires

Group	Mean weight initial	Mean weight final	Mean difference	*P* value
Group 1a	0.0290±0.0020	0.0292±0.0021	−0.0002	0.37
Group 1b	0.0292±0.0008	0.0306±0.0015	−0.0014	0.04*
Group 2a	0.0236±0.0013	0.0242±0.0014	−0.0006	0.07
Group 2b	0.0254±0.0032	0.0266±0.0037	−0.0012	0.03*

*Statistically significant

Table 3: Comparison of colony counts in coated and uncoated wires

Group	Range	Mean±SD	*P* value
Group 3a	806-964	866.40±46.95	0.001*
Group 3b	202-298	257.80±32.74	0.647
Group 4a	786-887	808.20±34.66	0.001*
Group 4b	192-332	271.20±40.97	0.848

*Statistically significant, SD: Standard deviation

self-cleaning coating and said that dust on the wall will be abscised together with TiO_2.[19] Qin prepared TiO_2/Al by liquid phase deposition and then blended it with coating. The self-cleaning performance was evaluated in the end. Oleic acid was the pollution stimulant.[20]

Shah *et al*. found the photocatalytic TiO_2 surface modified stainless steel bracket has an antiadherent and antibacterial property against *Lactobacillus* acidophilus.[1] Mhaske *et al*. found the same result with the stainless steel and NiTi wires against *Lactobacillus acidophilus*.[2] Study done by Chattani *et al*. on photocatalytic TiO_2 coated NiTi and stainless steel wires demonstrated antiadherent and antibacterial property against *S. mutans*.[3]

In this study, surface modified NiTi and SS wires with photocatalytic TiO_2 showed effective antiadherent and antibacterial property against *S. mutans*.

CONCLUSION

- Photocatalytic TiO_2 coating on SS and NiTi wire prevented the adhesion of *S. mutans*, hence demonstrating antiadherent property.
- The photocatalytic TiO_2 also revealed antibacterial effects against *S. mutans*.
- Surface modification of orthodontic wires with photocatalytic TiO_2 can be used to prevent the development of dental plaque and dental caries during orthodontic treatment.

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