Inhibition of the adhesive ability of Streptococcus mutans on hydroxyapatite pellet using a toothbrush equipped with TiO₂ semiconductor and solar panel

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Abstract TiO₂ has an antibacterial effect against Streptococcus mutans (S. mutans). The objective of this study was to show the Inhibition of the adhesive ability of S. mutans on hydroxyapatite pellet using a toothbrush equipped with TiO₂ semiconductor and solar panel. Gradual brushing of 9 hydroxyapatite pellets was done by the same person using a solar toothbrush under 750/LUX light source in water. As a control, pellets brushed with a placebo toothbrush were done using the same procedure by the same person. All the pellets were inoculated in 10 ml Brain Heart Infusion (BHI) broth containing 100 µl of 10⁶ CFU/ml S. mutans and 1% sucrose and then incubated at 37°C for 12 hours. After incubation, the adherence conditions of S. mutans on each pellet were checked. The bacterial count of S. mutans on the brushed pellets using solar toothbrush were fewer than those using placebo toothbrush. The quantities of adherence of S. mutans decreased according to number of brushing and adhesion on pellets brushed more than 50 times could not be recognized. However, adhesion of S. mutans on the pellets brushed using placebo toothbrush were recognized and the adhesive quantities were visually the same. These results occurred because of some electronic charges on the brushed pellets using solar toothbrush. Soladey-3 toothbrush prevented S. mutans adhesion to the hydroxyapatite pellet under a light source.

Key words Hydroxyapatite pellet, Soladey-3 toothbrush, Streptococcus mutans

Introduction Dental caries is a progressive damage to teeth exposed to the saliva and acid production initiated by bacterial plaque. In microbiological terms, plaque is biofilm. Biofilms consists of a hydrated viscous phase formed from bacteria and their extracellular polysaccharide matrices¹. Streptococcus mutans (S. mutans) is considered to play an initiative role in the development of dental caries in animals and humans. Mutans Streptococci are the most cariogenic pathogens as they are highly acidogenic, producing short-chain carboxylic acids which dissolve hard tissue such as enamel and dentine². In addition, they ferment sucrose and produce insoluble polysaccharides, which are adherent to the tooth surface and form biofilm³. The first stage involves deposition of an acquired enamel pellicle (AEP) on the tooth. This acellular coating (AEP) includes salivary components and bacterial constituents such as bacterial glucosyltransferase (GTFs) and fructosyltransferase (FTF) which synthesize exopolysaccharides. The second stage involves the adherence and co-adherence of bacteria from the oral cavity with the aid of polysaccharides on the
AEP. Proliferation occurs in the third stage, while in the final stage, the biofilm reaches a steady state in relation to the surrounding environment⁴. Colonization of Mutans Streptococci on tooth occurs soon after tooth eruption. If followed by the colonization in fissures in their depths, this will inevitably lead to caries⁵. Tooth brushing is one of the most effective methods for removing dental plaque and preventing caries. Presently, there are several hundred varieties of toothbrushes on the market, making selection of an appropriate toothbrush very difficult.

Solar energy conversion has recently attracted special interest and there are many studies of the photo catalysis induced by TiO₂-powdered semiconductors. Among these photo catalysts, powdered titanium dioxide (TiO₂) possesses a strong oxidizing power for almost all kinds of organic substances⁶-⁸.

A new toothbrush Soladey-3® (Shiken Co., Ltd., Osaka, Japan) has been recently introduced and in the monitors questionnaire, many monitors fells that their teeth maintains to be smooth for long time after brushing using Soladey-3® than using conventional toothbrush.

The objectives of the study were to investigate the effect of TiO₂ semiconductor Soladey-3 toothbrush, especially the prevention of S. mutans adherence on hydroxyapatite and to verify how many times brushing was most effectively.

**Materials and Methods**

**Materials**

Nine hydroxyapatite pellets were used (10×10×2 mm in diameter; APP-100, PENTAX, Tokyo, Japan). The Soladey-3® toothbrush was equipped a TiO₂ rod in neck of the brush and a solar panel in the body. The TiO₂ (positive charge) and stainless rod were connected with a copper wire to produce electric circulation in this experiment. The solar panel is the energy source to produce electron (Fig. 1). As

**Fig. 1 Soladey-3 toothbrush contained TiO₂ rod and positive charge containing wire**

**Fig. 2 Placebo toothbrush contained stainless steel rod and false solar panel**
a control, a placebo toothbrush, which contains a stainless steel rod in the neck of the brush was used without a solar panel on top of the body (Fig. 2).

Organism and growth media
The *S. mutans* strain (ATCC25175) was used for this experiment. The strain was grown overnight in brain heart infusion (BHI) broth (Becton, Dickinson Co., Maryland, USA).

Methods
Aliquots of the overnight cultures were inoculated into fresh medium and incubated at 37°C anaerobically until a stationary growth phase (10⁶ CFU/ml). The growth was monitored by measuring optical density (OD). Then, 100 μl of bacterial suspension was added into a new test tube that contained 10 ml BHI broth with 1% sucrose.

Photocatalytic reaction:
The hydroxyapatite pellet was brushed using Soladey-3 toothbrush. Briefly, 9 hydroxyapatite pellets were used as experimental model and 9 pellets were used as control. Gradual brushing of the apatite pellet was done for 1, 5, 10, 15, 20, 25, 30, 50 and 70 times using Soladey-3®. The brushing procedure was done in a jar containing distilled water and under 750 LUX light source. The photo catalytic reaction was started by irradiating the water with light (40 W and 750 LUX) and stopped by turning off the light. In the same way, the control apatite pellets were gradually brushed with the placebo brush. The same person brushed all apatite pellets for keeping the same brushing pressure. All the pellets were suspended into previously prepared bacterial suspension and incubated at 37°C for 12 hours. After incubation, the pellets were dyed by Erythrosin (Red-cote, Chicago, USA) to check the adhesion of *S. mutans* on the brushed and non brushed surfaces of the pellet and were washed twice with sterilized saline. After drying, all pellets were examined under a microscope (Olympus DP-11, Olympus Optical Co., Ltd., Osaka, Japan). The pellets found *S. mutans* colony on the brushed pellet surface was judged by a person who didn’t brush and didn’t know the brushing technique. Colony presence were scored as plus (+) and no colony as minus (−). This experiment was done four times in the same manner.
Results

Brushing with TiO$_2$ semiconductor Soladey-3® toothbrush

After 12 hours incubation the brushing pellets, it was observed that with increased number of brushings, amount of plaque adherence decreased. No plaque adherence was observed on the pellets with 50 and 70 times brushing (Fig. 3H and 3I). However, brushing 25 and 30 times showed small patches of plaque over the pellet surface (Fig. 3F and 3G). In 15 and 20 times brushing, pellets contained few amounts of plaque that gradually increased with the decreasing number of brushings (Fig. 3D and 3E). Pellets that were brushed 10 times showed greater amount of plaque (Fig. 3C). Pellet brushed 1 and 5 times showed huge amounts of plaque (Fig. 3A and 3B). From 35 to 45 times brushing, sometime we found out that the colonies on pellets and sometime couldn’t. We couldn’t find out the colonies from pellets at 50 times brushing.

Brushing with placebo toothbrush

On all brushing times on the pellets’ surfaces, huge amounts of plaque adherence were observed. In the case of brushing with the placebo toothbrush, pellets showed huge amounts of bacterial adherence and there was no effect on increasing the number of brushings (Fig. 4). All the pellets visually showed the same amount of bacterial adherence. The plaque accumulation is listed in Table 1.

Discussion

Sucrose is the only sugar that S. mutans can use to form sticky polysaccharide$^{13}$. S. mutans is gram-positive, facultatively anaerobic bacteria commonly found in the human oral cavity and is a significant contributor to tooth decay.

Toothbrushes with a semiconductor have been gaining a lot of attention lately. The TiO$_2$ semiconductor is located in the neck region of the toothbrush.

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Table 1 Relationship between brushing time and plaque adherence

<table>
<thead>
<tr>
<th>Number of brushing</th>
<th>Soladey-3 toothbrush</th>
<th>Placebo toothbrush</th>
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<tbody>
<tr>
<td>70 times</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>50 times</td>
<td>–</td>
<td>+</td>
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<td>30 times</td>
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<td>+</td>
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<tr>
<td>5 times</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1 time</td>
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(–) No adhesion of S. mutans
(+ ) presence of S. mutans adhesion
In the presence of light, saturated low energy electrons in the wet semiconductor are transformed into energy electrons resulting in a reaction of H\(^+\) ions as shown below.

\[
\begin{align*}
\text{H}^+ + \text{O}_2 + e^- &\rightarrow \text{HO}_2 \\
2\text{HO}_2 &\rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \\
\text{H}_2\text{O}_2 &\rightarrow 2\text{HO}^- + \text{O}_2 \\
\end{align*}
\]

This reaction results in the reduction of H\(^+\) ions from the organic acid in the plaque causing its decomposition. The reaction could also have an effect on plaque formation\(^9\). There is also evidence that the powdered TiO\(_2\) semiconductor irradiated with visible light has a bactericidal effect against *Escherichia coli* and *S. mutans*\(^10\). On the control of dental plaque, using a photo energy conversion toothbrush has an effect on plaque formation\(^11,12\). The TiO\(_2\) rod of the new Soladey-3 toothbrush produces a negatively-charged electron (e\(^-\)) in the presence of light during tooth brushing and acts against oral bacteria.

Enamel is the most highly mineralized tissue known, consisting of 96% mineral and 4% organic material and water. The inorganic content of enamel consists of a crystalline calcium phosphate known as hydroxyapatite.

Titanium dioxide (TiO\(_2\)) in the anatase crystalline form behaves as a classical semiconductor. Illumination of TiO\(_2\) in water with light of less than 400 nm generates excess electrons in the conduction band (e\(^-\)\(_{\text{cb}}\)) and positive “holes” in the valence band (h\(^+\)\(_{\text{vb}}\)): TiO\(_2\) + hv \rightarrow e\(^-\)\(_{\text{cb}}\) + h\(^+\)\(_{\text{vb}}\). At the TiO\(_2\) particle surface the holes react with either adsorbed H\(_2\)O or surface OH\(^-\) groups to form HO\(^-\) radicals: h\(^+\)\(_{\text{vb}}\) + H\(_2\)O (ads.) \rightarrow HO\(^-\) + H\(^+\) or h\(^+\)\(_{\text{vb}}\) + OH\(^-\) (sur.) \rightarrow HO\(^-\). Excess electrons in the conduction band react with molecular oxygen to form superoxide ions, e\(^-\)\(_{\text{cb}}\) + O\(_2\) \rightarrow O\(_2\)^{2-}, which further disproportionate to form more HO\(^-\) radicals: 2O\(_2\)^{2-} + 2H\(_2\)O \rightarrow 2HO\(^-\) + 2OH\(^-\) + O\(_2\)^{4+}. It was speculated that positive “holes (h\(^+\)\(_{\text{vb}}\))”, have on bacteria a direct bactericidal effect\(^15\). The negatively charged e\(^-\)(ion) decomposed the bacterial plaque by the reduction of H\(^+\) ions and HO\(^-\) radicals have a higher bactericidal efficiency\(^9,14\). It also produces superoxide ions, which may produce a layer over the tooth surface. This layer may protect the tooth surface from the bacterial plaque adhesion. Gelover *et al.* reported that the disinfection process using TiO\(_2\) kept treated water free of coliforms for at least seven days after sun irradiation\(^16\).

After comparing experiment results four times, all results were the same. That means, by the Soladey-3 toothbrush, more numbers of brushing resulted in less amount of bacterial adherence while less number of brushing resulted in more amount of bacterial adherence. This experiment was carried out *in vitro*. This manuscript showed that we might remove the dental plaque not only physical function but also chemical reaction by using Soladey-3 toothbrush.

It is hypothesized that the solar panel (light source), TiO\(_2\) rod and saliva consists of electronic circle and then the teeth surface change minus charge. As a result, *S. mutans* cannot adhere on the teeth surfaces (Fig. 5).

We want to emphasize that more than 50 times brushing using Soladey-3 could completely inhibit the *S. mutans* adhesion on the hydroxyapatite pellet for 12 hours. The results indicated that this brush could be a great therapeutic impact in dental caries prevention.
Conclusion

In this study it was observed that, in the presence of light and water, more than 50 times brushing using Soladey-3® toothbrush produced could prevent S. mutans adhesion on hydroxyapatite pellet in vitro, which will have a great therapeutic effect in dental caries prevention.

References