The Effect of Light Emitting Diode Toothbrush on *Streptococcus mutans* Biofilm *In Vitro*

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Abstract

Streptococcus mutans is one of the most significant contributors to tooth decay. Recently, blue light was reported to have a bactericidal effect. This study aims to evaluate the bactericidal effect of an LED toothbrush and determine the effect of duration of LED exposure on *Streptococcus mutans* biofilm *in vitro*. The LED light source is from an LED toothbrush (WHITENGO™, UK). *S. mutans* biofilms were assigned to 5 groups with 6 samples each, depending on the duration of LED exposure, which are 15 seconds, 30 seconds, 60 seconds, 120 seconds, and the control with no LED exposure. Kruskal-Wallis test was performed to compare the percentage of viability among groups. The percentage of bacterial viability in the 120-second group was significantly lower than those of other groups. The LED toothbrush effectively reduced *S. mutans* viability when the biofilm was exposed to the light for at least 120 seconds. This information may help consider the use of the LED toothbrush for oral care.

Keywords: *Blue light, Biofilm, LED toothbrush, Streptococcus mutans*

1. Introduction

Streptococcus mutans (*S. mutans*) is a common type of microorganisms found in dental plaque. Mutans streptococci, as well as lactobacilli, are the potential pathogens for demineralization that cause dental caries because they metabolize dietary sugar to acids. According to the ecological plaque hypothesis, these microorganisms normally exist in a small amount even within a healthy host. However, when the oral environment critically changes, such as high fermentable sugar or low salivary flow, the pathogen will outgrow and cause dental caries. The prevention of dental caries highlights dental plaque removal, together with managing the supplementary risks, for instance, reducing the fermentable sugar, increase salivary flow, and promote remineralization by using fluoride (Marsh, 2006).

Blue light, with a wavelength of about 400-500 nm, was proved by numerous studies to have antimicrobial activity (De Lucca et al., 2012; Tzung & Huang, 2004). The investigations about the bactericidal effect of blue light on *S. mutans* and oral biofilms suggested that it could reduce the *S. mutans*' viability and inhibit biofilm development (Chebath-Taub et al., 2012; De Sousa et al., 2015). Light-emitting diode (LED) generates the light by allowing the currents to pass through the semiconductors, which determine the color of the light. Gallium nitride (GaN) is commonly used to generate blue light. The LED is recently incorporated into electric toothbrushes for various purposes, such as improvement of oral hygiene or tooth whitening. However, there is no study yet that examined the effect of an LED light source from the LED toothbrush on the oral biofilm even though the clinical studies have shown that the LED toothbrush tended to reduce gingival inflammation (Genina et al., 2015; Lee, 2017).

Our study aims to investigate the lethal effect of LED light originated from the LED toothbrush on *S. mutans* biofilm and to demonstrate the duration of LED exposure that affects the vitality of the bacteria.

2. Objectives

1) To examine the bactericidal effect of an LED toothbrush on *Streptococcus mutans* biofilm *in vitro*

2) To compare the bactericidal effect of different exposure times of an LED toothbrush on *Streptococcus mutans* biofilm *in vitro*

3. Materials and Methods

3.1 LED toothbrush

The LED light source was from the LED toothbrush (WHITENGO™, UK). The product was certified by European Conformity (CE marking). The specifications indicated 460-480 nm wavelength, 840 mW of power. The radiance measured by spectroradiometer CS-2000 (Konica Minolta, INC, Japan) was 0.0176 W/Sr.m^2. The LED light located at the toothbrush head was covered by the silicone bristles that were 9 mm long. (Figure 1)

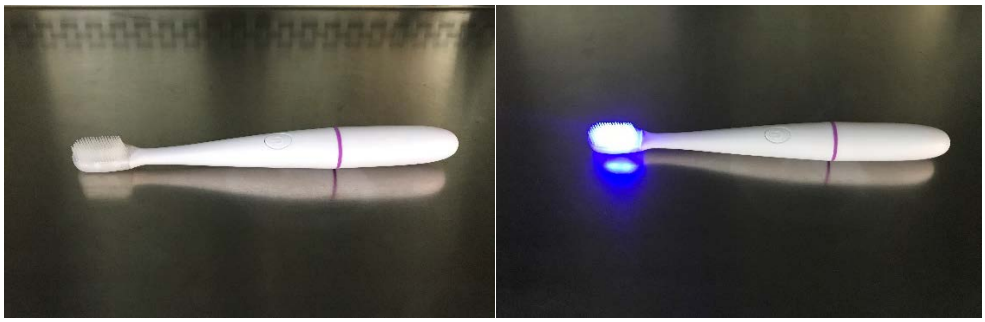

Figure 1 LED toothbrush (WHITENGO™, UK)

3.2 Bacterial growth

S. mutans UA 159 from bacterial glycerol stocks were inoculated in Brain-Heart Infusion (BHI) agar, and then were incubated at 37°C with 5% CO_2 for 24 hours. The isolated colony was regrown overnight in BHI broth with sustained shaking at 240 RPM. After that, its optical density at 600 nm (OD 600 nm) was evaluated and adjusted to 0.1. The culture was then incubated at 37°C with 5% CO_2 for 3 hours to reach the determined logarithm phase of growth (OD 600 nm ≈ 0.4-0.6), which would be used for the biofilm formation.

3.3 Formation of biofilms

The bacterial cells at the log phase were harvested by centrifugation (12,000 x g, 4°C, 15 minutes). The cells were then re-suspended in BHI broth with 1% sucrose. 3 mL of suspension that contains 3x10^8 bacterial cells were added to each culture plate and were incubated at 37°C with 5% CO_2 for 24 hours.

3.4 Exposure of the LED toothbrush to biofilms

The biofilms were divided into 5 groups: the control group with no LED exposure, and the experimental groups exposed to the LED light for 15, 30, 60, and 120 seconds. Each group contained two plates of *S. mutans biofilms*. In the experimental groups, the LED electric toothbrush was set at 2 mm above the biofilms and switched on. The blue LED light was exposed to the biofilm in each group with specific durations of 15, 30, 60, and 120 seconds, respectively. The supernatant fluid above the biofilms was removed and the biofilms were then scraped off and put into 1mL of phosphate-buffered saline (PBS).

3.5 Bacterial cells count

The bacteria in PBS suspensions were sonicated and serially diluted (10^{-1} to 10^{-8}). An aliquot of 100 µl of each concentration was spread on BHI agar in triplicate and were incubated for 48 hours at 37°C with 5% CO_2. The concentration that yielded 30-300 colonies will be used to count the number of bacteria. The experiments were repeated 3 times with duplicate sample/group in each experiment (total N=6/group) (Figure 2). The percentage of survival was calculated relative to the control.

3.6 Statistical analysis

Shapiro-Wilk test is used to test the normality of the data. The Kruskal-Wallis test was performed to compare the bactericidal effect of blue light from the LED toothbrush among groups. Statistically, a

significant difference was set at a *p*-value < 0.05. Post hoc comparisons were carried out by Mann-Whitney U test with the Bonferroni correction of the significance level of multiple pairwise comparisons ($p < 0.005$).

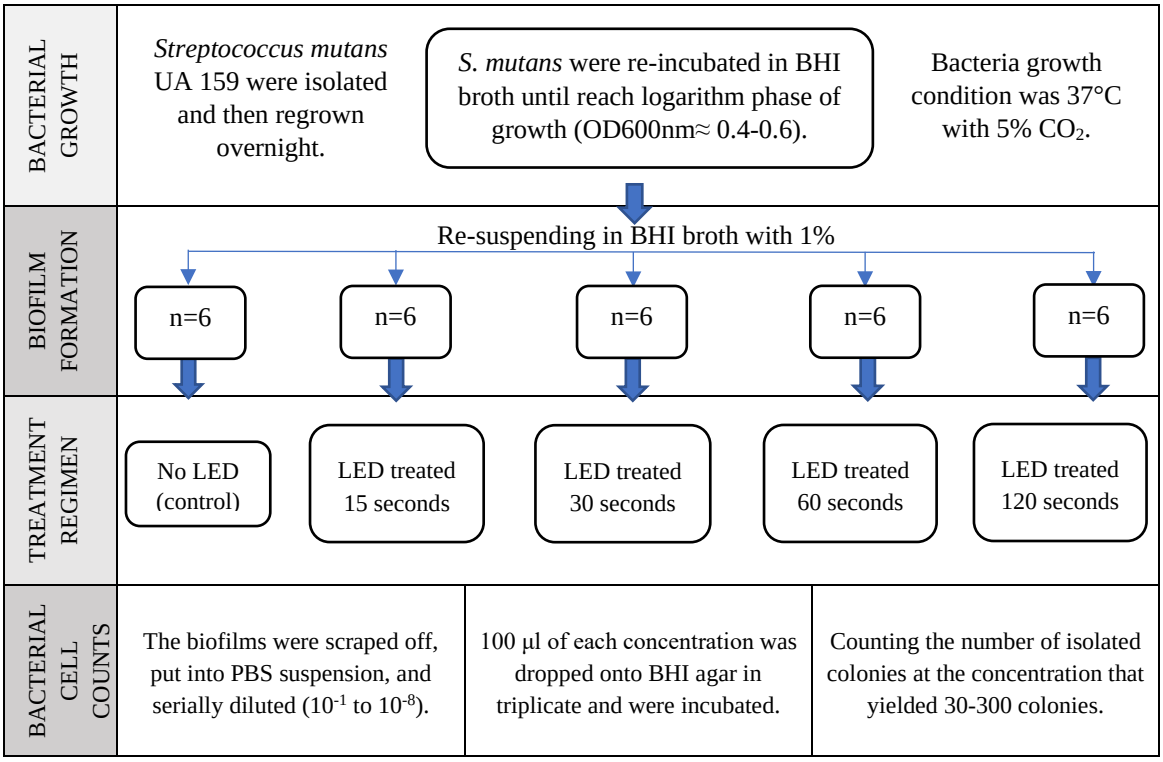

Figure 2 Flow chart of the study design.

4. Results and Discussion

4.1 Results

Table 1 showed the percentage of bacteria viability between the groups in mean and 95% confidence interval. Our results indicated that exposure to LED for 120 seconds significantly decreased the percentage of bacterial viability, compared with those exposed for 15, 30, and 60 seconds, and the control group ($p < 0.005$) (Figure 3).

Table 1 Percentage of bacterial viability [a]

		% Bacteria viability
Intervention	**n**	**Mean (95% CI)**
No LED exposure	6	100.02 (25.75, 174.29)
LED 15 seconds	6	68.05 (25.49, 110.60)
LED 30 seconds	6	52.57 (39.16, 65.98)
LED 60 seconds	6	63.15 (50.10, 76.20)
LED 120 seconds	6	21.16 (15.00, 27.32)
P value[b]		0.004

[a] % Bacteria viability calculated from the number of bacteria colonies in form of colony-forming unit per milliliter (CFU/mL) when control was adjusted to 100%; CI, confidence interval

[b] Kruskal-Wallis test (Statistically significant difference was set at *p*-value < 0.05.)

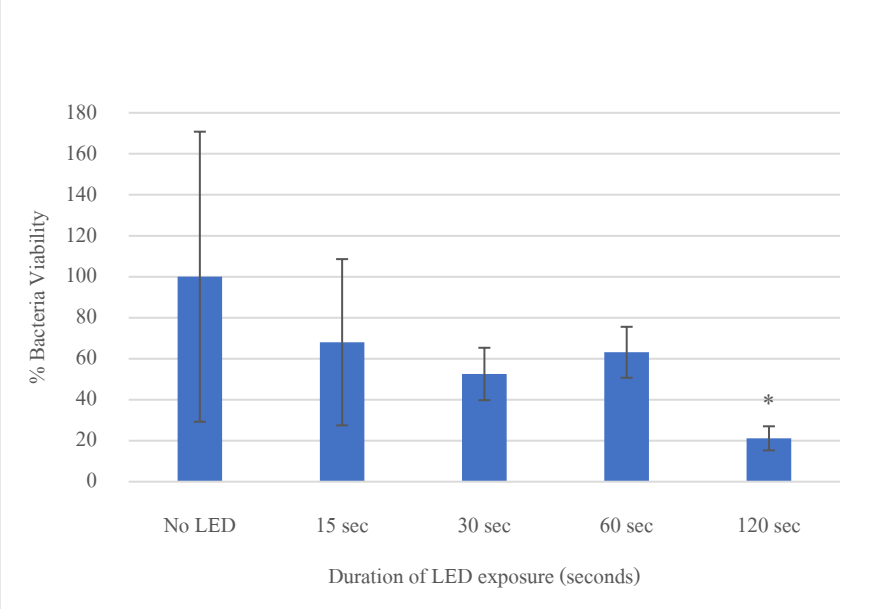

Figure 3 Percentage of bacterial viability. Post hoc comparisons were carried out by Mann-Whitney U test with Bonferroni correction of the significance level of multiple pairwise comparisons ($p < 0.005$). Asterisk (*) marked that the 120-sec group had a significantly lower percentage of bacterial viability than the other 3 groups ($p = 0.004$).

4.2 Discussion

The mechanism of the bactericidal effect of light is based on the photosensitizer, the agent that can absorb the light (Soukos & Goodson, 2011). Bacteria can take up external photosensitizers, while some bacteria have endogenous photosensitizers. When the photosensitizer is activated by light with its preferable wavelength, the electrons are transferred to produce the radical ions that react with oxygen and result in cytotoxic species (Athar et al., 1988). Blue light has been reported to reduce bacterial viability (Felix Gomez et al., 2018). Moreover, a previous study revealed that blue light reduced *S. mutans* biofilm re-formation rather than anti-biofilm directly (Chebath-Taub et al., 2012). *S. mutans* was tested with several exogenous photosensitizers (Rolim et al., 2012), but its specific endogenous photosensitizer remains unclear. The result of our study demonstrated that the visible blue light from the toothbrush could reduce the viability of *S.mutans* in biofilm. Although the activity of blue light against *S.mutans* has been shown in previous *in vitro* studies, the blue light was tested without any barrier between the light source and the bacteria (Chebath-Taub et al., 2012; Cohen-Berneron et al., 2016; Felix Gomez et al., 2018). In contrast, the blue light in LED toothbrushes has to pass through the silicone bristles. Thus, it is important to test whether the LED light in the toothbrush could still have an antibacterial effect. Our results suggest that the light from the LED toothbrushes can significantly reduce *S. mutans* viability in biofilm after a 2-minute exposure.

The exposure time of blue light on bacteria is one of the most important factors for the bactericidal effect of the LED toothbrush. Previous *in vitro* studies (Chebath-Taub et al., 2012; Cohen-Berneron et al., 2016; Felix Gomez et al., 2018) set the exposure duration from 1 to 10 minutes. People usually brush their teeth for 2-3 minutes at a time, and the toothbrush does not stay at one position for minutes in the patient's mouth. Therefore, our study varied the duration of blue light exposure to find the shortest time that decreased the viability of *S. mutans*. Our data showed that a significant reduction in bacterial viability occurred after the biofilm had been exposed to LED blue light for 120 seconds. The other durations, 15 seconds, 30 seconds, and 60 seconds showed the tendency of reduction, but the difference was not statistically significant. According to the recommendation of the American Dental Association, toothbrushing should be performed for 2 minutes, twice a day (American Dental Association, 2020). Thus, if an LED toothbrush was applied for 2 minutes, it may help to reduce bacterial viability in the oral cavity. However, the LED toothbrush in our

study was applied to the biofilm formed in 35x10 mm culture plates, which have a smaller surface area compared with the oral cavity.

The appearance of *S. mutans* indicates dental plaque accumulation, which is the major cause of dental caries and gingival inflammation. More anaerobic condition in older plaque consequently favors the growth of *S. mutans* (Fine, 1988). Our study supports the clinical study that the blue-light LED toothbrushes with 412 nm wavelength significantly reduced dental plaque, gingival bleeding, and inflammation more than the manual toothbrushes (Genina et al., 2015). A controlled trial by Kwon *et al* in 2019 revealed that the gingival index and bleeding on marginal probing were significantly lower in the LED electric toothbrushes group than the non-LED group after 6 weeks (Kwon et al., 2019). More future experiments with other bacteria, such as periodontal pathogens, and clinical studies would be useful for clinical implications. Overall, current evidence suggests that LED toothbrushes may be beneficial to improve oral hygiene and may help to reduce the risk of dental diseases such as dental caries and periodontal disease. Thus, further studies are warranted.

5. Conclusions

The LED blue light from the LED toothbrush significantly reduced the number of *S.mutans* in biofilm *in vitro* when the biofilm was exposed to the light for at least 120 seconds.

6. Acknowledgements

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