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RESEARCH PAPER

Antimicrobial Activity of TiO_2-coated Orthodontic Ceramic Brackets against *Streptococcus mutans* and *Candida albicans*

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Abstract Polycrystalline alumina ceramic orthodontic brackets were coated with anatase TiO_2 film *via* a sol-gel dip-coating method. The surface structure morphology and composition of the films were evaluated *via* scanning electron microscopy, atomic force microscopy, and X-ray diffraction. The antimicrobial activity of the ceramic brackets was assessed against two oral pathogens, *S. mutans* and *C. albicans*. The results demonstrated that TiO_2-coated brackets exposed to low energy UV-A illumination efficiently reduced the populations of test microorganisms relative to the uncoated brackets. The reduction efficiencies were 98% for *S. mutans* ATCC 10449 and 93% for *C. albicans* ATCC 60193.

Keywords: TiO_2, orthodontic brackets, antimicrobial activity, *Streptococcus mutans*, *Candida albicans*

1. Introduction

TiO_2 is one of the most widely used photocatalysts in applications involving organic material degradation- -including, for example, air purification, water treatment, self-cleaning, and bacterial degradation. TiO_2 is chemically stable under UV light and in water, and is transparent to visible light. Moreover, TiO_2 films can be readily synthesized *via* comparatively inexpensive methods, with relatively good adherence to substrates with intricate geometry. In dentistry, the photocatalyst applications of TiO_2 have included, among others: The disinfection of dental mirror surfaces [1], the production of dental implants with antibacterial activities [2], and the manufacture of antibacterial endodontic wires [3].

The mouth harbors diverse populations of microorganisms comprising over 350 taxa, including at least 37 bacterial genera. Virtually all oral bacteria harbor surface molecules that function in cell-to-cell interaction. The earliest colonizers are overwhelmingly *Streptococci*, which constitute 47 to 85% of the cultivable cells detected in the first 4 h after a professional tooth cleaning [4]. *Streptococcus mutans* is the primary causative agent of dental caries and is a prodigious producer of the glucosyltransferase enzyme, which catalyzes the formation of soluble and insoluble α-linked glucans from sucrose [5]. Glucans promotes the adherence and accumulation of cariogenic microorganisms to the tooth surface, resulting in the formation of dental plaques. Eventually, dental plaque contributes to caries-forming activity [6]. *Candida albicans* is the fungal species that is most frequently detected in the oral cavities of both healthy and medically compromised individuals. It can colonize tongue, mucosa, dentin, root, subgingiva, and periodontal pockets [7]. *C. albicans* co-aggregates with certain oral bacteria making use of surface molecules, which promote adhesion to the host tissues. *C. albicans* can produce biofilms on dental surfaces, which is resistant to antifungal agents up to 100-fold [8], and participate in primary, secondary, and persistent root canal infections [7]. In this

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study, the antimicrobial activity of a TiO_2 coated orthodontic ceramic bracket was assessed against two important oral pathogens, *S. mutans* and *C. albicans*.

2. Materials and Methods

2.1. Sol-gel preparation and dip-coating

As-received polycrystalline alumina ceramic Intrique™ brackets were manufactured by Lancer Orthodontics (Fig. 1). Anatase TiO_2 thin-film coating was conducted using a sol-gel dip-coating method. In this method, the sol was prepared with 12 mL of titanium (IV) isopropoxide (97%, Aldrich), 170 mL 2-proponol, and 0.4 mL of hydrochloric acid (2M) [9]. Polyethylene glycol (PEG, Mw = 600, Aldrich) at an amount of 3 wt% was added. The selected wt% PEG has been shown previously to induce a relatively crack-free and strongly adherent TiO_2 coating layer to the substrates after calcination [10,11]. The solution was then stirred continuously for 3 h at room temperature. After stirring, the sol was aged for 24 h in a refrigerator (4°C). Prior to dip-coating, the surfaces of the brackets were cleaned in an ultrasound bath, with a sequence of acetone and distilled water. The brackets were then dipped into the solution inside a steel cage with a dipping and removal rate of 76 mm/min. After coating, the brackets were dried at room temperature, followed by 1 h of furnace drying at 120°C. The dip-coating procedure was then repeated 3 times in an effort to increase the thickness of the films. Finally, the coated brackets were calcined for 1 h at 500°C in a furnace with a heating and cooling rate of 2°C/min. The brackets were maintained in desiccators until being used in the microbial activity tests. Microscopic analysis of the coated brackets was conducted using a scanning electron microscope (SEM) (Philips XL30-SFEG, Holland) with an energy dispersive X-ray (EDX) analyzer (Philips X'Pert Pro, Holland) and a nanoscope-IV atomic force microscope (AFM) (Digital Instruments- MMSPM Nanoscope IV, USA) in contact mode. In a separate experiment, a glass (silica) slide was coated with TiO_2 using the same procedure as previously outlined and used for phase identification. The crystal structure of the TiO_2 thin film was determined via grazing incidence X-ray diffraction (GIXRD) with an incident angle of 0.5°.

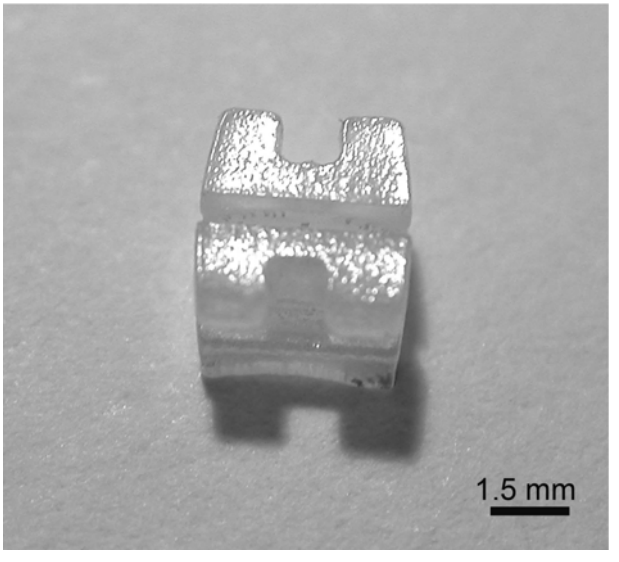

Fig. 1. Picture of a TiO_2-coated ceramic bracket.

2.2. Microorganisms and antimicrobial activity assay

S. mutans ATCC 10449 and *C. albicans* ATCC 60193 were cultured in Brain Heart Infusion Broth (Difco) [12] and Sabouraud Dextrose Broth (Difco) at 35 ± 1°C, respectively [13]. The cells were washed twice in NaCl solution (0.9 wt%) after 16 h of incubation. The initial microbial concentrations were adjusted by dilution with NaCl solution to McFarland 0.5 [14]. To determine the initial microbial counts (IMC), the microbial solutions were serially diluted and 100 µL suspensions were spread onto microbial media (Sample A).

Seven coated (Sample B) and five uncoated (Sample C) brackets were transferred to sterile petri dishes containing 1 mL initial concentrations of microorganisms and illuminated with UV-A (black light, 400 ~ 315 nm) for 1 h at 1.0 mW/cm^2. A group of coated brackets (n = 6) were maintained in darkness (negative control) for each microorganism (Sample D). After the specified time course, 100 µL of culture liquid from each sample was serially diluted and the final microbial counts (FMC) were determined as described above. The reductions in the microbial counts were evaluated in terms of residual colony forming units per milliliter (cfu/mL) after the treatment. The mean cfu values for each group were calculated. The decreases in the cfu's were calculated using Reduction Efficiency % = IMC-FMC/IMC. The cfu data were analyzed using Turkey's and LSD tests in ANOVA [15]. A $p < 0.05$ was considered significant.

3. Results

An SEM micrograph of a TiO_2-coated bracket surface is shown in Fig. 2. The coating layer was found to be continuous and to harbor uniformly distributed surface microcracks of 1 ~ 10 µm in length. It was noted that the coated film detached in sections of very high surface roughness, revealing underlying alumina grains of ~ 20 µm in size (Fig. 2). The thickness of the film was also measured from the SEM micrographs of the cracked sections, and was found to vary between 0.5 and 1 µm depending on the

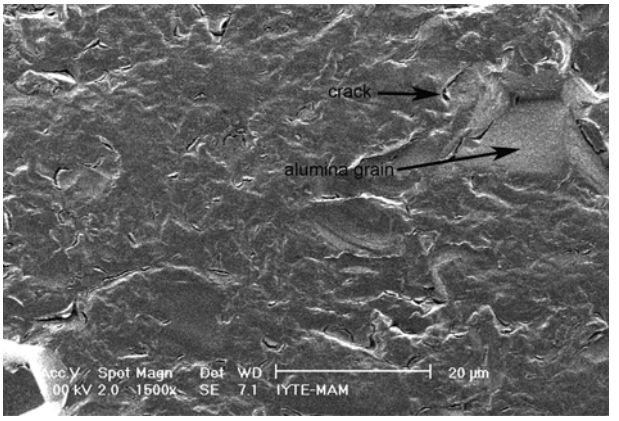

Fig. 2. SEM micrograph showing TiO_2 film at the bracket surface.

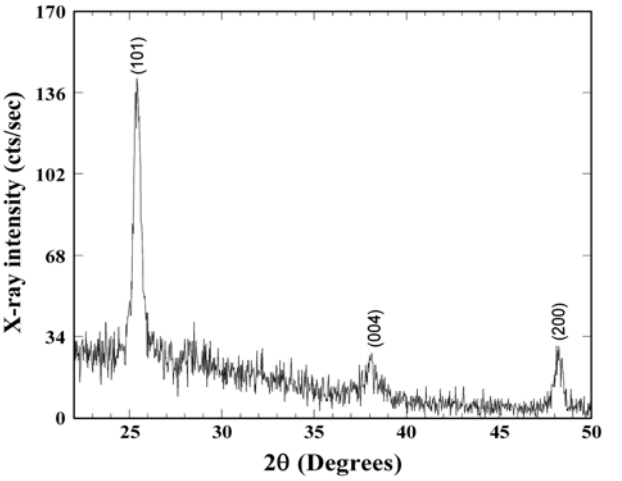

Fig. 3. GIXRD spectra of TiO_2-coated glass substrate after heat treatment at 500°C.

surface roughness. Typical GIXRD spectra of the films coated on a glass substrate after heat treatment at 500°C are provided in Fig. 3. The diffraction lines at $2\theta = 25.4°$, 38°, and 48.1° confirm the anatase coating layer. 3 and 2D AFM micrographs of an uncoated and a coated sample are shown sequentially in Figs. 4A and 4B. The upper micrographs in these figures are 3D AFM surface profiles of the brackets, and the lower micrographs show 2D AFM surface profiles of the brackets. The micrographs were obtained using a silicon nitride contact tip in a scanning area of 20 × 20 μm^2. In these figures, the 2D images provide information regarding the height of the surface roughness prior to and after coating. TiO_2 coating, as anticipated, reduces

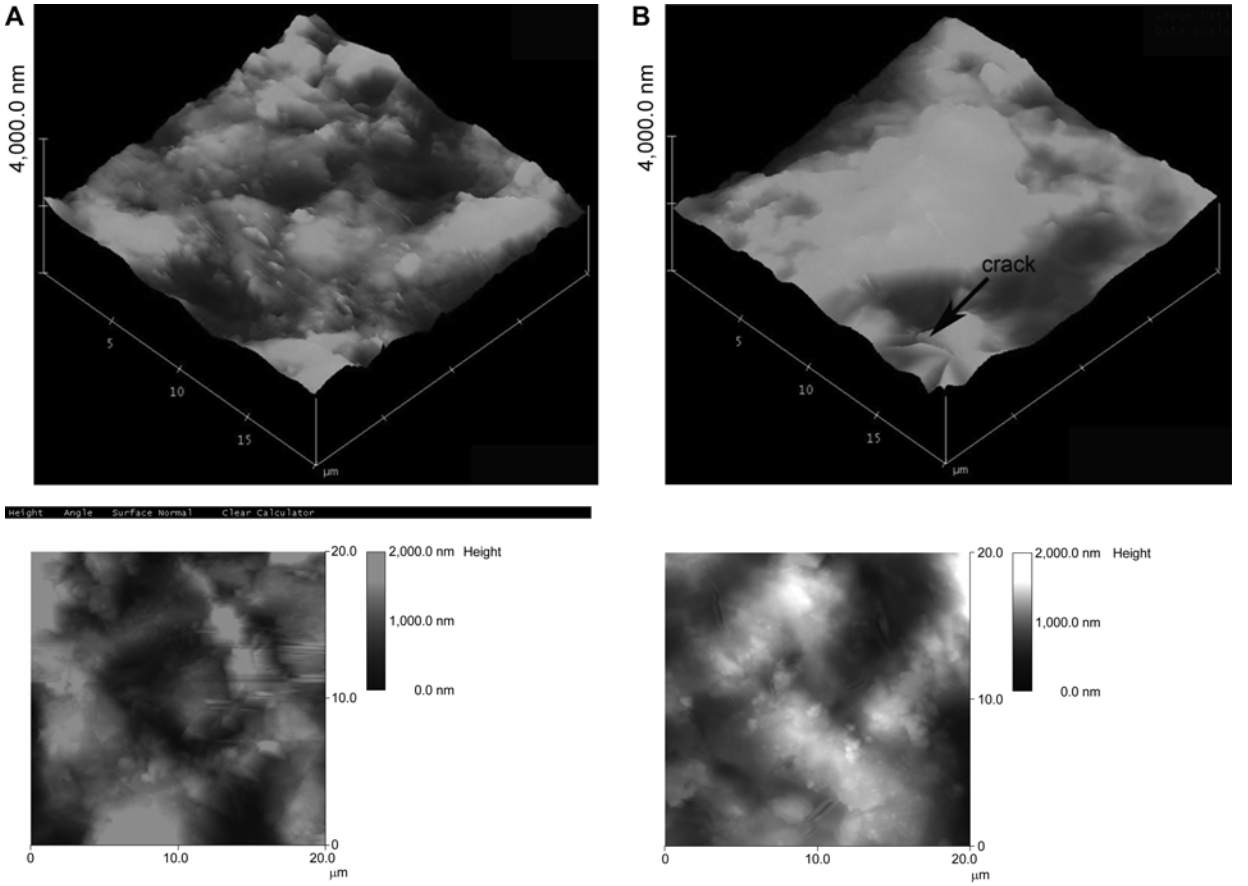

Fig. 4. 3 and 2D AFM contact mode surface images of (A) uncoated and (B) TiO_2-coated bracket.

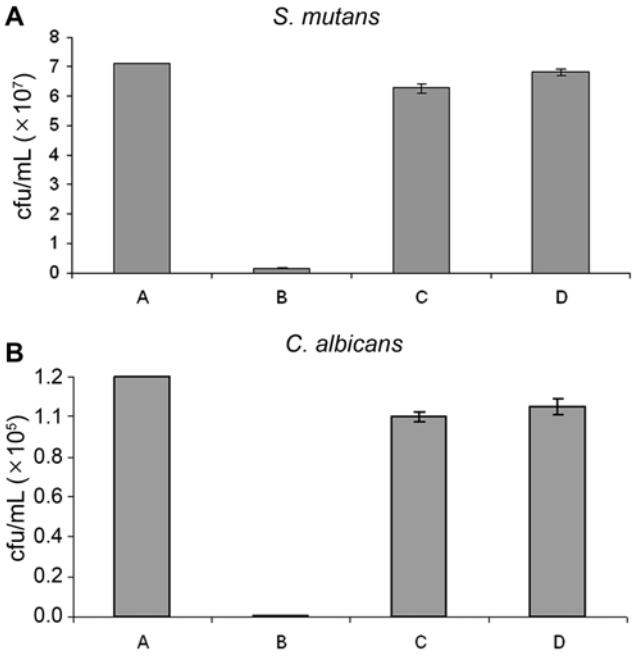

Fig. 5. (A) The effect of photocatalytic activity on *S. mutans* ATCC 10449 and (B) *C. albicans* ATCC 60193.

the surface roughness of the brackets, as can be seen in Figs. 4A and 4B. Furthermore, the cracks on the film can be clearly observed in the 3 and 2D AFM micrographs shown in Fig. 4B. The surface roughness of the coated bracket shown in the 2D image of Fig. 4B is lower than that of the uncoated bracket shown in the 2D image of Fig. 4A. The surface roughness of the coated and uncoated brackets was measured as 197.99 and 330.18 nm, respectively.

The IMC and FMC values for *S. mutans* and *C. albicans* were shown in Fig. 5. Statistically significant decreases were detected between each group ($p < 0.05$). Although the decreases in the IMC values between each group are important, TiO_2 film coating exerts a significant effect on the microorganisms used under UV-A, as compared to the other groups (Figs. 5A and 5B). Figs. 6A, 6B, 6C, and 6D show *S. mutans* ATCC 10449 and *C. albicans* ATCC 60193 microorganisms attached to the TiO_2 coating prior to and after UV-A illumination, respectively. The number of attached cells is reduced and the cells break down after UV illumination as seen in Figs. 6B and 6D which is consistent with the results of the bacterial activity tests. These findings indicate that TiO_2 coating can effectively reduce the microbial activities of both oral pathogens.

4. Discussion

The marked photo-catalytic activity of TiO_2 is suggestive

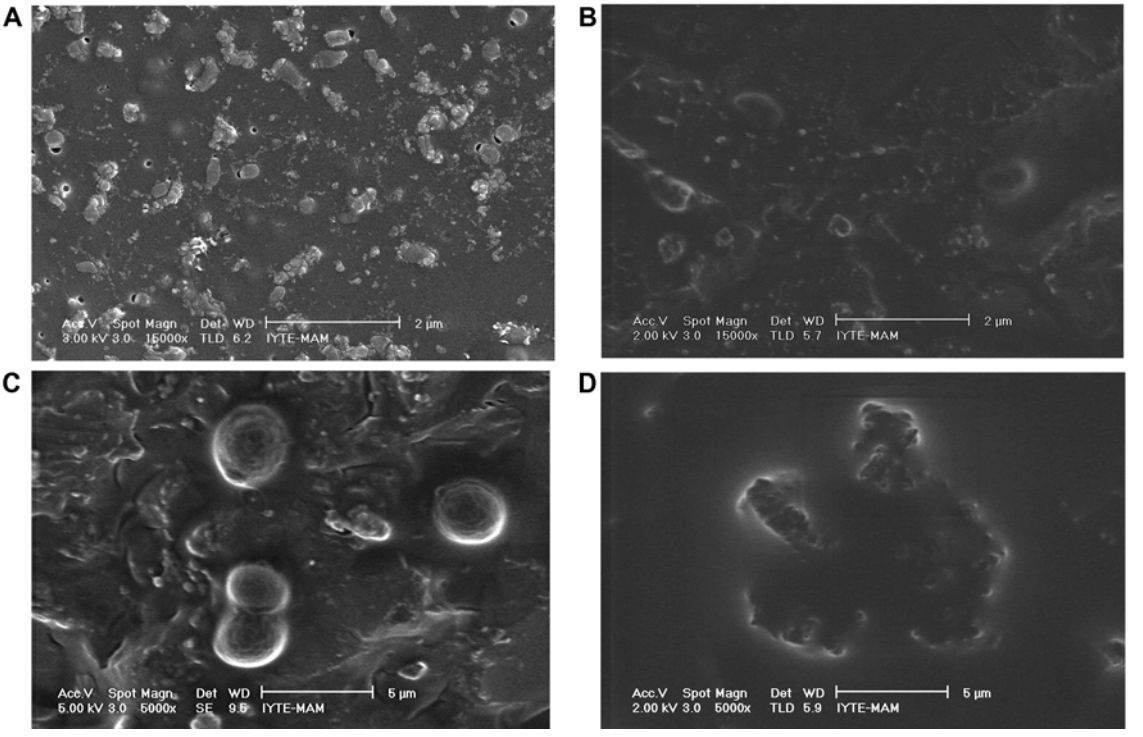

Fig. 6. SEM micrographs of microorganisms attached to the TiO_2-coated brackets: *S. mutans* ATCC 10449 (A) before and (B) after UV-A illumination and *C. albicans* ATCC 60193 (C) before and (D) after UV-A illumination.

of its sizeable potential for anti-bacterial reactions [10], and TiO_2 is also superior to other such materials due to its profound chemical stability in aqueous solutions under UV irradiation [16]. The sol-gel method used to prepare TiO_2 film is both simple and suitable for the deposition of substrates of complex geometry. It was demonstrated previously that, when the substrate was removed from the solution, the alcohol (or sometimes water) in the solution began to gel due to rapid evaporation [17]. A constant dip-coating immersion and removal speed, 76.2 mm/min, was therefore employed in this study in order to ensure a uniform film thickness and to minimize cracks at the film's surface. Furthermore, the addition of PEG to the coating solution delays the hydration reaction, hence reducing the extent of film cracking, and leading to the formation of a relatively homogeneous coating layer [18,19]. Polycrystalline alumina grains on the bracket surface increase the surface roughness, potentially accounting for the mechanical gripping between the TiO_2 film and the bracket.

Hamid and Rahman [20] evaluated the crystal structure of the TiO_2 thin film calcined for 1 h at 500°C. The XRD results confirmed the formation of an anatase TiO_2 film. The loss of vividness during the photo-catalytic reaction was determined previously in a study using *Enterococcus faecium*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *C. albicans* as test organisms [21]. After 30 min of UV irradiation, bacterial reduction was noticed with the TiO_2 coating, whereas the bacterial reduction occurred after 90 min without TiO_2 coating. The reduction efficiency of the TiO_2-coated brackets was 98% for *S. mutans* ATCC 10449 and 93% for *C. albicans* ATCC 60193 when the brackets were illuminated for 1 h with 1.0 mW/cm^2 UV-A. However, a significant reduction in uncoated brackets was also detected at 11% for *S. mutans* ATCC 10449 and 16% for *C. albicans* ATCC 60193. It has been well established that although UV-A has relatively low energy compared to UV-B, it can still cause cellular or DNA damage as the result of oxidative stresses caused by oxygen radicals [21,22]. Nevertheless, TiO_2-coated brackets maintained in the dark were found to be less efficient in the reduction of both microorganisms, 0.04% for *S. mutans* ATCC 10449 and 12.5% for *C. albicans* ATCC 60193.

The antimicrobial activity of TiO_2-coated substrates can be simply explained by the photo-induced oxidative power of TiO_2 photocatalysts. Its bactericidal function, however, has yet to be thoroughly understood, even though numerous reports have described the photo-killing of bacteria [23], viruses [24], and tumor cells [25]. Illuminated TiO_2 photocatalysts decompose organic compounds by oxidation and with hydroxyl radicals generated by the oxidation of the water [18]. The antimicrobial activity of the illuminated TiO_2 in solutions is associated with the formation of free hydroxyl radicals (HO·). The HO· radical is highly toxic towards microorganisms and very reactive in the oxidation of organic substances. These substances damage nucleic acids and cell walls. In a previous study, the reduction efficiency of TiO_2 films was found to be superior in Gram (+) bacteria relative to *C. albicans*, ostensibly owing to the cell wall composition of the organisms [18]. The results of this study are consistent with this finding. The reduction efficiency of the TiO_2-coated brackets was higher in *S. mutans* ATCC 10449 (98%) than in *C. albicans* ATCC 60193 (93%).

5. Conclusion

In this study, the antimicrobial activity of ceramic orthodontic brackets (polycrystalline alumina Intrique™) coated with anatase TiO_2 film were assessed against two relevant oral pathogens: *S. mutans* and *C. albicans*. The results demonstrated that the TiO_2-coated brackets, when exposed to low energy UV-A illumination, efficiently reduced the populations of test microorganisms relative to the uncoated samples. This demonstrated the potential of TiO_2 film coating for the prevention of oral pathogens during endodontic treatments.

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