The Online
ANGLE ORTHODONTIST
An International Journal of Orthodontics and Dentofacial Orthopedics
78th Anniversary 1930–2008

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doi: 10.2319/0003-3219(2007)077[0483:SMOOWW]2.0.CO;2
***The Angle Orthodontist:* Vol. 77, No. 3, pp. 483–488.**

Surface Modification of Orthodontic Wires with Photocatalytic Titanium Oxide for its Antiadherent and Antibacterial Properties

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ABSTRACT

Objective: To test the antiadherent and antibacterial properties of surface modification of orthodontic wires with photocatalytic titanium oxide (TiO_2).

Materials and Methods: TiO_2 was coated on the surface of the orthodontic wires by a sol-gel thin film dip-coating method. Bacterial adhesion to the wires was evaluated by the weight change of the wires. The antibacterial activity of the surface-modified orthodontic wires was demonstrated by the dilution agar plate method for *Streptococcus mutans* and spectrophotometry for *Porphyromonas gingivalis*.

Results: The orthodontic wires coated with the photocatalytic TiO_2 showed an antiadherent effect against *S. mutans* compared with the uncoated wires. The bacterial mass that bound to the TiO_2-coated orthodontic wires remained unchanged, whereas that of the uncoated wires increased by 4.97%. Furthermore, the TiO_2-coated orthodontic wires had a bactericidal effect on *S. mutans* and *P. gingivalis*, which cause dental caries and periodontitis, respectively. The antiadherent and antibacterial mechanisms of TiO_2 to break down the cell wall of those bacteria were revealed by scanning electron microscopy.

Conclusion: The surface modification of orthodontic wires with photocatalytic TiO_2 can be used to prevent the development of dental plaque during orthodontic treatment.

KEY WORDS: Photocatalytic, TiO_2, Orthodontic wire, Antiadhesion, Antibacterial, *Streptococcus mutans*.

Accepted: July 2006. Submitted: May 2006

INTRODUCTION Return to TOC

The photocatalytic degradation of organic compounds in water-treatment processes and the decomposition of air pollutants have been studied for several decades.[1] Semiconductor particles such as titanium oxide (TiO_2), zinc oxide, tungsten oxide, cadmium sulfide, zinc sulfide, strontium titanate oxide, and iron oxide are considered ideal photocatalysts for these reactions. Among these, TiO_2 has attracted considerable attention and has been reported to be the most useful substance in organic degradation processes on account of its chemically stable properties and absence of harmful effects on humans. TiO_2 is found as either rutile or anatase crystalline structures. A rutile structure is more thermodynamically stable than an anatase structure, but an anatase structure is more photoactive. At more than 900°C, an anatase crystalline structure can also be converted to rutile.[2,3] Therefore, the crystalline structure of TiO_2 is considered an important factor in the photocatalytic activity.

The application of TiO_2 as an antibacterial agent has been reported based on its photocatalytic properties. In a mixture of *Escherichia coli* with the anatase crystalline form of titanium dioxide, rapid cell death was observed.[4] Cho et al[5] reported that there is an excellent linear correlation between HO· radicals and the rate of *E. coli* inactivation, which indicates that the HO· radical is the primary oxidant species responsible for inactivating *E. coli* in the ultraviolet (UV)/TiO_2 process. These reports suggest that photocatalytic TiO_2 may provide a novel tool for the prevention of bacterial contamination and disinfection.

The orthodontic wires used in dental treatments supply a good habitat for oral microorganisms sufficient to cause dental caries or even periodontal diseases. Accordingly, orthodontic patients might have a higher risk of contracting other dental diseases.[6–8] More successful results can be achieved by reducing the chances for oral microorganisms to adhere to the surfaces of teeth and orthodontic wires. Therefore, the surface modification of orthodontic wires with photocatalytic TiO_2 may provide more effective results in orthodontic treatments.

In this report, TiO_2 was coated on stainless steel orthodontic wires by the sol-gel dip-coating method in an attempt to impart a photocatalytic activity to the wires. The antiadherent and antibacterial effects of photocatalytic TiO_2 were measured against *S. mutans* or *P. gingivalis*.

MATERIALS AND METHODS Return to TOC

Bacteria Strains

S. mutans ATCC 31989 and *P. gingivalis* 381 were used for the adhesion and viability tests. *S. mutans* was inoculated in 5 mL of a brain heart infusion (BHI) broth (Difco Co Ltd, Franklin Lakes, NJ) and incubated for 24 hours at 37°C. For the adhesion test, 10% of an overnight-cultured broth was transferred to 10 mL of the BHI broth containing 10% sucrose and incubated for 24 hours. The BHI broth containing 0.1% vitamin K_1 (Sigma Co Ltd, St. Louis, Mo) and 1% hemin (Sigma Co Ltd) was used to cultivate *P. gingivalis*. *P. gingivalis* was grown at 37°C; in an anaerobic chamber (Forma Co, Marietta, Ohio) with 85% nitrogen, 10% hydrogen, and 5% carbon dioxide (CO_2) mixed gas.

Preparation of Photocatalytic TiO_2-coated Orthodontic Wire

The surface modification of the orthodontic wires with photocatalytic TiO_2 was carried out by a sol-gel thin film dip-coating method.[9–11] Before the coating of the wires, a precursor solution was prepared. In the preparation of the titanium-precursor solution, 4.8 g of titanium (IV) tetrabutoxide (Aldrich Chemical, St. Louis, MO, USA) was hydrolyzed with 100 mL of distilled water. The resulting titanium hydroxide precipitate was separated and washed with water. The precipitate was dissolved in 75 mL of aqueous hydrogen peroxide to obtain a transparent orange solution of a titanium peroxo complex. Precleaned stainless steel orthodontic wires (STSS-1622, G&H Wire Co, Greenwood, IN, USA) were dipped in the precursor solution and pulled out with a uniform pulling rate of 3 cm/min and then dried at room temperature. The film formed was further heated at 500°C for 5 hours in an electric furnace in air. This procedure was repeated twice more.

Evaluation of Bacterial Adhesion to Orthodontic Wires

Before the adhesion test, 5 cm of the stainless steel wires was ultrasonicated for 5 minutes in 2-propanol (UVASOL, Merck, Glattbrugg, Switzerland) to remove adventitious macroscopic contaminations and was dried in a desiccator. After cleaning and sterilizing in an autoclave, the wires were placed immediately in culture tubes containing 10 mL of a BHI broth with or without 10% sucrose. An overnight-cultured *S. mutans* culture broth was then inoculated into the culture tubes to a final concentration of 10% and incubated for 24 hours at 37°C under illumination of UV-A black light (1.0 mW/cm^2). Quartz tubes were used for the bacterial cultures to allow better transparence of UV-A light. Wires on which the bacteria were adhered to were carefully removed and immersed in a 10% formaldehyde solution for 30 minutes to immobilize the cells. After a careful rinse with distilled water, the wires were dried in a desiccator for 24 hours. The weight change of the wires during the bacterial adhesion test was recorded with an analytical balance (Mettler Toledo B154, Greifensee, Switzerland).

Antibacterial Activity Assay of Orthodontic Wires

The antibacterial activities of the surface-modified orthodontic wires were demonstrated against *S. mutans* and *P. gingivalis*. First, the *S. mutans* or *P. gingivalis* culture broth was diluted with BHI broth to make an optical density of 1.0 at 660 nm. Ten milliliters of the diluted bacterial suspension was transferred onto petri dishes containing either the uncoated stainless steel wires or TiO_2-coated wires. These dishes were illuminated with a UV-A black light (2 W/m^2, F10T8BLB, Sankyo Denki, Kanagawa, Japan) on a clean bench with an intensity of 1.0 mW/cm^2 for 60 minutes. After illumination, 100 μL of the bacterial suspension was serially diluted and plated onto BHI agar plates. The antibacterial activity was described as survival rate by colony-forming unit (CFU) for *S. mutans* or optical density at 660 nm for *P. gingivalis*. In the case of *P. gingivalis*, antibacterial activity assay was carried out in an anaerobic chamber.

Statistical Analysis

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

RESULTS Return to TOC

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

The photocatalytic activity of TiO_2 is generally known to be activated by UV light (<380 nm).[4] Therefore, TiO_2-coated orthodontic wires were immersed in a bacterial suspension and illuminated by UV-A light. For the preliminary test of the adhesion ability of *S. mutans*, the TiO_2-coated orthodontic wires were immersed into a BHI broth with or without 10% sucrose. Without UV-A illumination, *S. mutans* cultivated in BHI broth containing 10% sucrose showed prominent aggregation and adhesion to the orthodontic wires. The weight of the orthodontic wires was increased by 6.1%. However, any noticeable change was not observed when the wires were immersed in the *S. mutans* culture broth without sucrose. To compare the photocatalytic activity, the stainless steel wires precoated with TiO_2 and the intact stainless steel wires were immersed in a *S. mutans* culture broth containing 10% sucrose. After 24 hours incubation with UV-A light illumination, there was only a 0.33% change in weight observed in the TiO_2-coated wires, whereas there was a 4.97% increase in weight in the intact wires (Figure 1).

Antibacterial Activity of Surface-modified Orthodontic Wires on *S. mutans* and *P. gingivalis*

Besides the antiadherent ability of the photocatalytic TiO_2-coated wire, antibacterial activities against *S. mutans* and *P. gingivalis* were demonstrated. In the dilution agar plate method, the survival rate of *S. mutans* was 100 CFU in the case of the TiO_2-coated wires when illuminated with UV-A light for 60 minutes, whereas it was 720 CFU in the intact wires (Figure 2).

Because *P. gingivalis* is a strict anaerobe, the dilution agar plate method is not appropriate for the antibacterial activity assay. Therefore, the decrease in optical density at 660 nm was measured instead. As the UV-A illumination time increased the optical density of the culture broth was decreased in the case of the TiO_2-coated wire. However, after 30 minutes, the optical density reached a steady state (Figure 3).

Bacterial Surface Change

For further investigation of the antibacterial effect of TiO_2-coated orthodontic wire, the bacterial surface was observed by scanning electron microscopy. When the TiO_2-coated wires were used with UV-A illumination, severe damage was observed in almost all the Streptococcal cells. However, there was relatively low damage observed in *P. gingivalis* (Figure 4).

DISCUSSION Return to TOC

The photocatalytic activity of illuminated TiO_2 has been actively investigated in diverse areas such as water-treatment processes, air-cleaning agents, and antibacterial agents.[12] TiO_2 shows strong oxidation and reduction activity when irradiated with various wavelengths of UV-light and mineralizes a wide variety of organic compounds to CO_2, water, and inorganic constituents.[13]

Illuminated TiO_2 in water with light at a wavelength less than 380 nm generates excess electrons in the conduction band (e^-_{cb}) and positive “holes” in the valence band (h^+_{vb}). At the TiO_2 particle surface, the holes react with either adsorbed water or surface hydroxyl (OH^-) groups to form HO· radicals. The excess electrons in the conduction band react with molecular oxygen to form superoxide ions, which form more HO· radicals.[4] In aqueous systems, the complete mineralization of many organic substances is possible when a sufficient HO· flux can be generated in situ.[13] Therefore, suspended TiO_2 particles have largely been used as efficient catalysts for the decomposition of

organic contaminants.[14]

Several attempts have been made to apply the photocatalytic activity of TiO_2 to microorganisms.[4,5,9] Microbial cell components that are mainly composed of organic compounds might be sufficient to be degraded by the photocatalytic TiO_2. Although many attempts to apply the photocatalytic properties of TiO_2 to various infectious microorganisms have been made, the actual efficacy was reported to differ according to the species or to the structural components of the cell wall.

Among the various infectious microorganisms, *S. mutans* is one of the most closely investigated organisms in dentistry.[15–19] *S. mutans* degrades sucrose to make insoluble glucan through the action of glycosyl transferase.[20–22] This insoluble glucan also attaches to the tooth surface, providing other oral bacteria places to inhabit. The resulting complex of glucan and various oral bacteria then creates an oral biofilm, which is the mature stage of dental plaque. At this stage, other oral pathogenic bacteria, such as *P. gingivalis* and *Treponema denticola*, form a major part of the oral biofilm. As dental plaque accumulates, acidic compounds, such as fructose and other fatty acids, degrade the enamel surface of the teeth through a process known as dental caries.[23,24]

One of the main causes of failure in orthodontic treatment is the development of dental plaque, which is initiated by the adhesion of *S. mutans* to the tooth surface or orthodontic devices.[6–8] The first step of dental caries is the formation of insoluble glucan followed by the growth of *S. mutans*.[25] The application of photocatalytic TiO_2 to the surface of orthodontic wires and brackets can efficiently prevent the adhesion of *S. mutans* and the development of dental plaque.

In this report, TiO_2 coated on the surface of orthodontic wires showed effective antiadherent properties against *S. mutans* with the illumination of UV-A light. From the data shown in Figure 1, it is possible that a sufficient amount of reactive oxygen species such as OH· were released from the TiO_2-coated wires as a result of the UV-A light. The reduced adhesion of *S. mutans* on the TiO_2-coated wires might be a result of the decomposition of the surface organic molecules of *S. mutans* such as the M-protein. This phenomenon might further influence the cell wall of the bacteria to become more fragile. Indeed, an excessively damaged cell wall of *S. mutans* was observed by scanning electron microscopy. As shown in these results, it is obvious that OH· radicals released from the photocatalytic TiO_2 could directly influence the growth of this oral bacterium, which is consistent with a previous report.[4] Similar results were observed in *P. gingivalis*, which is a major pathogenic bacterium in periodontitis. The photocatalytic TiO_2-coated orthodontic wires effectively reduced the survival rate of *P. gingivalis*.

On the other hand, the fact that the OH· radicals released from the photocatalytic TiO_2 can decompose organic compounds and damage the cell walls of microorganisms may imply a negative supposition. Hydroxyl radicals from TiO_2 may also affect normal oral epithelial cells. However, this serious defect could be excluded by a simple solution. The low efficiency for the utilization of visible light and the relatively low intensity of UV light in normal daylight are the major limiting factors for the use of TiO_2 but are an advantage in this case. As described above, the photocatalytic activity of TiO_2 is usually effectively activated by UV light with a wavelength less than 380 nm. Therefore, the photocatalytic activity can be modulated by manually controlling the illumination time and period in dental clinics. Nevertheless, the toxicity of TiO_2 will need to be determined before it can be applied to orthodontic treatments. The photocatalytic TiO_2-coated orthodontic wires did not show any remarkable effect on KB cells when illuminated with UV-A light of the same intensity for 60 minutes, as described in the antibacterial activity assay (data not shown).

Because orthodontic wires bear diverse mechanical loads, the mechanical stability of the TiO_2 coating is an additional subject that needs to be addressed. However, from a variety of reports about surface modification not only in dental materials but also in biomedical industries, it is believed that the optimization of a TiO_2 coating can be readily achieved.

CONCLUSION Return to TOC

- Surface modification of orthodontic wires with photocatalytic TiO_2 can be used to prevent the development of dental plaque during orthodontic treatment.

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FIGURES Return to TOC

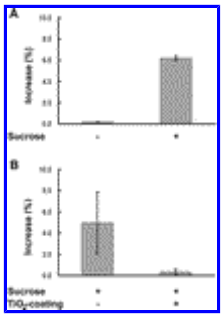

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Figure 1. Increase in the wire mass as a result of the adsorption of *Streptococcus mutans*. (A) 5 cm of the titanium oxide (TiO_2)-coated wires was placed in culture tubes containing 10 mL of a brain heart infusion (BHI) broth with or without 10% sucrose. The overnight-cultured *S. mutans* culture broth was then inoculated into the culture tubes to a final concentration of 10% and incubated for 24 hours at 37°C. $P < .001$. (B) Either the stainless steel wires or the TiO_2-coated wires were placed in a tube containing 10 mL of the BHI broth with 10% sucrose. An overnight-cultured *S. mutans* culture broth was then inoculated into the culture tubes to a final concentration of 10% and incubated for 24 hours at 37°C under illumination of ultraviolet-A black light (1.0 mW/cm^2). The data represent mean values of five independent experiments. The error bar indicates the standard deviation. A paired *t*-test was used to analyze the correlation between each group. $P < .05$

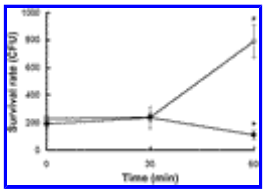

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Figure 2. Survival rate of *Streptococcus mutans* under ultraviolet(UV)-A irradiation. *S. mutans* culture broth was diluted with brain heart infusion (BHI) broth to make an optical density 1.0 at 660 nm. Ten milliliters of the diluted bacterial suspension was transferred onto petri dishes containing either the stainless steel wires or the titanium oxide (TiO_2)-coated wires. The dishes were illuminated with UV-A black light (2 W/m^2, F10T8BLB, Sankyo Denki) on a clean bench with an intensity of 1.0 mW/cm^2 for 60 minutes. After illumination, 100 µL of the bacterial suspension was serially diluted and plated onto a BHI agar plate. The survival rate of *S. mutans* is described by the colony-forming unit. ○ indicates bacterial culture with the uncoated wire; ●, bacterial culture with the TiO_2-coated wire. The data represent mean values of five independent experiments. The error bar indicates the standard deviation. The Kruskal-Wallis H test was used to analyze the control and TiO_2-coated groups. $P < .01$. *Paired *t*-test, $P < .0005$

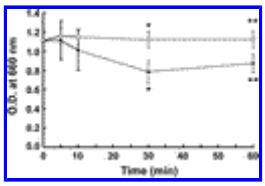

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Figure 3. Survival rate of *Porphyromonas gingivalis* under ultraviolet (UV)-A irradiation. *P. gingivalis* culture broth was diluted with brain heart infusion broth to make an optical density of 1.0 at 660 nm. Ten milliliters of the diluted bacterial suspension was transferred onto petri dishes containing either stainless steel wires or titanium oxide (TiO_2)-coated wires. These dishes were illuminated with UV-A black light (2 W/m^2, F10T8BLB, Sankyo Denki) in an anaerobic chamber with an intensity of 1.0 mW/cm^2 for 60 minutes. The survival rate of *P. gingivalis* was analyzed by the optical density at 660 nm. The data represent mean values of five independent experiments. The error bar indicates the standard deviation. The Kruskal-Wallis H test was used to analyze the control and TiO_2-coated groups. $P > .10$ for control

group and $P < .50$ for TiO_2-coated group. *Paired *t*-test, $P < .01$, **Paired *t*-test, $P < .05$. ○ indicates bacterial culture with the uncoated wire; ●, bacterial culture with the TiO_2-coated wire

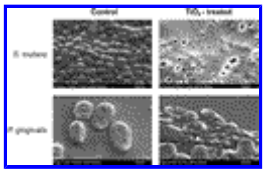

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Figure 4. Scanning electron microscopy analysis of *Streptococcus mutans* and *Porphyromonas gingivalis*. The *S. mutans* or *P. gingivalis* culture broth was diluted with the brain heart infusion broth to make an optical density 1.0 at 660 nm. Ten milliliters of the diluted bacterial suspension was transferred onto petri dishes containing either the stainless steel wires or titanium oxide–coated wires. These dishes were illuminated with ultraviolet-A black light (2 W/m^2, F10T8BLB, Sankyo Denki) for 60 minutes. Magnification: 20,000×

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