An Antibacterial Surface on Dental Implants, Based on the Photocatalytic Bactericidal Effect

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ABSTRACT

Background: It is well known that the moderately roughened surfaces of dental implants enhance direct bone-implant contact. However, rough implant surfaces, as compared to smooth surfaces, are thought to pose a higher risk of bacterial infection when exposed to the oral cavity.

Purpose: This study was focused on evaluating the photocatalytic bactericidal effects of anatase titanium dioxide (TiO_2) on gram-negative anaerobic bacteria known to be associated with periimplantitis.

Materials and Methods: A film of photocatalytic anatase TiO_2 was added onto the surface of commercially pure titanium disks by plasma source ion implantation (PSII) followed by annealing. The photocatalytic properties of the film were confirmed by the degradation of methylene blue. *Actinobacillus actinomycetemcomitans* and *Fusobacterium nucleatum* cells were incubated anaerobically and seeded on the disk. The disks were then exposed to ultraviolet A (UVA) illumination from black light in an anaerobic environment. After illumination, the number of viable cells was counted in terms of colony-forming units.

Results: The anatase TiO_2 film added by the PSII method and annealing exhibited a strong photocatalytic reaction under UVA illumination. The viability of both types of bacteria on the photocatalytic TiO_2 film was suppressed to less than 1% under UVA illumination within 120 minutes.

Conclusion: The bactericidal effect of the TiO_2 photocatalyst is of great use for sterilizing the contaminated surface of dental implants.

KEY WORDS: bactericidal effect, dental implant, gram-negative bacteria, periimplantitis, photocatalyst, plasma source ion implantation, ultraviolet A

Anatase titanium dioxide (TiO_2) displays photocatalytic activity under ultraviolet A (UVA) illumination. Much research on this phenomenon has been carried out since it was discovered (during the 1960s) that anatase TiO_2 electrodes can directly split the water molecule.[1] It is well known that a photocatalyst can decompose various organic compounds under UVA illumination by generating active-oxygen species such as $\cdot OH$, $O_2^-\cdot$, $HO_2\cdot$, and H_2O_2.[2] This photodecomposition of organic compounds is also useful for killing bacteria. It has been confirmed that these active-oxygen species can destroy the outer membrane of the *Escherichia coli* cell, finally leading to cell death.[3–6] Numerous reports have also described this photocatalytic bactericidal effect on other types of bacteria.[7–10]

The clinical success of oral implants has been improved by surface alterations that enhance direct bone-implant contact. Moderately increased surface roughness is one of these alterations.[11,12] At the same time, however, rough implant surfaces have potential disadvantages from a hygienic viewpoint. The long-term presence of plaque on the surface of an implant may lead to periimplantitis, a destructive inflammatory

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process affecting the soft and hard tissues around osseointegrated implants, leading to the formation of periimplant pockets and loss of supporting bone.[13] It has been demonstrated that rough surfaces harbor significantly higher amounts of microorganisms than smooth surfaces harbor.[14–16] Normally the roughened surface of a successfully osseointegrated implant is embedded in bone, as long as the marginal bone level around the implant is maintained. However, it might happen that the rough surface is exposed to the oral cavity because of a degradation of supporting tissues, and then it becomes contaminated. As mentioned above, rough implant surfaces have been reported to harbor considerably larger amounts of bacteria than smooth surfaces harbor, and they must be cleaned immediately to avoid the rapid development of infection and loss of supporting tissues. To make matters worse, plaque attached to a rough surface cannot be removed easily because it is protected against naturally occurring removal forces or oral hygiene measures.[15,17] Therefore the development of an alternative sterilizing method for treating contaminated rough surfaces is urgently needed, and such a method may well benefit many patients.

We have been studying the application of anatase TiO_2 as a photocatalyst on rough-surfaced dental implants, aiming at a bactericidal effect. The bactericidal effect of a photocatalyst strongly depends on the active oxygen produced on the surface when the surface is illuminated with UVA. An increase in the surface area to which the photocatalyst is applied enlarges the area in which active oxygen is produced. It is known that photocatalytic activity tends to be enhanced by an increase in surface area.[18] This is favorable for dealing with the roughened implant surface, whose area has been increased. The purpose of the current study was to evaluate the photocatalytic bactericidal effect of rough-surfaced titanium disks coated with anatase TiO_2 on *Actinobacillus actinomycetemcomitans* and *Fusobacterium nucleatum*, which are known periodontal pathogens and which are thought to play important roles in the etiology of periimplantitis.

MATERIALS AND METHODS

Preparation of Test Disk

A commercially pure (CP) titanium disk, 8 mm in diameter and 1.5 mm in thickness, was prepared. The disk was blasted with aluminum oxide (Al_2O_3) particles of an average size of 50 μm (control disk). The plasma source ion implantation (PSII) apparatus used in this study has been previously described.[19] A TiO_2 film was deposited onto another blasted CP titanium disk from titanium tetraisopropoxide plasma in the PSII apparatus chamber. The radiofrequency discharge generated plasma, and a pulsed high-negative-bias voltage of −20 kV was applied to the disk. The disk was annealed at 873 K for 1 hour (anatase TiO_2–covered disk).

Surface Analyses

The crystallinity of the film deposited by PSII and annealing was confirmed as consisting of anatase by glancing-angle x-ray diffraction, a procedure that has been described previously.[20] Micrographs of the surfaces of the control disk and the anatase TiO_2–covered disk were obtained by using a scanning electron microscope (S-3500N™, Hitachi High-Technologies Corporation, Ibaragi, Japan). The surface morphology of the control and the anatase TiO_2–covered disks was analyzed with an interferometer (MicroXAM™, ADE Phase Shift, Tucson, AZ, USA). Three disks of the control and the anatase TiO_2–covered specimens were measured. Five sample areas sized 300 × 400 μm were measured for each disk. We presumed that the form and waviness of the disks might affect the roughness parameters. Therefore a gaussian filter sized 50 × 50 μm was used before roughness was calculated, to separate form and waviness from roughness. The following four three-dimensional parameters were calculated for the numeric description of surface roughness:

- S_a: arithmetic-average height deviation from the mean plane, in micrometers
- S_q: root-mean-square height deviation from the mean plane, standard deviation for the height distribution, in micrometers
- S_{ds}: number of summits in the unit sample area, in negative square millimeters
- S_{dr}: surface area enlargement, in percent

Degradation of Methylene Blue

The photocatalytic property of the anatase TiO_2–covered disk and the control disk was determined by degradation of methylene.[21,22] The concentration of the standard methylene blue solution used was set to 10 ppm. To eliminate reduction in the concentration of methylene blue owing to absorption by the

specimens, the specimens were soaked in 300 mL of standard methylene blue solution for 24 hours prior to the test. Each disk was immersed in 300 μL of standard methylene blue solution. The specimens were then illuminated with UVA for 2 hours with a type FL15BL-B black light source (NEC, Tokyo, Japan) set above the specimens. The intensity of the light was 2.0 mW/cm^2 at a peak wavelength of 352 nm. UVA illumination was performed according to the following protocol:

- Anatase TiO_2–covered disks were illuminated with UVA (group 1).
- Anatase TiO_2–covered disks were used, but aluminum foil was placed above the disks to block UVA (group 2).
- Control disks were illuminated with UVA (group 3).
- Control disks were used, but UVA was blocked by aluminum foil (group 4).

After UVA illumination the solution was retrieved, and the optical density (OD) was measured at a wavelength of 664 nm. Two disks were each evaluated, and the mean OD value was recorded.

Evaluation of Bactericidal Effect

Actinobacillus actinomycetemcomitans HK 921 and *Fusobacterium nucleatum* ATCC 10953 cells were incubated anaerobically for 24 hours at 37°C in 10 mL of Todd-Hewitt (TH) broth. TH broth is a nonselective basal broth medium suitable for the culturing of *Actinobacillus actinomycetemcomitans* and *Fusobacterium nucleatum*.[23] The bacterium cells were harvested by centrifugation at 2,800 rpm for 10 minutes and then suspended in sterilized distilled water to a concentration of 1×10^6 cells/mL. A total of 50 μL of the suspension was added onto the anatase TiO_2–covered disks and control disks. The disks were illuminated with UVA by black light in an anaerobic environment, at an intensity of 0.5 mW/cm^2, according to the same protocol as that applied for the methylene blue reduction test (ie, in groups 1, 2, 3, and 4). UVA illumination was continued for up to 180 minutes. The viable cells in each group were counted by quantitation of colony-forming units (CFUs) at 0, 60, 120, and 180 minutes after the commencement of UVA illumination. Following UVA illumination the bacterial suspension on the disks was retrieved and was diluted with 5 mL of sterilized water. After being stirred, 200 μL of the diluted suspension was placed onto TH agar plates; three TH agar plates were used for each cell-counting. The bacteria were incubated anaerobically for 120 hours, and the formed colonies were counted. The bactericidal effect in each group was represented by the bacterial survival ratio at 60, 120, and 180 minutes, which was calculated as follows:

$$\text{Survival ratio at } \alpha \text{ minutes (\%)} = 100 \times \text{CFUs at } \alpha \text{ minutes/CFUs at 0 minutes}$$

Three replicate experiments were performed.

Statistical Analyses

All statistical analyses in this study were performed with SPSS® software (SPSS Inc., Chicago, IL, USA). The mean and standard deviation values of the topographic parameters of the disks ($n = 3$) and of the survival ratio of the bacterium cells ($n = 3$) were calculated. The average values were compared by paired *t*-test and analysis of variance (ANOVA) followed by a post hoc Duncan's test, with the value of statistical significance set at .05.

RESULTS

Figure 1 displays the findings of scanning electron microscopy of the control and anatase TiO_2–covered disks. The anatase TiO_2–covered disk had a number of global microstructures with diameters of 1 to 2 μm. These structures were not seen on the control disk. The interferometric image (a hybrid three-dimensional image) and surface roughness parameters for each disk are shown in Figure 2. The roughness parameters of the anatase TiO_2–covered disk were similar to those of the control disk. The paired *t*-test showed no significant differences between the S_a, S_q, S_{ds}, and S_{dr} values of the anatase TiO_2–covered disk and those of the control disk.

Figure 3 shows the result of the methylene blue reduction test. No considerable changes were observed in methylene blue degradation when no UVA illumination was given. Only the methylene blue that was applied to the anatase TiO_2–covered disk was degraded under UVA illumination with time, indicating that the disk showed photocatalytic properties.

With time and under UVA illumination, the survival ratio of *Actinobacillus actinomycetemcomitans* and *Fusobacterium nucleatum* on the anatase TiO_2–covered disk decreased, as shown in Figure 4. When the disks were stored in the dark, the reduction of the survival

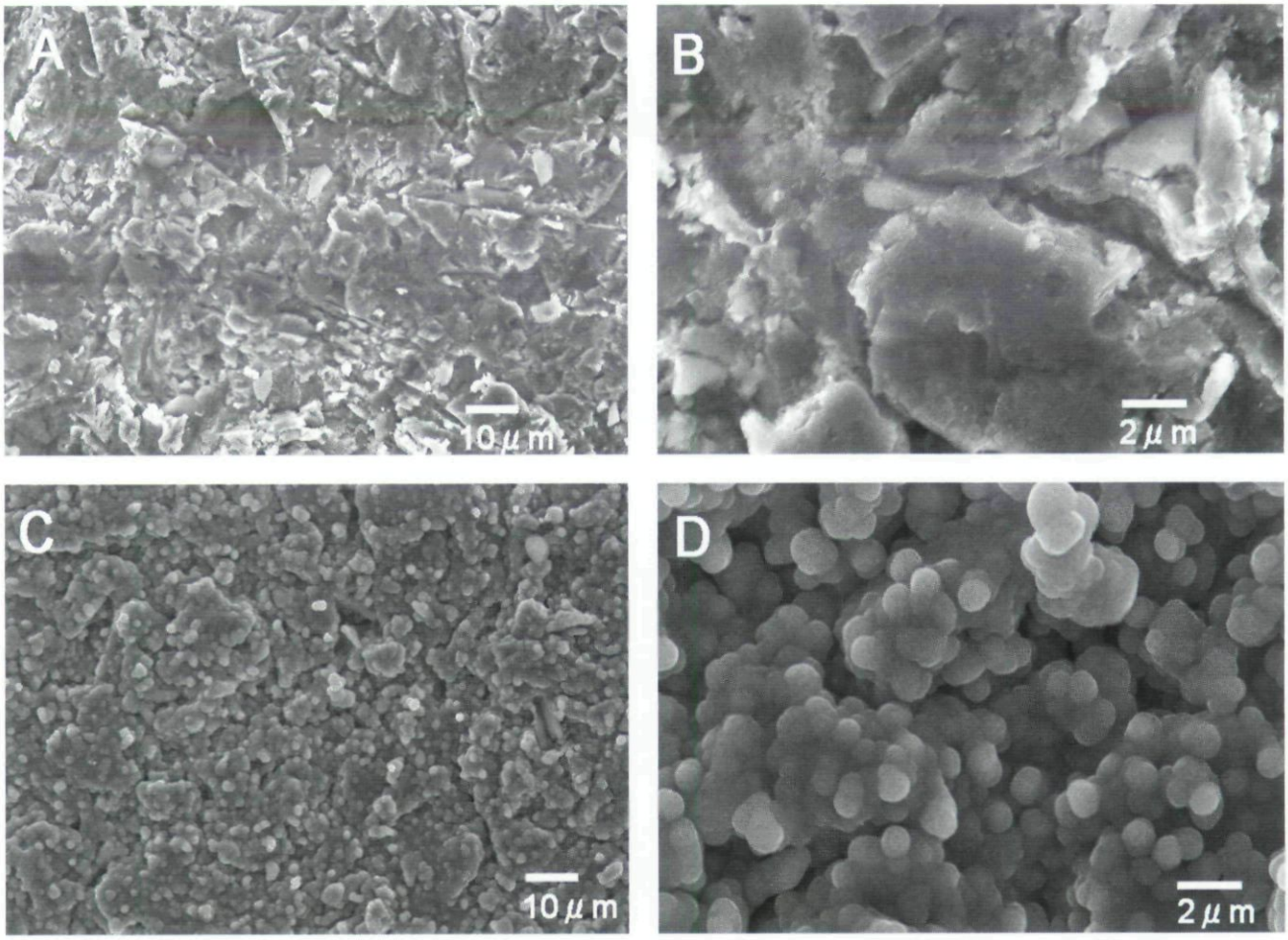

Figure 1 Scanning electron microscopy images showing the surface structures of the control disk (*A* and *B*; ×1000 original magnification) and the anatase TiO_2–covered disk (*C* and *D*; ×4000 original magnification).

ratio of both bacteria was less than 20% after 180 minutes of UVA illumination (groups 2 and 4). In group 3 the viability of *Actinobacillus actinomycetemcomitans* and *Fusobacterium nucleatum* placed on the control disks was reduced to 60% and 70%, respectively, after 120 minutes of UVA illumination. In this group anatase TiO_2–covered (ie, photocatalytic) disks were not used, meaning that no photocatalytic reaction occurred, which implies that the bacteria were killed by other factors. The most probable factor is the direct harmfulness of UVA itself to the bacterium cells. Viability in group 1 was suppressed to less than 1% at 120 minutes and 180 minutes. ANOVA showed significant differences in viability between group 1 and group 3 at 120 minutes and 180 minutes, for both bacteria.

DISCUSSION

In this study, we introduced new decontaminating methods for rough implant surfaces, based on photocatalytic reactions. The photocatalytic anatase TiO_2 surface revealed a bactericidal effect, which cannot be seen on conventional CP titanium surfaces.

Wennerberg and colleagues stated that there was no significant increase in the accumulation and adhesion of bacteria on rough (blasted) abutment surfaces when compared to smooth (turned) surfaces.[24] The Wennerberg study presented negative findings regarding the supposed increase in the risk of infection with rough-surfaced implants. However, only short-term evaluations were performed in this study. The authors have speculated that differences might have appeared if observation had continued for a longer period. Hence the necessity of developing effective decontaminating methods for rough implant surfaces is still acute, especially with regard to the long-term stability of implants.

It is known that UVA itself has deleterious effects on bacterium cells, depending on the type of bacterium and the bacterium's degree of sensitivity.[25,26] About

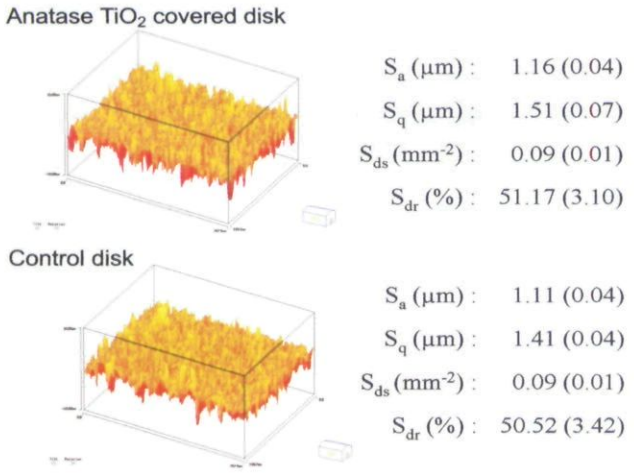

Figure 2 Interferometric images and surface topography parameters of the anatase TiO_2–covered disk and the control disk. The values presented are mean values, with standard deviations in parentheses. (S_a = arithmetic-average height deviation from the mean plane; S_{dr} = surface area enlargement; S_{ds} = number of summits in the unit sample area; S_q = root-mean-square height deviation from the mean plane, standard deviation for the height distribution)

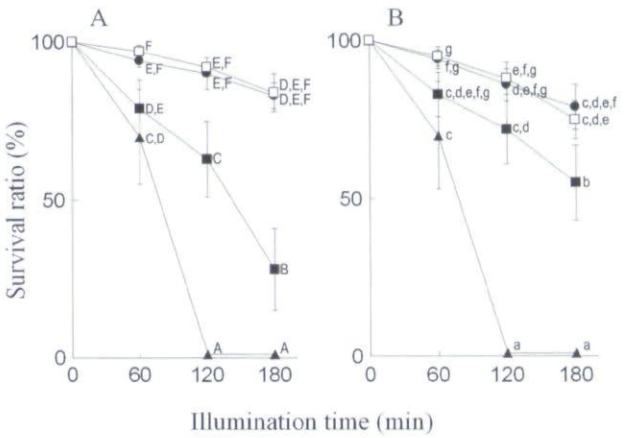

Figure 4 The change in the survival ratios of *Actinobacillus actinomycetemcomitans* (*A*) and *Fusobacterium nucleatum* (*B*) in the four groups. The mean values and standard deviations were calculated on the basis of three measurements; the *error bars* represent standard deviations. Identical letters indicate that the difference is not statistically significant. (▲ = group 1; ● = group 2; ■ = group 3; □ = group 4)

40% of *Actinobacillus actinomycetemcomitans* cells and 30% of *Fusobacterium nucleatum* cells that were put onto the control disk were inactivated after 120 minutes of UVA illumination in the current study. These findings show that UVA has deleterious effects on these two types of bacterium. Therefore there is no doubt that UVA killed not only the bacteria in group 3 but also those in group 1. However, the fact that statistically larger numbers of bacteria were killed in group 1 than were killed in group 3 at 120 minutes and 180 minutes suggests the presence of photocatalytic bactericidal action against *Actinobacillus actinomycetemcomitans* and *Fusobacterium nucleatum* cells. This effect may be clinically useful for killing bacteria on rough-surfaced implants. It has been confirmed that the dead bodies of the bacteria and the toxic organic compounds (such as the lipopolysaccharide released from the cell walls of the bacteria) can be decomposed as well.[10,27] These properties may serve to restore surfaces to a bacterium-free and toxicity-free state after contamination, which would potentially prevent the rapid development of periimplantitis and promote re-osseointegration.

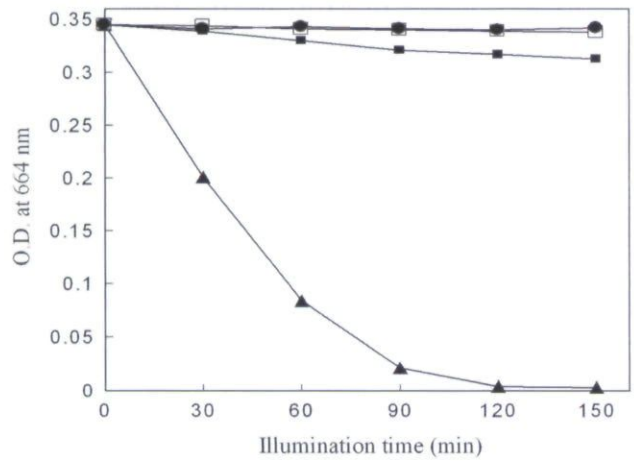

Figure 3 Photocatalytic degradation of methylene blue under UVA illumination. (▲ = group 1; ● = group 2; ■ = group 3; □ = group 4; OD = optical density)

Although such a photocatalytic surface offers new and favorable properties from a hygienic viewpoint, it is very important that there should be no disadvantages in regard to achieving osseointegration. When UVA does not reach the surface, no active oxygen is produced, and the photodecomposition of organic compounds does not occur. It has been confirmed that implants with thick TiO_2 surfaces fabricated by the PSII method did not prevent osseointegration in a rabbit.[28] In addition, the topographic data indicated that the anatase TiO_2–covered disk had a moderately rough surface, a characteristic presumed to be necessary for the enhancement of bone anchorage. Therefore it can be said that the photocatalytic surface prepared in this study was not disadvantageous to osseointegration.

With regard to clinical applications, the implant surface should be coated with anatase TiO_2 by PSII

and annealing, prior to implantation. In the sterilization of the contaminated surface, flap elevation will be essential before UVA illumination because UVA cannot penetrate thick tissues such as the human gingiva. For the same reason, any thick biofilm around the contaminated implant should be removed mechanically in advance. In this study 120 minutes of UVA illumination were necessary to suppress the viability of the bacteria to less than 1%; this duration is obviously too long for clinical use and must be shortened. It is known that more-intense UVA illumination can shorten the illumination time. However, this is unfavorable for clinical use because excessive UVA illumination can damage human tissue. Photocatalyst technology is rapidly developing, and much research is ongoing. Many modifications of the TiO_2 photocatalyst have been attempted in order to enhance the catalytic performance. Numerous articles have stated that some kinds of ion implantation improved the efficiency of photocatalysis and that a new type of photocatalyst that can function with visible light (a visible-light photocatalyst) has been produced by nitrogen doping.[29–33] These newly developed TiO_2 photocatalysts are of great interest and will be discussed in further research.

In this study we tested the photocatalytic bactericidal effect of anatase TiO_2 on only *Actinobacillus actinomycetemcomitans* and *Fusobacterium nucleatum*. The effect on the other types of pathogenic bacteria that are associated with periimplantitis should be confirmed.

CONCLUSION

The results of this study show that a photocatalytic anatase TiO_2 film can be applied to a commercially pure titanium surface and that the photocatalytic bactericidal effect is of great use for sterilizing the contaminated surface of dental implants.

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