

Photocatalytic Antibacterial Effect of TiO₂ Film of TiAg on *Streptococcus mutans*

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ABSTRACT

Objective: To test through various oxidation procedures the differences in antibacterial activities against *Streptococcus mutans* (*S mutans*) of Titanium (Ti) and Titanium silver (TiAg) metals coated with TiO₂.

Materials and Methods: This study examined the photocatalytic antibacterial effects on *S mutans* of Ti and TiAg substrates coated with two crystalline forms of TiO₂ by thermal and anodic oxidation. A bacterial suspension of *S mutans* was pipetted onto TiO₂-coated metal specimens and uncoated specimens with ultraviolet A (UVA) illumination for 20 to 100 minutes. The same specimen without UVA was used as the control. The level of colony-forming units of *S mutans* after UVA illumination was compared with that of the control.

Results: The level of colony-forming units of *S mutans* was significantly lower on TiO₂-coated Ti and TiAg metal specimens after UVA illumination than on uncoated Ti and TiAg specimens. The level of colony-forming units of *S mutans* was significantly lower on the metals coated by anodic oxidation than on those coated by thermal oxidation. The TiO₂ coating on TiAg had a significantly higher and more rapid antibacterial effect than did the TiO₂ coating on Ti.

Conclusions: The antibacterial effect of a TiO₂ film formed by anodic oxidation was superior to that formed by thermal oxidation. The addition of Ag to the Ti specimen indicated a synergistic effect on the photocatalytic antibacterial property against *S mutans*. (*Angle Orthod.* 2009;79: 528–532.)

KEY WORDS: TiAg; TiO₂; Photocatalytic antibacterial effect; Anodic oxidation; *Streptococcus mutans*

INTRODUCTION

Titanium (Ti), because of its good corrosion resistance, biocompatibility, and self-cleaning effect, is

commonly used for orthodontic brackets, wires, and temporary anchorage devices such as miniscrews.¹ The corrosion resistance and biocompatibility of Ti alloys are maintained by the surface oxide layer, which mainly consists of Titanium dioxide (TiO₂). The surface oxide film on Ti alloys generally exists in an amorphous state, which does not indicate photocatalytic activity.^{2,3} However, three crystal structures of TiO₂—anatase, rutile, and brookite—can be produced by various oxidation methods to induce a photocatalytic reaction.^{2,3} Hydroxyl radicals that are extremely reactive to organic compounds are produced by the irradiation of TiO₂ at wavelengths >385 nm, resulting in photocatalytic antibacterial activity.^{4,5} TiO₂ was reported to have antibacterial activity against two major bacteria in the dental field—*Lactobacillus acidophilus*⁵ and *Streptococcus mutans* (*s mutans*)⁶—indicating its possible clinical applications.

TiO₂ can be produced by the oxidation of Ti itself⁷ or by a sol-gel method by dipping the metal into a liquid-coating agent followed by solidification, which results in surface crystallization.⁸ It is interesting to note that the photocatalytic effects can be enhanced

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by the addition of noble metals such as platinum (Pt) and silver (Ag) to the TiO₂ surface.^{5,9} Recently, Ag has attracted attention, and now it is widely used in medical materials owing to its antibacterial effects.¹⁰ Therefore, it was hypothesized that a TiO₂ coating formed on a Titanium silver (TiAg) alloy might exhibit superior antibacterial activity against oral microorganisms to that formed on a Ti substrate alone.

This study investigated the antibacterial activity of metals coated with TiO₂ through various oxidation procedures against *S mutans* and examined whether or not the addition of Ag to Ti can improve antibacterial activity.

MATERIALS AND METHODS

Preparation of Metals

Ti (Grade III, ASTM F67, Supra Alloys Inc, Camarillo, Calif) and a TiAg (Ti-2.0at%Ag, Biomaterialskorea, Seoul, Korea) plate were cut into 5 × 5 mm² specimens. TiO₂ exists in three crystal structures: rutile, anatase, and brookite. In this study, anodic oxidation (AO) and thermal oxidation (TO) were used to produce anatase and rutile, respectively.⁵

For TO, the Ti and TiAg alloys were treated in a muffle furnace at 400°C for 1 hour in air and were cooled. For AO, the samples were anodic-oxidized at 250 V for 3 minutes in a solution that contained 0.04 M β-glycerophosphate disodium salt pentahydrate and 0.4 M calcium acetate.⁵ The x-ray diffraction (XRD) patterns on the uncoated specimens (Ti and TiAg) and those on the thermally oxidized Ti (TO) and TiAg (TO) were similar, as was previously reported.⁵ Metastable anatase peaks were identified on the surfaces of Ti (AO) and TiAg (AO) after anodic oxidation; this event is identical to that described in previous reports.^{5,7}

Cell Culture

S mutans (KCTC 3298) was cultured in 20 mL of a brain heart infusion (BHI) broth for 12 hours, centrifuged, and adjusted to 4.8 × 10⁶ colony-forming units (CFU) by dilution with phosphate-buffered saline (PBS).

To investigate photocatalytic antibacterial activity, 100 μL of a bacterial solution was pipetted onto the metal specimens (Ti, TiAg, Ti [TO], TiAg [TO], Ti [AO], and TiAg [AO]). The samples were illuminated with ultraviolet A (UVA) light for 60 minutes (Philips Electronics, Seoul, Korea) 2 × 15 W, black light, 356 nm peak emission). The light source was placed 7 cm above the sample. For the control group, the light was covered with a black cloth to confirm the viability of germs during the experiment. The samples were placed on cold water-filled Petri dishes to prevent them from drying.

After illumination, each reaction solution was diluted in a 10-fold series to 10⁵ and was shaken for 10 minutes (vortex 200 rpm). A total of 100 μL of each sample was plated onto BHI agar and was incubated for 36 hours at 37°C; the CFU then were counted. The experiments performed for each specimen, including those of controls, were repeated four times.

To determine time dependence on photocatalysis, 100 μL of each bacterial solution was used to examine antibacterial performance at 20, 40, 60, 80, and 100 minutes.

Statistical Evaluation

Differences in CFU for each specimen were examined by one-way analysis of variance (ANOVA) with the use of the Statistical Package for the Social Sciences (SPSS) software (SPSS Inc, Chicago, Ill). All results were reported as mean ± standard deviation (SD). *P* < .05 was considered significant.

RESULTS

The viability of *S mutans* was decreased after the photocatalytic reaction had occurred. After UV illumination for 60 minutes, the level of CFU of *S mutans* on Ti, Ti (TO), and Ti (AO) was significantly lower than that found on the surfaces of controls (blocked UV illumination) (*P* < .05) (Figure 1a). The TiO₂-coated groups, both TO and AO, showed a significantly lower level of CFU than Ti after UV illumination. Between the TiO₂-coated groups, the antibacterial activity of the Ti (AO) samples was significantly greater than that of the Ti (TO) samples (*P* < .05) (Figure 1a).

The level of CFU of *S mutans* on the TiAg, TiAg (TO), and TiAg (AO) substrates after 60 minutes of illumination was significantly lower than that of controls (*P* < .05) (Figure 1b). The level of CFU was significantly decreased in the TiO₂-coated TiAg specimens, even under control conditions (without UV illumination). Antibacterial activity after UV illumination was significantly greater in TiO₂-coated TiAg specimens than in uncoated TiAg specimens. However, the antibacterial activities of TiAg (TO) and TiAg (AO) after UV illumination were similar (Figure 1b).

Time-dependent analysis indicated that the level of CFU of all Ti specimens decreased with increasing illumination time. Ti (AO) was sterilized completely after 60 minutes, but this process took 100 minutes for Ti and Ti (TO) (Figure 2a). A similar trend was noted for the TiAg specimens. The CFU level of *S mutans* in TiAg (AO) reached near 0 after 20 minutes, compared with 80 minutes for TiAg (TO) and 100 minutes for TiAg (Figure 2b). Compared with the Ti specimens, TiAg specimens showed a rapid decrease in level of

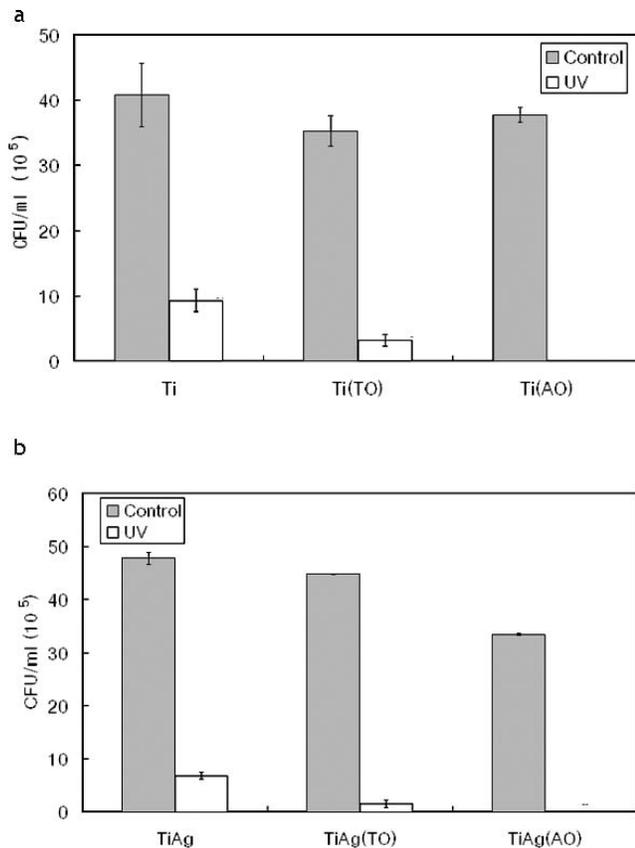


Figure 1. The viability of *Streptococcus mutans* cells was decreased after ultraviolet A (UVA) illumination. The viability of *S mutans* cells (4.8×10^6 colony-forming units [CFU]/mL) after 60 minutes UVA illumination for Ti (a) and for TiAg (b). The controls were covered with black cloth. Data are expressed as the mean \pm standard deviation (SD). * Statistical significance compared with the control was noted at $P < .05$.

CFU, particularly in the early stages (<20 min) of illumination (Figure 2).

DISCUSSION

The use of fixed orthodontic appliances can accelerate the rate of plaque accumulation, which can result in enamel decalcification and dental caries without proper maintenance.^{11,12} The main bacterial species that may cause dental caries is *S mutans*.¹³ The number of oral bacteria can be decreased by brushing, or with the additional use of fluoride, chlorhexidine, and antibiotics.^{14–16} In addition, considerable effort has been made to produce antibacterial dental materials in the oral environment.

According to these results, UV illumination alone revealed a decrease in CFU in all types of metal specimens after 60 minutes. However, the photocatalytic activities of both Ti and the TiAg alloy significantly decreased the CFU level of the surface-coated *S mutans* in vitro. The addition of Ag resulted in significant en-

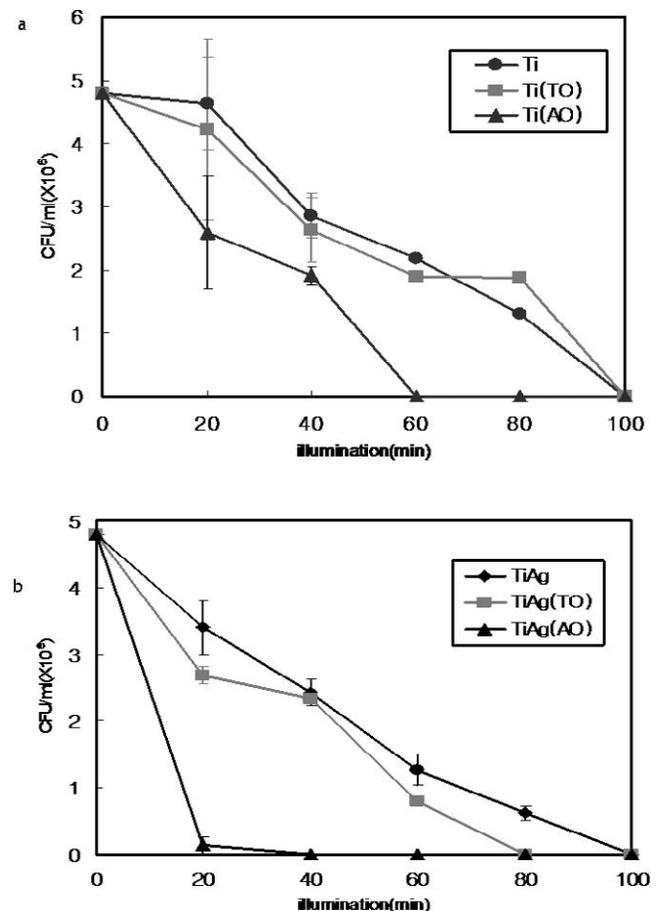


Figure 2. Kinetics of *Streptococcus mutans* inactivation induced by photocatalysis of TiO_2 on Ti (a) and on TiAg (b). TO indicates thermal oxidation; AO, anodic oxidation. Data are expressed as the mean \pm standard deviation (SD). * Statistical significance compared with the control was noted at $P < .05$.

hancement of the antibacterial effect within 20 minutes, indicating its effectiveness. Irradiation of the TiO_2 surface with a wavelength >385 nm generates an electronic hole pair on the TiO_2 surface. This hole reacts with H_2O to produce hydroxyl radicals, which are extremely reactive to organic compounds.⁴ It was reported that the photocatalytic effect can be enhanced by preventing the recombination of electrons and holes through the addition of a noble metal.^{6,9} A similar mechanism might also apply in the case of Ag.

Similar to previous reports, the photocatalytic reaction induced a relatively mild decrease in germ counts for Ti and TiO_2 (TO) after the first 20 minutes of UV illumination and showed a rapid decrease in subsequent stages.^{1,17} In case of TiAg alloys, germ counts decreased rapidly in the initial 20 minutes. The primary step in photocatalytic decomposition consists of hydroxyl radical attack on the cell wall. This leads to increased permeability, which allows radicals to reach and damage the cytoplasmic membrane, causing lipid

peroxidation. It was reported that the antibacterial effect of TiO₂ is based on the disorder of the cytoplasmic membrane.^{1,6,17} The photocatalytic ability is lost if the electronic hole pairs of TiO₂ recombine before they can be used for photocatalysis. The high levels of antibacterial efficiency of TiAg compared with that of Ti may be explained by the role of Ag in preventing the recombination of electrons and holes, which in turn, facilitates disruption of the cell wall. The antibacterial effect of the TiO₂ coating for various organisms is determined primarily by the complexity and density of the cell walls,¹⁸ as well as by the types of microorganisms.¹⁹ Therefore, additional studies on the effects of Ag on various organisms are needed not only under in vitro conditions but also under in vivo and intraoral conditions. In addition, to clarify the direct and indirect effects of improved photocatalysis of TiAg, time course results of the CFU level without UV illumination would be helpful.

Among the two types of oxidation procedures, a greater and more rapid photocatalytic antibacterial effect was observed for the Ti and TiAg specimens coated by anodic oxidation—Ti (AO) and TiAg (AO)—compared with the specimens that were thermally oxidized—Ti (TO) and TiAg (TO). This enhancement of photocatalytic activity of the AO type may be due to differences in the reducing power of AO compared with TO. TiO₂ absorbs light with energy greater than the band gap, which causes electrons to jump to the conduction band to create holes in the valence band. In both rutile (TO) and anatase (AO), the position of the valence band is deep. However, the position of the conduction band in anatase is more negative than in rutile, which results in stronger reducing power.⁷

Several reports have showed that the physical properties of Ti brackets, including friction and strength, are comparable with those of stainless steel brackets.^{20,21} TiAg alloys also have been used in dental implants to supplement the weak strength and wear resistance of Ti.²² Given these results, a TiO₂ film coating may assist in the oral hygiene of orthodontic patients. However, for a photocatalytic reaction to occur, the TiO₂ surface must be exposed to UV radiation. This means that the brackets in oral environments should be exposed to UV light, possibly through smiling or widely opening outdoors. Although UVA has a lower impact on humans than does UVB or UVC, long-term exposure to UVA can be harmful.²³ Therefore, for clinical applications, outdoor UV or UVA lamps at an intensity of 1 mW/cm².²⁴ or the development of a visible light photocatalyst that reacts in indoor light is advisable.²⁴ In addition to studies undertaken to explore limitations in its light source, studies of the intraoral environment should be conducted to clarify fully the photocatalytic

antibacterial effect and its potential clinical applications in the orthodontic field.

CONCLUSIONS

- Photocatalyst reactions of TiO₂-coated metals showed antibacterial activity against *S mutans*.
- Synergistic enhancement of photocatalytic antibacterial effect against *S mutans* was achieved by the addition of Ag to Ti metal.
- Anatase produced by anodic oxidation (AO) showed greater and more rapid antibacterial activity against *S mutans* than did rutile produced by thermal oxidation (TO).

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