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Influence of saliva-coating on the ultraviolet-light-induced photocatalytic bactericidal effects on modified titanium surfaces

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ABSTRACT

The purpose of this study was to investigate the ultraviolet-light-induced photocatalytic bactericidal effects of titanium surfaces on *Streptococcus sanguinis* in the presence of saliva-coating. Three different titanium disks were prepared: machined (MA), heat-treated (HT), and anodized surfaces (AO). Each disk was incubated with whole saliva or phosphate-buffered saline for 2 h. Antibacterial tests were performed by incubating a *S. sanguinis* suspension with each disk for 90 or 180 min under ultraviolet (UV) illumination. The viable counts of bacteria were enumerated from the cell suspension and the UV-light-induced photocatalytic bactericidal effects were determined by the bacterial survival rate. Without saliva-coating, AO disks exhibited significantly decreased bacterial survival rates compared to MA disks. The bacterial survival rates of the HT disks were intermediate between MA and AO in the absence of saliva-coating. However, saliva-coating significantly increased bacterial survival rates in all surface types. There was no significant difference in bacterial survival rates among the three surface types after saliva-coating. This study suggests that Ti-based antibacterial implant materials using TiO_2 photocatalyst may have a limitation for intraoral use.

1. Introduction

Titanium (Ti) is one of the most commonly used biomaterials in the dental field. An important feature of Ti-based biomaterials is its outstanding biocompatibility. The air-induced passivation due to the spontaneous formation of highly inert and tenacious oxide film creates a protective and stable layer of titanium dioxide (TiO_2), which minimizes ion release from the implant to the surrounding tissues. TiO_2 is found as many different crystalline forms of oxide, rutile, anatase, or brookite [1–3]. The rutile or anatase crystalline forms are most commonly seen depending on the titanium surface treatment method [4,5].

The TiO_2 layer has been a main focus in dental research due to its photocatalytic bactericidal action [6]. Upon excitation with ultraviolet (UV) light below 385 nm, the photon energy generates electron–holes pairs in the TiO_2 surface in water, which induces a series of photocatalytic reactions. The hole in the valence band can react with H_2O or hydroxide ions adsorbed on the surface to produce hydroxyl radicals (OH^-). The electron in the conduction band can reduce O_2 to produce superoxide ions (O_2^-). Both the holes and radicals have a very short lifespan, but are extremely reactive with organic compounds, leading to the degradation of organic matter and bacterial death [7–10]. Recently, there have been many efforts to create antibacterial implant materials by enhancing the photocatalytic bactericidal effect of the TiO_2 layer by treatment of the titanium surface [11,12].

The titanium surfaces of implant materials are covered by saliva when exposed in the oral cavity. Given that organic film-coating, such as blood, has been cited as one of the main reasons for the clinical failure of antibacterial medical devices [13], the clinical usefulness of the UV-light-induced photocatalytic bactericidal effects of Ti-based dental materials may be significantly influenced by saliva-coating. However, there have been few reports about the UV-light-induced photocatalytic effects of Ti-based materials in the presence of saliva-coating. The purpose of this study was to analyze the influence of saliva-coating on the UV-light-induced photocatalytic bactericidal effects of titanium disks against *Streptococcus sanguinis*, one of the primary colonizers of dental plaque on teeth. The null hypothesis of this experiment was that saliva-coating would not influence the UV-induced photocatalytic bactericidal activities of the titanium disks.

Abbreviations: MA, machined titanium surface; HT, heat-treated titanium surface; AO, anodized titanium surface; CFUs, colony forming units.

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2. Materials and methods

2.1. Material preparation

The disks (10.0 mm in diameter and 3.0 mm thick) were fabricated from grade 4 commercially pure titanium. Three different surfaces were prepared: machined titanium surface (MA, no surface treatment), heat-treated titanium surface (HT), and anodized titanium surface (AO). The HT disks were heat-treated at 300 °C for 100 min in air. The AO disks were anodized in an electrolyte solution containing 0.35 M sulfuric acid and 0.35 M phosphoric acid at 50 V. All disks were packed, sealed and sterilized with ethylene oxide gas. For each surface treatment group, 40 disks were fabricated.

2.2. Surface analysis

The surface roughness of each sample was measured using a confocal laser scanning microscope (Axiovert 200M, Carl Zeiss, Thornwood, NY, USA). The multi-argon laser emits light at a wavelength of 633 nm and allows for the calculation of the arithmetic mean surface roughness from a mean plane within the sampling area ($900 \times 900 \times 80\ \mu m$). The system provides the numerical values for the surface roughness parameter, which is defined as the arithmetical mean deviation of the assessed profile. Each surface roughness reading was performed three times on three different areas for each disk. The phase components were analyzed using thin-film X-ray diffractometry (TF-XRD), which was used to identify the crystal structure of TiO_2 in a scanning range of $2\theta = 20–70°$.

2.3. Saliva preparation

Unstimulated whole saliva was collected from 6 healthy volunteers. The volunteers had no acute dental caries or periodontal lesions. Saliva collection was routinely performed between 9:00 A.M. and 11:00 A.M. to minimize the effects of diurnal variability on salivary composition. The saliva samples were centrifuged at $3500 \times g$ for 10 min to remove any cellular debris and the resulting supernatant was used after filter-sterilization through a Stericup & Steritop (Millipore, Billerica, MA, USA). The saliva was stored at −20 °C before use.

2.4. Bacterial culture

Overnight cultures of the *S. sanguinis* SL1 strain were transferred to pre-warmed brain heart infusion (BHI) (Difco, Sparks, MD, USA) medium and grown at 37 °C in a 5% CO_2 to OD_{600} of 0.5. Cells were washed twice with phosphate-buffered saline (PBS, pH = 7.2) and resuspended to an OD_{600} of 0.5 (approximately 3.5×10^7 colony forming units/mL).

2.5. Saliva treatment

Each titanium disk was placed in polystyrene 48-well cell culture clusters (Corning Inc., Corning, NY, USA). The disks were divided into two groups: saliva-coating or no saliva treatment. For the saliva-coating group, each disk was conditioned with 500 μL saliva in the well at 37 °C for 2 h with gentle shaking, followed by two washes with PBS. After air drying for 30 min, 500 μL of the cell suspensions ($OD_{600} = 0.5$) was inoculated into the wells. For the group that received no saliva treatment, the same procedure was performed with sterile PBS instead of saliva.

2.6. Evaluation of bactericidal effects under UV illumination

The cell culture clusters were monitored with or without UV illumination. For the UV illumination group, the cell culture clusters were incubated for 90 or 180 min under UV light using a type F15T8BLB black light blue lamp (SANKYO DENKI, Kanagawa, Japan). The light intensity was 2.0 mW/cm^2 at a peak wavelength of 352 nm. The light source was placed 10 cm above the samples. For the group without UV illumination, UV light was blocked with aluminum foil during the experiment.

After incubation, each bacterial suspension was transferred into a 1.5 mL Eppendorf tube. The collected cell suspensions were serially diluted, plated on BHI agar, and incubated at 37 °C for 2 days before viable cells were counted. Cell counts recorded in colony forming units (CFUs). All assays were performed in duplicate and repeated seven times.

The UV-light-induced photocatalytic bactericidal effects were determined by the bacterial survival rate using the following equation: bacterial survival rate (%) = (viable cell count with UV irradiation/viable cell count without UV irradiation) × 100.

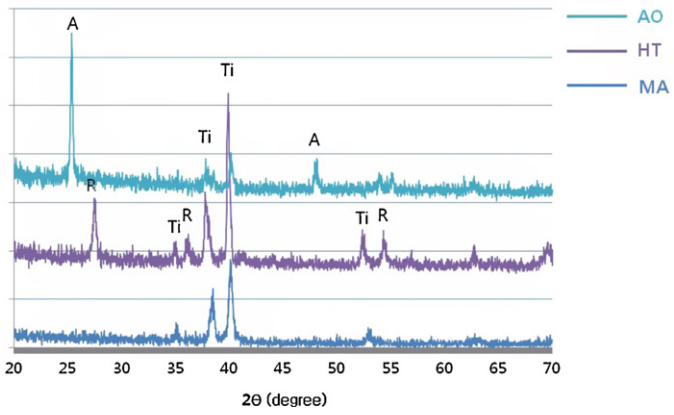

Fig. 1. The X-ray diffractometry spectra of the titanium disks with the machined titanium surface (MA), heat-treated titanium surface (HT) and anodized titanium surface under 50 V (AO). Rutile peaks (R) appeared on the HT disks and anatase peaks (A) were observed on the AO disks. MA disks exhibited only Ti peaks (Ti).

2.7. Preparation of the blank group

The same experiments were performed using the same cell clusters without titanium disks (blank group). Each well of the cell culture clusters was conditioned with 500 μL of saliva or PBS for 2 h in the absence of titanium disks. The bacterial survival rates in the blank group were compared to those in the experimental groups containing titanium disks.

2.8. Statistical analysis

Surface roughness was analyzed using one-way ANOVA to compare the differences among the three surface types. Factorial ANOVA was used to analyze the differences in viable cell counts and bacterial survival rates on cell suspension with respect to surface types, saliva-coating, and incubation time. Bonferroni's *t* test was used as a post hoc test. All values were considered significant when $P < 0.05$.

3. Results

There were significant differences in surface roughness among the three surface types. HT and AO disks had rougher surfaces than MA disks (Table 1). The TF-XRD spectra of the titanium disks are shown in Fig. 1. The AO group exhibited TiO_2 peaks corresponding to a 2θ value of 25.2 indicating the presence of an anatase structure, while the HT group showed TiO_2 peaks corresponding to the