Efficacy of a solar-powered TiO_2 semiconductor electric toothbrush on *P. gingivalis* biofilm

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ABSTRACT

Purpose: To reveal the efficacy of a solar-powered TiO_2 semiconductor electric toothbrush on *Porphyromonas gingivalis* biofilm.

Methods: *P. gingivalis* cells were cultivated on sterilized coverslips under anaerobic conditions and were used as a biofilm. To evaluate the efficacy of the solar-powered TiO_2 electric toothbrush on the *P. gingivalis* biofilm, the bacterial cell biofilm coverslips were placed into sterilized phosphate buffered saline (PBS) and brushed for 1 minute. Following mechanical brushing, the coverslips were stained with 1% crystal violet (CV) for 10 seconds at room temperature. The efficacy of *P. gingivalis* biofilm removal by the solar-powered TiO_2 electric toothbrush was measured through the absorbance of the CV-stained solution containing the removed biofilm at 595 nm. The antimicrobial effect of the solar-powered TiO_2 semiconductor was evaluated by the *P. gingivalis* bacterial count in PBS by blacklight irradiation for 0 to 60 minutes at a distance of 7 cm. The electrical current though the solar-powered TiO_2 semiconductor was measured by a digital multimeter. The biofilm removal by the solar-powered TiO_2 semiconductor was also evaluated by scanning electron microscopy (SEM).

Results: The biofilm removal rate of the solar-powered TiO_2 electric toothbrush was $90.1 \pm 1.4\%$, which was 1.3-fold greater than that of non-solar-powered electric toothbrushes. The solar-powered TiO_2 semiconductor significantly decreased *P. gingivalis* cells and biofilm microbial activity in a time-dependent manner ($P< 0.01$). The electrical current passing through the solar-powered TiO_2 semiconductor was 70.5 ± 0.1 μA, which was a 27-fold higher intensity than the non-solar-powered brush. SEM analysis revealed that solar-powered TiO_2 semiconductor caused a biofilm disruption and that cytoplasmic contents were released from the microbial cells.

Clinical Significance: *P. gingivalis* biofilm removal by the solar-powered electric toothbrush was significantly greater than that by the non-solar-powered electric toothbrush and the electric control brush. TiO_2 semiconductors within the solar-powered electric toothbrush can enhance the antimicrobial activity against an oral biofilm and contribute to the elimination of periodontal pathogens.

Introduction

Dental plaque is a multispecies biofilm that grows on the hard and soft tissues of the oral cavity. Biofilms consist of bacterial cells embedded in an exopolysaccharide. Over 500 species of bacteria have been identified in the oral cavity, all of which have been shown to trigger periodontal diseases.[1] Periodontal diseases are chronic inflammatory diseases characterized by alveolar bone loss and connective tissue destruction.[2] *Porphyromonas gingivalis* is a Gram-negative anaerobic rod frequently isolated from human periodontal pockets,[3] and is known to invade and survive in host cells, inducing a network of inflammatory responses.[4] Moreover, it has been implicated in multiple systemic diseases.[5] Therefore, this bacterium is considered an important target organism in the prevention of periodontal and systemic diseases.

Microbial biofilms have an inherent mechanism that protects microorganisms from the host's immune system and antimicrobial therapies. Mechanical removal methods are effective in the destruction of biofilms.[6] A good toothbrush is an essential tool in the removal of biofilms and maintaining good oral health. Electric toothbrushes use rotating, oscillating, or sonic action that achieve plaque removal primarily through direct physical contact between the bristles and the tooth surface.[7,8] Several studies have demonstrated that electric toothbrushes are effective for plaque removal and reduction of gingival inflammation.[7-10] Recently, a new electric toothbrush has been introduced for the improvement of plaque removal efficacy. This electric toothbrush has a solar-powered titanium oxide (TiO_2) semiconductor. TiO_2 is a chemically stable, non-toxic, biocompatible, and inexpensive material with a very high dielectric constant and interesting photocatalytic activities.[11] TiO_2 photocatalysts have been demonstrated to exert bactericidal effects[12-15] and biofilm reduction[15,16] by ultraviolet (UV) light activation. However, the UV light is damaging to human eyes and skin,[17] which limits the use of TiO_2 under UV light in the home environment.[18] Several studies have reported an increased antimicrobial activity of TiO_2 by fluorescent light (FL) irradiation.[19-21] These results show that low UV light emitted by FL irradiation activates TiO_2 and induces bacterial growth inhibition by the TiO_2 photocatalyst.

The purpose of this study was investigated the efficacy of *P. gingivalis* biofilm removal using the solar-powered TiO_2 electric toothbrush in the presence of FL irradiation. In addition, the antimicrobial activity and biofilm removal by the solar-powered TiO_2 semiconductor was evaluated against *P. gingivalis* cells in the presence of UV irradiation.

Materials and methods

Bacterial cultures and growth conditions

P. gingivalis ATCC 33277 was grown in brain heart infusion (BHI[a]) broth supplemented with yeast extract (5 mg/mL), hemin (5 μg/mL), and vitamin K_1 (0.2 μg/mL). Bacterial cells were grown under anaerobic conditions (85% N_2, 10% H_2, and 5% CO_2) at 37°C for 18 hours.

Electric toothbrushes and semiconductors

The solar-powered TiO_2 electric toothbrush was used with a device (Soladey Rhythm[b]). An electric toothbrush connected to a stainless semiconductor was used as the electric control brush. To evaluate the antibacterial activity and biofilm removal efficacy of the semiconductors, an electrode (3.0 mm in diameter × 68 mm in length) comprising a TiO_2 rod, a stainless steel rod, and a solar battery was used as the solar-powered TiO_2 semiconductor. The stainless steel semiconductor used as control semiconductor consisted of stainless steel rods and a solar battery; however, the entire battery was covered with aluminum foil to inactivate the solar power.

Biofilm removal of the solar-powered TiO_2 electric toothbrush

Bacterial cells were grown on 24-well polystyrene plates with the sterilized coverslip at 37°C for 18 hours anaerobically. Following incubation, the coverslips were washed twice with PBS and brushed with the solar-powered TiO_2 electric toothbrush for 1 minute under fluorescent light irradiation[c] (6W, 505 LUX). Following mechanical brushing, the coverslips were stained with 1% crystal violet (CV). The biofilm removal ability was evaluated through the absorbance of the CV-stained solution containing the removed biofilm at an optical density of 595 nm. The results are expressed as the mean ± standard deviation (SD) of triplicate samples.

Antimicrobial activity of the solar-powered TiO_2 semiconductor

Bacterial suspensions (1.2×10^8 CFU/mL) were placed into polystyrene tubes. The solar-powered TiO_2 semiconductor was placed in sterilized phosphate buffered saline

(PBS) and was irradiation with a blacklight[d] (369 nm, 6 W) for 0 to 60 minutes at a distance of 7 cm. Bacterial suspensions were serially diluted and plated on BHI blood agar plates, and incubated anaerobically at 37°C for 7 days. After 7 days, antimicrobial activity was determined by counting the numbers of *P. gingivalis* cells. The electrical current of electrodes in the several solutions was measured with a digital multimeter.[e] The results are expressed as the mean ± standard deviation (SD) of triplicate samples.

Biofilm removal effect of the solar-powered TiO_2 semiconductor

P. gingivalis biofilm was prepared in 24-well polystyrene plates by inoculating an overnight starter culture. After incubation for 18 hours, non-adherent cells were removed by washing with PBS, and fresh PBS was then added into the biofilm-attached wells. The TiO_2 electrode and stainless electrode were placed into the wells and irradiation with a blacklight for 0 to 60 minutes at a distance of 7 cm. Bacterial suspensions following biofilm removal were scored at an optical density at 550 nm. The results are expressed as the mean ± standard deviation (SD) of triplicate samples.

SEM evaluation of biofilms removed by the solar-powered TiO_2 semiconductor

The round plastic coverslip[f] (15 mm in diameter) was placed in the well for the bacterial biofilm to grow on them. The biofilm coverslips were then washed with PBS and fixed overnight in 2% freshly prepared cold (4°C) glutaraldehyde in 0.2 M phosphate buffer (pH 7.2). For scanning electron microscopy, *P. gingivalis* biofilms were fixed in a 2.5% glutaraldehyde solution in 0.2 M cacodylate buffer (pH 7.2) for 1 hour. After rinsing and dehydration through a graded series of aqueous ethanol solutions, the biofilm was critical point-dried and mounted on copper stubs. Finally, it was coated with a thin layer of platinum and observed using a JSM-6301F SEM.[g]

Statistical analysis

Differences among experimental groups were analyzed by one-way analysis of variance and Tukey's test. P values less than 0.05 were considered statistically significant.

Results

Table 1 shows the efficacy of the solar-powered TiO_2 electric toothbrush on *P. gingivalis* biofilms. The efficacy of biofilm removal using the solar-powered TiO_2 electric toothbrush was significantly increased compared to those of the non-solar-powered TiO_2 electric toothbrush and the electric control brush ($P< 0.01$). The percentage of biofilm removal by the solar-powered electric toothbrush was 90.1 ± 1.4%, whereas the removal efficacies of the non-solar-powered brush and the electric control brush were 71.7 ± 3.9% and 44.2 ± 2.5%, respectively. Figure 1 shows the bactericidal effect of the solar-powered TiO_2 semiconductor against *P. gingivalis* cells. The electrical current of the solar-powered TiO_2 semiconductor was 70.5 ± 0.1 μA, which was 27 times more intense than that of the non-solar-powered semiconductor. The electrical current of the control semiconductor was 1.1 μA. The number of *P. gingivalis* cells was significantly decreased by the solar-powered TiO_2 semiconductor in a time-dependent manner ($P< 0.01$). The number of *P. gingivalis* cells decreased 74.5%, which was enhanced by the solar-powered TiO_2 semiconductor after 60 minutes. Figure 2 shows the removal efficacy of the solar-powered TiO_2 semiconductor on *P. gingivalis* biofilm. The biofilm was also removed by the solar-powered TiO_2 semiconductor in a time-dependent manner. Following treatment for 60 minutes, the efficacy of biofilm removal by the solar-powered TiO_2 semiconductor was 6.3-fold greater than that by the control semiconductor. Figure 3 shows SEM photomicrographs of *P. gingivalis* biofilms untreated and treated with the solar-powered TiO_2 semiconductor. Following incubation for 24 hours, *P. gingivalis* formed a thick biofilm on the coverslips (Figure 3A). Nevertheless, when the *P. gingivalis* biofilm was exposed to the solar-powered TiO_2 semiconductor, it broke down and was shown to release cytoplasmic and nuclear materials (Figure 3B).

Discussion

The TiO_2 semiconductor used in this study comprised rutile, which is a TiO_2 crystalline structure with a smaller band gap and excitation wavelengths that extend into the visible light range.[16] The photocatalytic action of rutile crystals has been reported to be less than that of anatase crystals. However, rutile crystals are characterized by the greatest stable physical chemical property[11] and the lowest cell toxicity.[22,23] Thus, rutile crystals are used in cosmetics, sunscreen, and food additives.

In this study, the TiO_2 semiconductor connects with the neck of the toothbrush. Hoover *et al.*[24] found that a manual toothbrush with a solar-powered TiO_2 semiconductor has improved plaque reduction compared to an electric control brush without a semiconductor. The authors concluded that the reduction in plaque was due to the photocatalytic effects in the presence of UV light. The antimicrobial effects of TiO_2 photocatalysts under UV light irradiation are considered to be caused by reactive oxygen species (ROS) released from the TiO_2 surface.[14-16] ROS attack the outer membrane of bacterial cells, induce oxidative stress, and lead to cell death. In previous studies on the solar-powered TiO_2 electric toothbrush [24,25], the electrons, which are released from the TiO_2 semiconductor in contact with saliva in the presence of light, attract positive ions from the organic acid in the dental plaque and promote the reduction of plaque formation. It is hypothesized that the solar panel (light source), TiO_2 rod, and saliva form an electrical circuit on the tooth surface. The ionic reactions shown below have been stipulated.

$H^+ + O_2 + e^- = HO_2 \quad 2H^+ + 2e^- = H_2$

$2HO_2 = H_2O_2 + O_2 \qquad H_2O_2 = 2HO$

In this study, SEM analysis revealed that the solar-powered TiO_2 photocatalyst induced the destruction of the *P. gingivalis* biofilm and release of cellular materials from the outer membrane under UV light irradiation. These results demonstrate that UV light irradiation stimulates the production of ROS from the TiO_2 semiconductor and induces the destruction of the *P. gingivalis* biofilm.

In contrast, the low electrical current enhances antimicrobial effects[26,27] and inhibits bacterial growth.[28] These antibacterial activity mechanisms are considered to be due to the electric current changing the bacterial cell surface polarity, inducing electrostatic and electrophoretic forces and desorption of the negatively charged bacterial cell surface.[27] Moreover, the low electrical current has no side effects against the human body,[29] increases the concentration of adenosine triphosphate in soft tissue,[30] and promotes anti-inflammatory effects[31] and wound healing.[32] Thus, the TiO_2 photocatalyst properties and the low electric current increase the efficacy of oral biofilm mechanical removal.

The present study has been shown that the mechanical effects by the electric toothbrush and chemical reactions induced by the TiO_2 semiconductor effectively remove the *P. gingivalis* biofilm. *P. gingivalis* biofilm removal by the solar-powered electric toothbrush was significantly greater than that by the non-solar-powered electric toothbrush and the electric control brush. The TiO_2 photocatalytic properties and electric current contribute to the reduction of bacterial biofilms and aid in the prevention of periodontal diseases. Therefore, the solar-powered TiO_2 electric toothbrush is an effective device for oral hygiene.

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Figure legends

Fig. 1. Bactericidal effect of the solar-powered TiO_2 semiconductor against *P. gingivalis* cells. The data are expressed as the mean ± standard deviation in triplicate samples. Circle, control semiconductor; Square, non-solar-powered TiO_2 semiconductor; Triangle, solar-powered TiO_2 semiconductor. *Indicates statistical significance of $P< 0.01$.

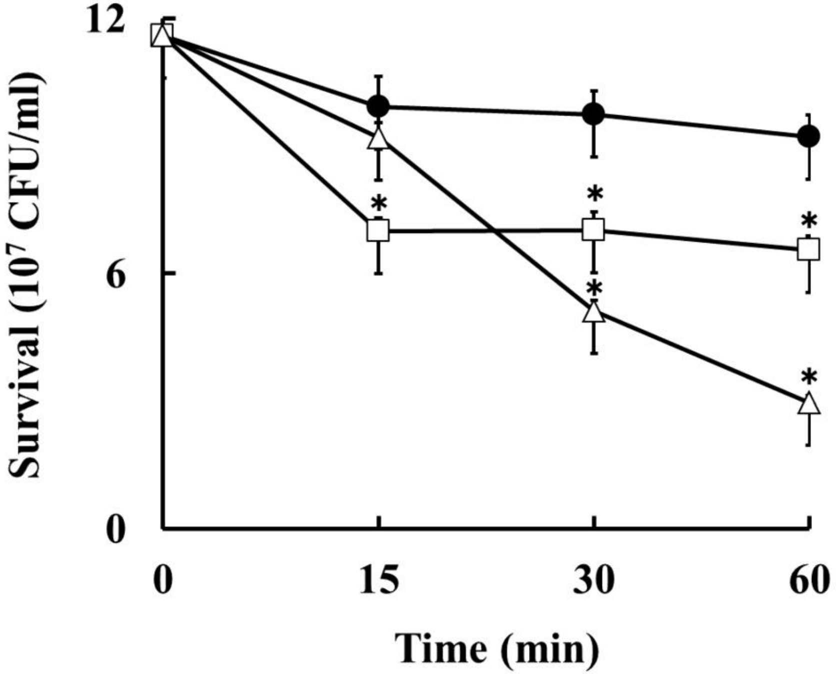
Fig. 2. The efficacy of the solar-powered TiO_2 semiconductor on *P. gingivalis* biofilm. Each point on the curves is the average optical density (O.D.) at 550 nm on a logarithmic scale measured in triplicate samples. The error bars are expressed as standard deviation. Circle, control semiconductor; Square, non-solar-powered TiO_2 semiconductor; Triangle, solar-powered TiO_2 semiconductor. *Indicates statistical significance of $P< 0.01$.

Fig. 3. Scanning electron photomicrographs of *P. gingivalis* biofilm untreated and treated with the solar-powered TiO_2 semiconductor. The scale bars indicate 2 μm.

Table 1. Percentage of biofilm removal by the electric toothbrushes.

Toothbrush	N	Mean ± SD
Electric control toothbrush	3	44.2 ± 2.5
Non-solar-powered TiO_2 electric toothbrush	3	71.7 ± 3.9*
Solar-powered electric toothbrush	3	90.1 ± 1.4*

N= number of subjects; SD = standard deviation.
Biofilm removal (%) = 100 × (treatment − untreated control) /untreated control
*Staistically singnificant reduction between three electric toothbrushes ($p < 0.01$)



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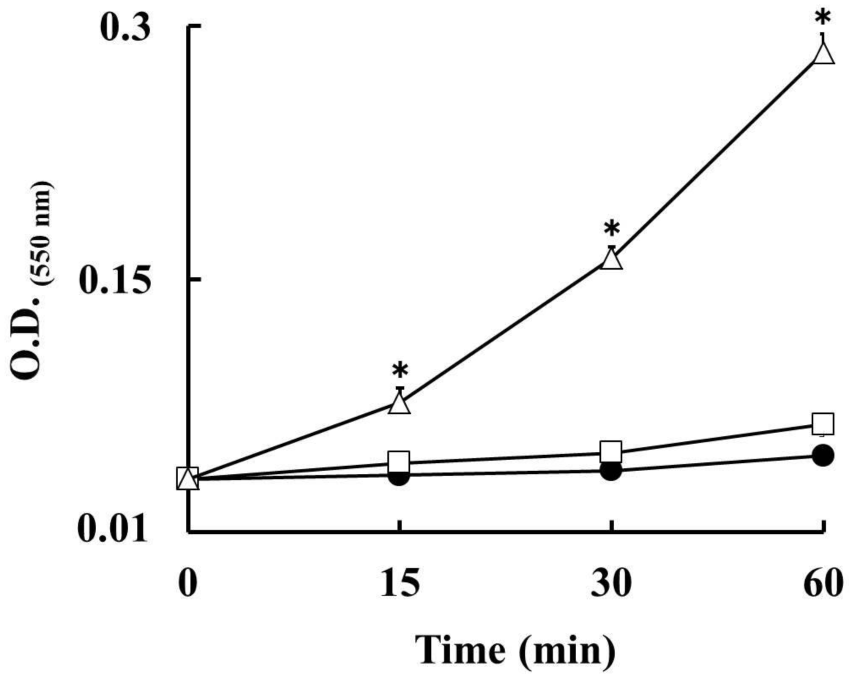

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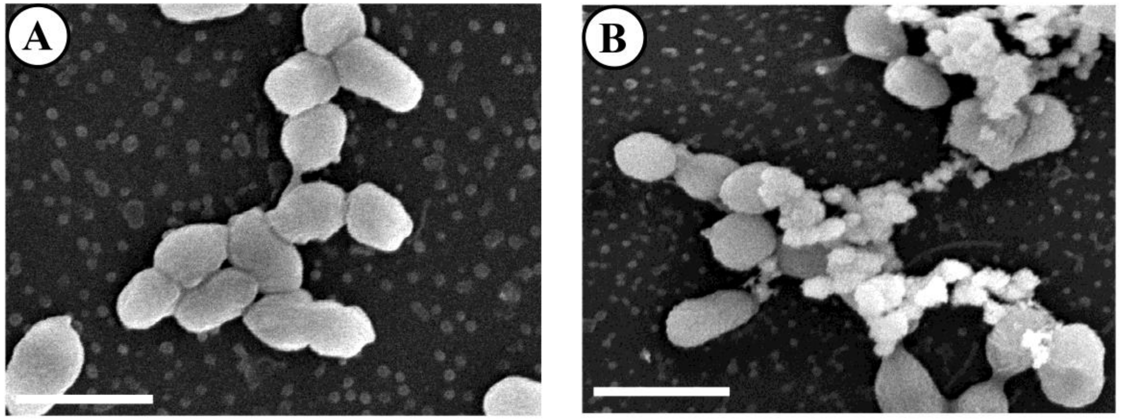

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