THE EFFECTIVENESS OF LED TOOTHBRUSH IN REDUCING DENTAL PLAQUE AND GINGIVITIS IN FIXED ORTHODONTIC PATIENTS: A RANDOMIZED CLINICAL TRIALS

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ประสิทธิภาพของแปรงสีฟันแอลอีดีในการลดคราบจุลินทรีย์และภาวะเหงือกอักเสบในผู้ป่วยที่รักษาด้วยเครื่องมือจัดฟันชนิดติดแน่น

น.ส.ชวิรกาญ แม้นพิบูลย์

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Thesis Title	THE EFFECTIVENESS OF LED TOOTHBRUSH IN REDUCING DENTAL PLAQUE AND GINGIVITIS IN FIXED ORTHODONTIC PATIENTS: A RANDOMIZED CLINICAL TRIALS
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ชวิรกาญ แม้นพิบูลย์ : ประสิทธิภาพของแปรงสีฟันแอลอีดีในการลดคราบจุลินทรีย์และภาวะเหงือกอักเสบในผู้ป่วยที่รักษาด้วยเครื่องมือจัดฟันชนิดติดแน่น. (THE EFFECTIVENESS OF LED TOOTHBRUSH IN REDUCING DENTAL PLAQUE AND GINGIVITIS IN FIXED ORTHODONTIC PATIENTS: A RANDOMIZED CLINICAL TRIALS) อ.ที่ปรึกษาหลัก : ผศ. ทญ. ดร.พินทุอร จันทรวราทิตย์, อ.ที่ปรึกษาร่วม : รศ. ทญ. ดร.อรนาฎ มาตังคสมบัติ

วัตถุประสงค์: เพื่อเปรียบเทียบประสิทธิภาพของแปรงสีฟันแอลอีดีและแปรงสีฟันธรรมดาต่อการลดคราบจุลินทรีย์และภาวะเหงือกอักเสบในผู้ป่วยที่จัดฟันด้วยเครื่องมือจัดฟันชนิดติดแน่น และเพื่อทดสอบประสิทธิภาพของแปรงสีฟันแอลอีดีรวมถึงผลของระยะเวลาได้รับแสงที่แตกต่างกันต่อแผ่นชีวภาพของแบคทีเรียสเตร็ปโตคอกคัสมิวแทนส์ในห้องปฏิบัติการ

วัสดุและวิธีการ: ผู้ป่วยที่จัดฟันด้วยเครื่องมือจัดฟันชนิดติดแน่น 15 รายได้รับการจัดกลุ่มแบบสุ่ม โดยแบ่งเป็นกลุ่มที่ได้รับแปรงสีฟันแอลอีดี และกลุ่มที่ได้รับแปรงสีฟันธรรมดา โดยจะได้รับการวัดค่าดัชนีคราบจุลินทรีย์และดัชนีสภาพเหงือกก่อนได้รับแปรงและหลังจากใช้แปรงไปแล้ว 28 วัน การทดลองในห้องปฏิบัติการแผ่นชีวภาพของแบคทีเรียสเตร็ปโตคอกคัสมิวแทนส์จะถูกแบ่งเป็น 5 กลุ่ม กลุ่มละ 6 ตัวอย่าง ตามระยะเวลาที่ได้รับแสงแอลอีดีได้แก่ 15 วินาที, 30 วินาที, 60 วินาที, 120 วินาที และกลุ่มที่ไม่ได้รับแสงแอลอีดีเลย

ผลการทดลอง: ค่าดัชนีคราบจุลินทรีย์และค่าดัชนีสภาพเหงือกระหว่างกลุ่มที่ใช้แปรงสีฟันแอลอีดีและกลุ่มที่ใช้แปรงสีฟันธรรมดาไม่แตกต่างกันอย่างมีนัยสำคัญ จากการทดลองในห้องปฏิบัติการพบว่ากลุ่มแผ่นชีวภาพที่ได้รับแสงแอลอีดีเป็นระยะเวลา 15 วินาที, 30 วินาที, 60 วินาที และ 120 วินาที มีการลดลงของความมีชีวิตของเชื้อสเตร็ปโตคอกคัสมิวแทนส์มากกว่ากลุ่มที่ไม่ได้รับแสงแอลอีดีอย่างมีนัยสำคัญ

สรุปผลการทดลอง: แปรงสีฟันแอลอีดีไม่มีประสิทธิภาพในการลดคราบจุลินทรีย์และภาวะเหงือกอักเสบในผู้ป่วยที่จัดฟันด้วยเครื่องมือจัดฟันชนิดติดแน่นมากกว่าแปรงสีฟันธรรมดา การทดสอบในห้องปฏิบัติการพบว่าแสงสีฟ้าจากแปรงแอลอีดีสามารถลดความมีชีวิตของเชื้อสเตร็ปโตคอกคัสมิวแทนส์ในแผ่นชีวภาพได้เมื่อได้รับแสงแอลอีดีเป็นระยะเวลาอย่างน้อย 15 วินาที

สาขาวิชา ทันตกรรมจัดฟัน ลายมือชื่อนิสิต .....
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KEYWORD: Dental plaque removal, Fixed orthodontic patients, Blue light, Biofilm, LED toothbrush, Streptococcus mutans

Chavirakarn Manphibool : THE EFFECTIVENESS OF LED TOOTHBRUSH IN REDUCING DENTAL PLAQUE AND GINGIVITIS IN FIXED ORTHODONTIC PATIENTS: A RANDOMIZED CLINICAL TRIALS. Advisor: Asst. Prof. PINTUON CHANTARAWARATIT, D.D.S., Ph.D. Co-advisor: Assoc. Prof. ORANART MATANGKASOMBUT, D.D.S., Ph.D.

Objective: To compare the effectiveness of LED toothbrush to manual toothbrush in reducing dental plaque and gingival inflammation in fixed orthodontic patients, and to investigate the effect of duration of LED toothbrush exposed to the *S.mutans* biofilm *in vitro*.

Materials and methods: Fifteen fixed orthodontic patients were recruited to this parallel-group analysis. The patients were randomly divided into 2 groups relying on brushing methods: manual toothbrush and LED toothbrush. Plaque index and gingival index were examined by a calibrated-blinded examiner at baseline and 28 days after brushing period. *In vitro* part, the *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.

Results: Between-group comparisons showed no significant difference in plaque index and gingival index. The LED toothbrush significantly reduced dental plaque at the gingival portion on the bracket side. *In vitro* part, the percentage of bacterial viability was significantly reduced in 15, 30, 60, 120 seconds group.

Conclusion: LED toothbrush did not more effective in reducing dental plaque and gingival inflammation than the manual toothbrush in fixed orthodontic patients. 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 15 seconds.

Field of Study: Orthodontics
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Student's Signature .....
Advisor's Signature .....
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Chavirakarn Manphibool

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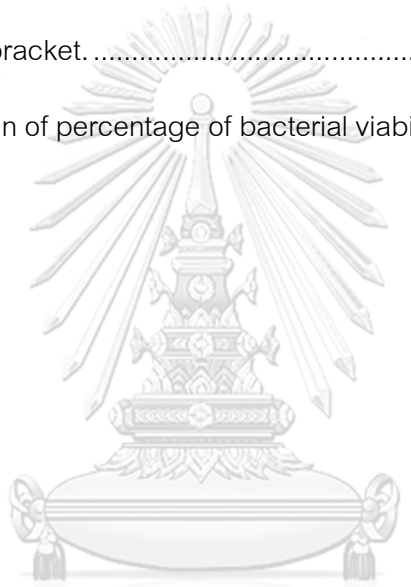

CHAPTER 1 INTRODUCTION

Background and Rationale

Dental plaque is the main etiology of dental caries and periodontal disease(1), which are challenging to a long time procedure like orthodontic treatment. Fixed orthodontic appliances have a complex shape of bracket wing and slot that are likely to be the plaque retentive area. Evidence showed that patients with fixed orthodontic appliances have higher plaque accumulation(2-6), the higher tendency of gingival inflammation, and bleeding on probing values(2, 4, 5, 7-9). Also, fixed orthodontic appliances cause the microbial shift towards more pathogenic types, for example, streptococci and lactobacilli(10) which are cariogenic bacteria, and the periodontopathogens like *T.forsythia*, *C.rectus*, and *P.nigrescens*(11). Hence, plaque control in fixed orthodontic patients is undeniably important. Due to the difficulties in plaque removal, a power toothbrush is one of the attempts trying to improve oral hygiene in fixed orthodontic patients. However, the evidence of its effectiveness is still in controversy(12, 13)

Recently, a light emitting diode (LED) toothbrush is trying to apply the photodynamic effect on the dental plaque in addition to mechanical effect. Because blue light was proved to have the bactericidal effect on *S. mutans* and oral biofilms(14-17), it is possible that an LED toothbrush would be more effective than a manual toothbrush, supported by a few studies. Clinically, the blue LED toothbrushes with 412 nm wavelength significantly reduced dental plaque, gingival bleeding, and inflammation more than the manual toothbrushes(18). However, the study in this area is still limited, and yet in orthodontic patients. The aim of this study is to investigate the effectiveness of LED toothbrush in dental plaque removal, reduction of gingival inflammation, and the number of mutans streptococci in fixed orthodontic patients.

Research Questions

1. Is an LED toothbrush more effective than a manual toothbrush in removing dental plaque in fixed orthodontic patients?
2. Is an LED toothbrush more effective than a manual toothbrush in reducing gingival inflammation in fixed orthodontic patients?
3. Does an LED toothbrush have a bactericidal effect on newly formed *Streptococcus mutans* biofilm *in vitro*?
4. Do the different exposure times of an LED toothbrush have the different bactericidal effect on *Streptococcus mutans* biofilm *in vitro*?

Research Objectives

1. To compare the effectiveness of LED toothbrushes and manual toothbrushes in dental plaque removal in fixed orthodontic patients.
2. To compare the effectiveness of LED toothbrushes and manual toothbrushes in the reduction of gingival inflammation in fixed orthodontic patients.
3. To evaluate whether an LED toothbrush have a bactericidal effect on newly formed *Streptococcus mutans* biofilm *in vitro*.
4. To evaluate whether the different exposure times of an LED toothbrush have the different bactericidal effect on *Streptococcus mutans* biofilm *in vitro*?

Research Hypothesis

Null hypothesis:

1. An LED toothbrush is not more effective than the manual toothbrush in dental plaque removal in fixed orthodontic patients.
2. An LED toothbrush is not more effective than the manual toothbrush in the reduction of gingival inflammation in fixed orthodontic patients.

3. An LED toothbrush does not have a bactericidal effect on newly formed *Streptococcus mutans* biofilm *in vitro*.
4. The different exposure times of an LED toothbrush have no different bactericidal effect on *Streptococcus mutans* biofilm *in vitro.*

Alternative hypothesis:

1. An LED toothbrush is more effective than the manual toothbrush in dental plaque removal in fixed orthodontic patients.
2. An LED toothbrush is more effective than the manual toothbrush in the reduction of gingival inflammation in fixed orthodontic patients.
3. An LED toothbrush has a bactericidal effect on newly formed *Streptococcus mutans* biofilm *in vitro*.
4. The different exposure times of an LED toothbrush have the different bactericidal effect on Streptococcus mutans biofilm in vitro.

Significance of the Study

The result of a randomized controlled trial with a parallel-group design which investigate whether the LED electric toothbrush is beneficial in reducing dental plaque and improving gingival condition in patients with fixed orthodontic appliances.

Conceptual Framework

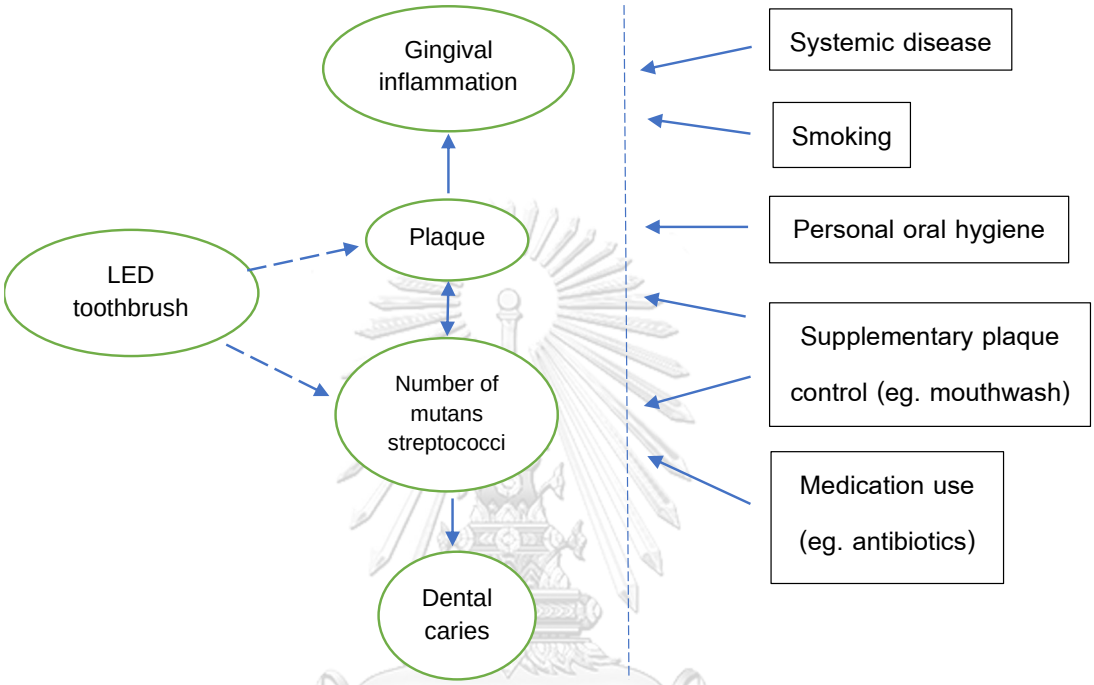

Figure 1 The conceptual framework

The conceptual framework shows independent variable (LED toothbrush), dependent variables (Reduction in plaque, gingival inflammation, and mutans streptococci), and confounders.

CHAPTER 2 LITERATURE REVIEW

Dental Plaque

Dental plaque is an accumulation of various microorganisms adhering to a tooth surface as a biofilm by embedding in the matrix of host pellicles and bacterial origin(19, 20). The mechanisms of dental plaque formation are divided into three steps sequentially: the formation of a pellicle, the occurrence of the pioneer microorganisms and their proliferation, and the collection of filamentous organisms and spirochetes (21). This contributes to host defense(22) while its structure limits the penetration of antimicrobial agents(23, 24). The understanding of dental plaque is crucial because it is also known as the main etiology of dental caries and periodontal disease(1).

Dental plaque genesis begins with the pellicular formation. The pellicle is an acquired acellular material secreting by the host. The major components of the pellicle are glycoproteins(1, 25) combined with other salivary components such as proline-rich proteins, statherin, and fibronectin which facilitate bacterial adhesion(26). The next step is the appearance of the pioneer microorganisms. Gram-positive aerobic bacteria, especially cocci, is the first colonizer, followed by the gram-positive rods and filament after a few days. Then, the bacterial plaque shift toward more anaerobic type like gram-negative organisms and fusiforms(1, 25). Gram-negative bacteria such as *Tannerella forsythia*(27), *Porphyromonas gingivalis*, *Treponema denticola*(19) are pathogenic to dental supportive tissue. When the new microorganisms arrive, they attach to the primary colonizer and implant themselves not only to the preceding salivary components but also to the exopolysaccharides of the bacteria(26). The adhesion between the cell surface and the pellicle is firstly a slight reversible physiochemical interaction which gradually leads to a stronger adhesin-receptor mediated attachment that promotes the secondary colonization(28). The final step is marked by the emergence of spiral forms and spirochetes. However, the final proportion of the species in dental plaque is mostly composed of filamentous organisms because of their abilities to overcome other surrounding cells(20, 21). In the normal conditions, this composition is stable and called

“microbial homeostasis” which endures small stresses such as host defense, oral hygiene, and salivary flow(29). However, whenever the stresses are so critical that the minor components are outgrowing and weaken this stability, the disease such as periodontal disease and dental caries may occur.

In the periodontal aspect, dental plaque induces host response and contributes to the disease. Commonly, if supragingival plaque is not removed for 2-3 weeks, the gingival inflammation will occur(30). During these 3 weeks, the compositions of the dental plaque are more complicated than the beginning. Because dental plaque usually accumulates over the gingival margin and gingival sulcus, the bacteria can utilize nutrients from gingival crevicular fluid together with saliva(31). Moreover, some species even metabolize by-products of another’s species. These factors massively exacerbate bacterial growth which is unfavorable to the periodontal tissue. The host response to the bacteria and bacterial products by the inflammatory process resulting in increased vascularity, swelling, leukocytic diapedesis, and the loss of connective tissues(32). Furthermore, once the dental plaque is mineralized and becomes calculus, it harbors more bacteria and strengthens the pathogenicity(25). Finally, if this circumstance is maintained long enough, it possibly results in the periodontal breakdown(33).

Another relevant disease is dental caries. In this point of view, there is a widely accepted concept named “Ecological plaque hypothesis”(34). This concept states that normally there are small amounts of potential pathogens for demineralization such as Mutans streptococci and Lactobacilli even within a healthy host. However, when the environment critically changes, the homeostasis is disturbed. To control the stability of the oral microorganisms, the important factors are host defense, including salivary flow, and composition of the diets(35). Regarding this, the prevention of dental caries highlights on dental plaque removal, together with managing the supplementary risks, for instance, reducing the fermentable sugar, increase salivary flow, and promote remineralization by using fluoride(20).

Orthodontic appliances have an advanced design that promotes plaque accumulation. Complex shapes of bracket wings and slots are likely to be the plaque

retentive area. Evidence showed that patients with fixed orthodontic appliances have higher plaque accumulation(2-6) which harmful to the gingival conditions. Patients with fixed orthodontic appliances were reported for the higher tendency of gingival inflammation and bleeding on probing values(2, 4, 5, 7-9). Some even found more clinical attachment loss in fixed orthodontic patients rather than no treatment group(2). Therefore, the key implication is that it is essential to emphasize plaque control and maintain good oral hygiene, especially in orthodontic patients.

Oral microorganisms in orthodontic patients

The orthodontic appliance is the critical environmental changes that disturb the oral microbial homeostasis, not only in terms of the higher plaque accumulation but also changing in the contents of the plaque. A study in 1970 by Balenseifen et al (10) showed that after banding and applying archwires for 4-5 weeks the plaque was significantly lower in pH, higher in a concentration of carbohydrates, and higher in the population of cariogenic bacteria which were streptococci and lactobacilli. Mutans streptococci, notified as an important pathogen for dental caries(36), was reported that it increased in the active phase of orthodontic treatment(37). Moreover, the composition of the periodontopathogens in dental plaque are also altered. A longitudinal study by Kim et al in 2012 discovered that there were increases in anaerobic pathogenic bacteria such as *T.forsythia*, *C.rectus*, and *P.nigrescens* in the leveling and alignment phase of fixed orthodontic treatment compared to before the treatment(11). This supported a cross-sectional study of Lee et al in 2005(38). *Aggregatibacter actinomycetemcomitans* was also investigated in several studies and showed the corresponding results(3, 39).

Overall, fixed orthodontic appliances cause higher plaque accumulation, and the microbial shift towards more pathogenic types. It seems that fixed orthodontic patients are vulnerable to the disease like gingival inflammation, periodontitis, and dental caries. With these concerns, orthodontists should motivate their patients to maintain good oral hygiene and evaluate their oral hygiene status regularly.

Plaque removal in orthodontic patients

There are both mechanical and chemical aspects for domestic plaque removal in orthodontic patients which are toothbrushing, complementary aids (e.g. dental floss, single-tufted brushes), and chemical cleaning.

Toothbrushing is a routine method to remove plaque deposits. It has several techniques based on directions and positions of the bristles such as roll method, horizontal scrub, vibration, and circulation(40). However, the horizontal techniques like scrub or Bass technique were considered to be the preferred method rather than the vertical one(41). A study in fixed orthodontic patients by Nassar et al in 2013 demonstrated that when comparing the efficacy of the reduction of the periodontal indices between brushing with scrub technique, modified Stillman technique, and Bass technique, the Bass technique showed the dramatic reduction of the gingival index rather than other groups(42). This result was sensible because the Bass techniques required placing the bristle on the gingival sulcus in an angular direction, gingival to an archwire, and brushed anteroposteriorly with the short strokes (approximately two teeth). The recommendation by the American Dental Association is that brushing should be performed twice a day with 2 minutes each time(43). Furthermore, the single-tufted brush may be used additionally in case of hard-to-reach areas such as ligature loops or area below springs(44).

Nowadays, power toothbrushes are trying to enhance the efficacy of plaque removal. Although the meta-analysis by Kaklamanos and Kalfas in 2008 showed that there were insufficient comparative studies to claim the effectiveness of power toothbrushes over the manual ones in orthodontic patients(12), a more recent systematic review and meta-analysis in 2017 by Makhmari et al revealed that the power toothbrushes tended to improve gingival condition better than the manual one(13). Furthermore, a study by Erbe et al in 2019 illustrated that the interactive power toothbrushes with Bluetooth technology motivated the adolescents with fixed orthodontic appliances to brush more frequently and reduced more dental plaque(45). However, the

additional comparative controlled trials in orthodontic patients are still necessary to confirm this issue.

Photodynamic therapy, Light-emitting diode (LED) - Blue light and antimicrobial activity

The inactivation of microorganisms by visible light has been studied for over a century, including in agriculture, industry, and medication. The mechanism of the bactericidal effect of light is based on the photosensitizer, the agent that can absorb the light(46). The bacteria can take the external photosensitizer, besides some strains, even have it endogenously. 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 such as superoxide, and hydroxyl radicals (type I reaction)(47). Besides, the type II reaction, the energy is transferred to the ground state triplet oxygen($^3O_2$) and causes the excited state singlet oxygen($^1O_2$) which can oxidize bacterial proteins, nucleic acids, and lipids, which are essential for bacterial viability(48). Some periodontal pathogens such as *Prevotella nigrescens* and *Prevotella intermedia* contain endogenous porphyrins(49), one of the photosensitizers. *Streptococcus mutans* had been experimented with many exogenous photosensitizers, for instance, erythrosine, toluidine blue, and malachite green(50-52), even though the specific endogenous photosensitizer has not been clearly revealed.

Light-emitting diode is a light generated by electroluminescence. It is the process that light is released after the current is flown through an inorganic material called semiconductor. In the LED circuit, there are two types of semiconductors; N-type and P-type. The N-type has a large number of electrons, while P-type contains holes. When the current circulates, it raises the energy of the electrons so that they move to combine with the holes, and then the energy is released as photons. The color of the light depends on the material of the semiconductors. For the blue light, gallium nitride(GaN) is mostly used(53).

Blue light is the visible light which its wavelength is about 400-500nm. The effect of blue light on antimicrobial activity was investigated by numerous studies. A study by

Tzung ef al in 2004 reported that a 420nm blue light was effective in the treatment of some types of acne. This could be explained by that the *Propionibacterium acne* contained porphyrins that caused the photodynamic effects(54). The comprehensive experiment about the effect of 470nm blue light on 3 bacterial strains and 2 fungi strains(55) indicated that the lethal effects depended on the microbial genus, energy levels, light purity, and temperature. This expanded our understanding that, practically, bacterial strains and environmental factors could affect the outcome. 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(14-17). Anyway, data showed that the blue light could have a lethal effect on the new biofilm formed by *S. mutans* only after 7- or 10-minutes exposure and destroy mostly on the outer surface of the biofilm(14). In 2018, an *in vitro* study by Gomez et al applied 405nm violet-blue light on the *S. mutans* biofilm specimens for 5 minutes twice a day for over 5 days. The result revealed the reduction of bacteria numbers in treated groups(17). Yet, there is no *in vitro* study that uses the blue-light LED toothbrush rather than using the blue light alone. Our question is that if the blue- light LED toothbrush apply on the *S. mutans* biofilm for 2 minutes, like the normal brushing duration, whether it still can inhibit the bacterial growth.

Light-emitting diode (LED) toothbrushes

Since light-emitting diode (LED) showed the antimicrobial effects(56, 57), it has been recently applied to toothbrushes in order to enhance the efficacy in reducing dental plaque and improve gingival health. The *in vitro* studies demonstrated that the LED toothbrushes were able to reduce the number of *Porphyromonas gingivalis* attached to titanium and zirconia surfaces(58, 59). In 2015, a pilot clinical study by Genina et al found that the blue LED toothbrushes with 412 nm wavelength significantly reduce dental plaque, gingival bleeding, and inflammation more than the manual toothbrushes(18). Another clinical study by Lee et al 2017 compared the antiplaque and antigingivitis effect between LED electric toothbrushes and the common electric toothbrushes in patients with gingival inflammation or mild periodontitis. The data

showed the significantly lower gingival index in the LED group after 2 weeks and 4 weeks whereas the plaque index did not show any significant differences(60). In this experiment, they used red and white LED lights. Recently, a controlled trial by Kwon et al in 2020 revealed the corresponding results(61). The data presented no significant difference in plaque index at any time points (3 weeks and 6 weeks). However, 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. This study used the advanced version called Electric 3-color LED toothbrush which is composed of 2 blue lights, 1 red light, and the other 1 white light.

At present, there is no data about the efficacy of the LED toothbrush in fixed orthodontic patients yet. Our study is willing to fulfill this gap of knowledge which may useful for orthodontic patients who have difficulties maintaining good oral hygiene.

CHAPTER 3 MATERIALS AND METHODS

Participants

The samples of this study were the patients submitted to orthodontic treatment at the Department of Orthodontics, Faculty of Dentistry, Chulalongkorn University. All participants eligible for the study were informed about the objectives and the procedures of the research. Informed written consents had been submitted by all participants before the experimental period. Candidates were recruited by the following criteria.

Inclusion criteria

1. Patients had been treated with fully bonded fixed appliances, at least from one first molar to another first molar in the same arch, and being treated for more than 1 month with the irregularity index not more than 1 mm(62).
2. Having a minimum of 20 fully erupted permanent teeth excluding the third molars.
3. No known systemic disease, medical condition, or under the medication that affects oral tissues.
4. No supplementary plaque control such as antiseptic mouthwashes or antibiotics for one month before the study.
5. No periodontitis

Exclusion criteria

1. Patients with systemic disease
2. Taking the medications that influence oral tissues, for example, corticosteroids and nonsteroidal anti-inflammatory drugs.
3. Smoking
4. Apply chemical supplementary plaque control.
5. Patients who have Parkinson's disease or other diseases that might affect hand control.

Sample size

The sample size was calculated based on gingival index data from a previous study(61) with an alpha of 0.05 and 0.8 power of the test. The result indicated 18

samples. However, to compensate for possible drop out of 30% during the study period, the sample size is adjusted to 24.

Allocation technique

Allocation of 24 subject into two groups was done by Block-of-4 randomization.

Group A- Manual/LED

Group B- LED/Manual

There were 6 possibilities to allocate participants to a block equally.

1= AABB, 2= ABAB, 3= BAAB, 4= BABA, 5= BBAA, 6=ABBA

2	6	0	5	8	3	9	9	4	7	6
7	7	0	9	4	4	9	9	5	6	8
1	5	9	5	0	8	2	9	6	3	0
1	7	1	9	9	6	0	8	3	2	9
6	5	4	3	4	4	5	3	5	5	2
2	8	6	2	6	5	7	7	8	8	1
7	4	8	9	0	2	9	0	6	9	8
0	9	3	2	1	6	3	7	5	2	7
0	7	5	0	3	7	8	3	6	6	0
3	6	3	8	0	2	5	2	6	0	6
2	6	4	5	9	9	7	9	4	2	1
3	1	9	0	1	8	5	4	0	2	8
8	3	4	0	5	8	1	9	0	2	3
6	4	3	5	2	7	7	0	8	4	3
7	4	2	7	2	9	9	5	2	4	7
7	5	6	2	7	9	0	9	1	5	2
9	0	9	0	8	0	5	2	4	9	4
2	0	2	7	1	8	7	4	0	8	3
5	2	5	7	2	8	6	0	2	8	5

Figure 2 Table of computer-generated random numbers

Subject	Group	Subject	Group	Subject	Group
1	B	9	A	17	B
2	A	10	A	18	A
3	A	11	B	19	B
4	B	12	B	20	A
5	A	13	B	21	A
6	A	14	B	22	B
7	B	15	A	23	A
8	B	16	A	24	B

Table 1 Group allocation of 25 subjects

A list of random numbers was randomly selected from a table (figure 2), any numbers other than 1 to 6 were excluded. For instance, if the highlighted numbers in figure 2 were selected, the results are 3 1 1 5 4 2. Random allocation was in blocks. (Subject:24, Block size: 4, Group: 2 (A,B)

Ethical consideration

The materials and methods of this study was approved by the Human Research Ethics Committee of the Faculty of Dentistry, Chulalongkorn University, Thailand (approval no.126/2020, study code: HREC-DCU 2020-116), and registered at the Thai Clinical Trials Registry (TCTR; TCTR 20210510004).

LED toothbrush

LED toothbrushes (WHITENGO™, UK) used in our study was certified by European Conformity (CE marking). The specifications include 460-480nm wavelength, 16,000 acoustic pulsations per minute, 840mW of power, and 1,000-3,000 MCD of light intensity. The bristles are made from silicone which were 9mm long. The radiance was 0.0176 W/Sr.m^2, measured by spectroradiometer CS-2000 (Konica Minolta, INC, Japan).

Research methodology

1. The clinical part

This clinical study was conducted as a crossover randomized controlled trial. Screening examinations were performed before starting the experiment. The 24 subjects were randomly allocated into two groups.

Group A: Manual/LED

Group B: LED/Manual

In both groups, the patients were instructed by one dentist for 5 minutes to brush with Bass techniques(42). On the bracket side, patients were also informed to place the bristles to the top and the bottom of the brackets, and brush with horizontal strokes. In group A, the manual toothbrushes were orthodontic toothbrushes by Systema OD (Japan). In group B, the patients brushed with LED toothbrushes (WHITENGO™, UK). The same standard toothpaste was used in both groups. All participants were assigned to brush for a minimum of 2 minutes at least twice a day, in the morning and the evening

after meals. Both groups had been brushing with the assigned toothbrushes for 28 days. After that, the patients will be under the washout interval for the other 28 days. During this period, they will be using their own normal toothbrushes. Then, the patients will switch to use another device for the other 28 days. The accuracy of the brushing method and compliance were confirmed by questionnaires. Other supplementary plaque control methods and scaling were prohibited during these periods.

Diagram of the study design

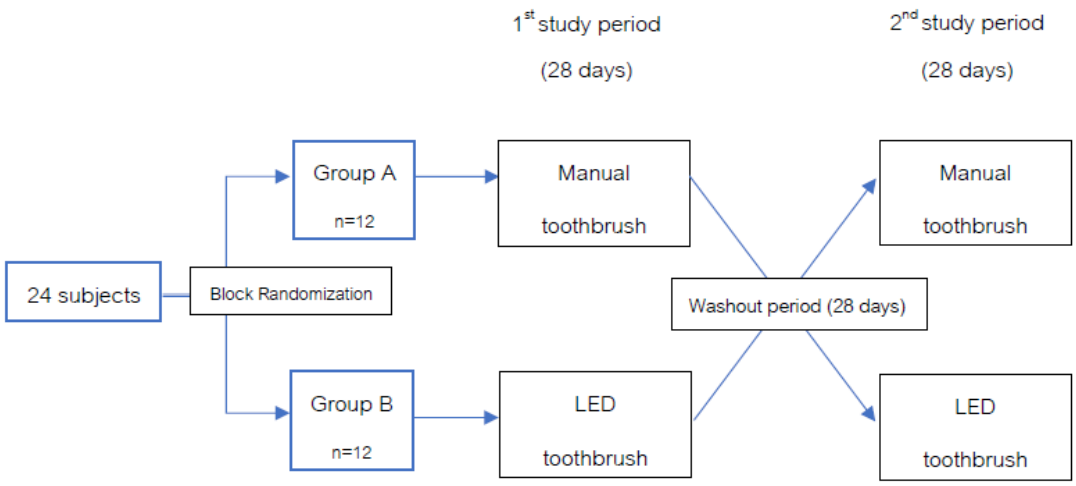

Figure 3 The cross-over study design flow chart

2. The in vitro part (modified from the study of Chebath-Taub et al (14).)

Our study demonstrated the effect of LED toothbrushes on newly formed *S. mutans* biofilms *in vitro* by these following methods.

Bacterial strains and growth condition

Streptococcus 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 600nm (OD600nm) was evaluated and adjusted until value 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 (OD600nm≈ 0.4-0.6) which would be used for the biofilm formation.

Formation of biofilms

The bacterial cells at 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. The of 3 mL of suspension which contains $3x10^8$ bacterial cells were added into each plate, and were incubated at 37°C with 5% CO_2 for 36 hours.

Exposure of the LED toothbrush to biofilm

After incubation, the supernatant fluid above the biofilm was removed. The biofilm samples were divided into 5 groups depending on the LED exposure time, 2 biofilm plates in each group. In the control group, there was no LED exposure. The other four experimental groups were exposed to the LED toothbrush for 15, 30, 60, and 120 seconds. The LED toothbrush was switched on and held 2 mm above the biofilm.

The remaining attached bacteria were scraped off and put into 100 μl of sterile PBS. The bacteria suspensions were then sonicated and serially diluted (10^{-1} to 10^{-8}). The 100 μl of each concentration were dropped onto BHI agar in duplicate, and then were incubated for 36 hours at 37°C with 5% CO_2. The highest concentration that the colonies can be counted separately in the range of 30-300 colonies were used to calculated the number of bacteria. The percentage of bacterial survival was calculated relative to the control.

The whole experiments were repeated 3 times (total N=6/group)

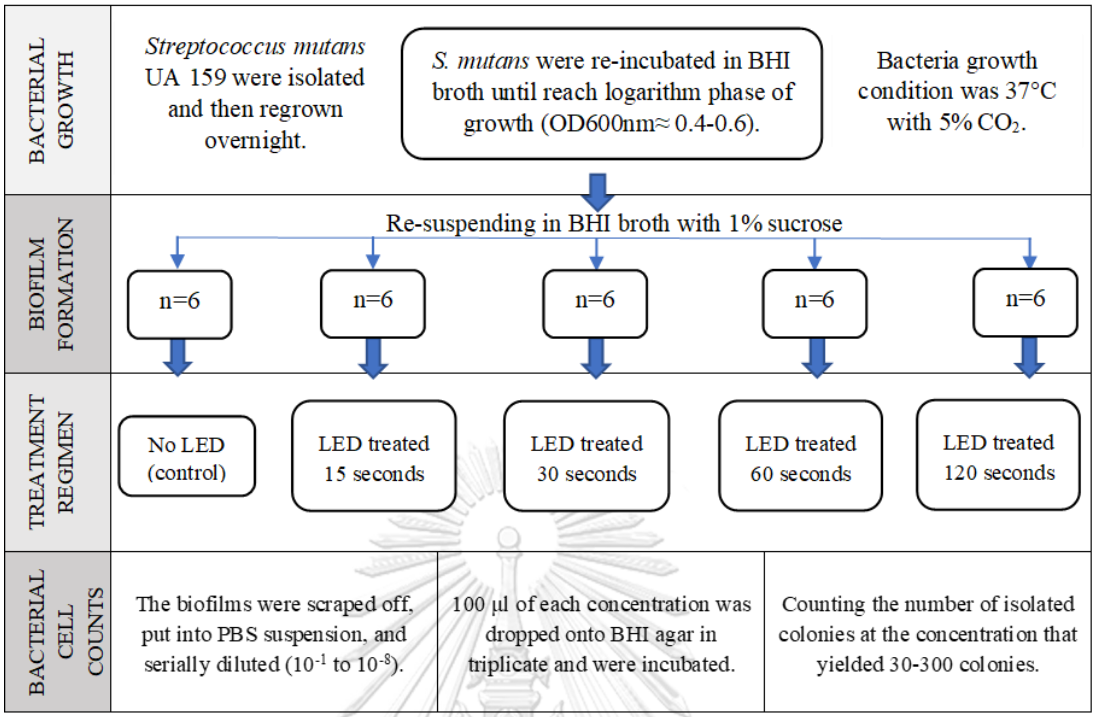

Figure 4 Flow chart of the in vitro study design

Outcome measurement

The plaque index and gingival index were assessed at baseline and after 28 days of each intervention.

Examiner calibration

Two trained examiners were calibrated for the evaluation of plaque index and gingival index by assessing both indices on 3 patients with fixed orthodontic appliances. The reliability of the intra-examiner and inter-examiner were evaluated by Kappa statistics. For the plaque index score, the Kappa of 0.861 and 0.887 were obtained for both examiners and a Kappa of 0.874 was obtained from inter examination agreement. For the gingival index score, the Kappa of 0.852 and 0.786 were obtained from both examiners and the Kappa of 0.792 was obtained from inter-examiner agreement.

Plaque index (PI)

On the non-bracket sides, Loe-Silness plaque index score(63) was taken. On the bracket sides, the surfaces were divided into 4 zones around the brackets: mesial(M), distal(D), gingival(G), and incisal(I). Each zone was evaluated following Loe-Silness score(64)(Figure 5). The assessment was performed on six teeth according to

Ramfjord's representative teeth(65): maxillary right first molar, maxillary left central incisor, maxillary left first premolar, mandibular left first molar, mandibular right central incisor, and mandibular right first premolar. In case of the first molar is absent or banded, the second molar can substitute. Likewise, the second premolar can replace the missing first premolar.

Score	Criteria
0	No plaque
1	A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen *in situ* only after application of disclosing solution or by using the probe on the tooth surface.
2	Moderate accumulation of soft deposits within the gingival pocket, or on the tooth and gingival margin which can be seen with the naked eye.
3	Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin

Table 2 The Plaque index system

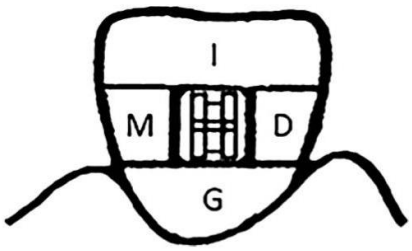

Figure 5 Plaque index bracket.

The modification of Loe-Silness index proposed by Williams. Plaque score of the tooth is divided into mesial (M), distal (D), gingival (G), and incisal (I) parts around the bracket. (Figure from SARUTTICHART, Thayika, et al. Effectiveness of a motionless ultrasonic toothbrush in reducing plaque and gingival inflammation in patients with fixed orthodontic appliances. *The Angle Orthodontist*, 2017, 87.2: 279-285.)(66)

Gingival index (GI)

Degree of gingival inflammation was rated referring to Loe-Silness gingival index score(67).

Score	Criteria
0	Absence of inflammation
1	Mild inflammation- slight change in color and little change in texture
2	Moderate inflammation- moderate glazing, redness, edema, and hypertrophy Bleeding on pressure
3	Severe inflammation- marked redness and hypertrophy Tendency to spontaneous bleeding Ulceration

Table 3 Criteria for the Gingival Index Score

Data collection and analysis

Data was collected and analyzed by using statistical software (IBM SPSS statistics 22, SPSS, Chicago, IL, USA).

In the clinical section, Shapiro-Wilk test was used to quality the normality of the data. Due to the limitation of the study, only 15 patients finished the first period. As a result, we used the available data to analyze as parallel-group. The difference between the value at baseline and after 28 days (within-group) was measured by Wilcoxon Signed Rank test whereas the difference of changes in value between the manual group and the LED group was performed by Mann-Whitney U test.

In the *in vitro* part, Kruskal-Wallis test was applied to test the difference of percentage of bacteria viability among groups.

Statistically significant differences were set at P-value < 0.05.

Expected benefit of this study

The result of randomized controlled trial with a parallel-group design which assess the effect of LED toothbrush would be beneficial to consider the use of the LED toothbrush for oral care in patients with fixed orthodontic patients.

Limitation

The result of this study can be applied only in fixed orthodontic patients.

CHAPTER 4 RESULT

The clinical part

A total of 22 subjects (5 males and 17 females) with ages ranged from 14 to 54 years (mean 26.91±SD 9.86) were enrolled in this study. Not all but 15 participants completed the assigned study period. During the experiment, one female from group B (LED group) had dropped out due to her parents' awareness of LED radiation. Finally, the number of participants included in data analysis was 15, 8 from group A (manual group) and the other 7 from group B (LED group).

Plaque index score

The presence of visible dental plaque was assessed at baseline and 28 days after brushing with the assigned toothbrush. Table 4 showed the mean PI scores (Pre_PI and Post_PI) of the bracket and non-bracket sides. In the manual group, there was no statistical difference between mean PI scores before and after the brushing period in both bracket and non-bracket sides (P=0.889 and P=0.228 respectively). Likewise, in the LED group, there was no statistical difference between mean PI scores before and after the brushing period in both bracket and non-bracket sides (P=0.128 and P=0.075 respectively). When comparing between groups, the changes of the mean PI score were not significantly different in both bracket and non-bracket sides (P=0.203 and P=0.412 respectively). However, when the different tooth surface area of the bracket site was analyzed separately, a significant reduction of the mean PI score was shown in the gingival portion in the LED group (P=0.048) whereas there was no significant difference

in the other surface area (Table5). There was also no significant difference in mean PI in the manual group at any surfaces.

Side	Brushing method	N	Pre_PI[a]	Post_PI[b]	*P-value*[c]	Post-Pre[d]	*P-value*[e]
Bracket	Manual	8	1.00 (0.22)	0.96 (0.37)	0.889	-0.04 (0.42)	0.203
	LED	7	1.19 (0.33)	0.90 (0.49)	0.128	-0.29 (0.45)	
Non-bracket	Manual	8	1.40 (0.24)	1.23 (0.34)	0.228	-0.08 (0.22)	0.412
	LED	7	1.60 (0.37)	1.19 (0.56)	0.075	-0.19 (0.29)	

Table 4 Mean (SD) Plaque Index Score (PI) of bracket and non-bracket sides before and after brushing by each method and their differences.

[a]Pre_PI = mean PI before brushing by each method

[b]Post_PI = mean PI after brushing by each method

[c]Wilcoxon Signed Rank test

[d]Post-Pre = mean difference of PI between after and before brushing by each method

[e]Mann-Whitney U test

	Site	Brushing method	N	Pre_PI[a]	Post_PI[b]	*P value*[c]	Post-Pre[d]	*P value*[e]
Bracket side	Proximal	Manual	8	1.25 (0.28)	1.35 (0.36)	0.326	0.11 (0.40)	0.164
		LED	7	1.50 (0.41)	1.25 (0.55)	0.248	-0.25 (0.56)	
	Gingival	Manual	8	1.25 (0.22)	1.06 (0.49)	0.256	-0.19 (0.44)	0.180
		LED	7	1.41(0.38)	1.00 (0.52)	0.048*	-0.41 (0.42)	
	Incisal	Manual	8	0.50 (0.30)	0.46 (0.46)	0.799	-0.04 (0.52)	0.486
		LED	7	0.67 0.35)	0.45 (0.49)	0.293	-0.21 (0.45)	

Table 5 Mean (SD) Plaque Index Score (PI) of a different tooth surface area around the bracket on the bracket side before and after brushing by each method and their differences.

[a]Pre_PI = mean PI before brushing by each method

[b]Post_PI = mean PI after brushing by each method

[c]Wilcoxon Signed Rank test

[d]Post-Pre = mean difference of PI between after and before brushing by each method

[e]Mann-Whitney U test

*Statistically significant difference (P<0.05)

Gingival index score

There was no significant difference detected in the comparisons of gingival index (GI) scores before and after each brushing method or the changes in GI scores between the two groups (Table 6).

Side	Brushing method	N	Pre_GI[a]	Post_GI[b]	*P-value*[c]	Post-Pre[d]	*P-value*[e]
Bracket	Manual	8	1.34 (0.20)	1.25 (0.20)	0.285	-0.08 (0.22)	0.553
	LED	7	1.33 (0.27)	1.14 (0.18)	0.121	-0.19 (0.29)	
Non-bracket	Manual	8	1.40 (0.24)	1.29 (0.21)	0.246	-0.11 (0.28)	0.521
	LED	7	1.55 (0.27)	1.31 (0.24)	0.111	-0.24 (0.31)	

Table 6 Mean (SD) Gingival Index Score (GI) of bracket and non-bracket side before and after brushing by each method and their differences.

[a]Pre_GI = mean GI before brushing by each method

[b]Post_GI = mean GI after brushing by each method

[c]Wilcoxon Signed Rank test

[d]Post-Pre = mean difference of GI between after and before brushing by each method

[e]Mann-Whitney U test

The *in vitro* part

The percentage of bacterial survival was calculated relative to the control. Table 7 showed the percentage of bacterial viability were significantly different among groups(P=0.006). After, post hoc comparisons were carried out by Mann-Whitney U test with Bonferroni correction of the significance level of multiple pairwise comparisons ($p < 0.05$), the data indicated that when comparing to the negative control group (no LED), the group with 15, 30, 60 and 120 seconds exposure had significantly lower percentage of bacterial viability than the control group. (Figure 6)

		% Bacteria viability
Intervention	n	Mean (95% CI)
No LED exposure	6	99.98 (62.31, 137.63)
LED 15 seconds	6	25.52 (-1.61, 52.65)
LED 30 seconds	6	17.37 (-5.43, 40.18)
LED 60 seconds	6	20.02 (-8.77, 48.81)
LED 120 seconds	6	11.28 (1.23, 21.34)
P value[b]		0.006

Table 7 Percentage of bacterial viability [a]

[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

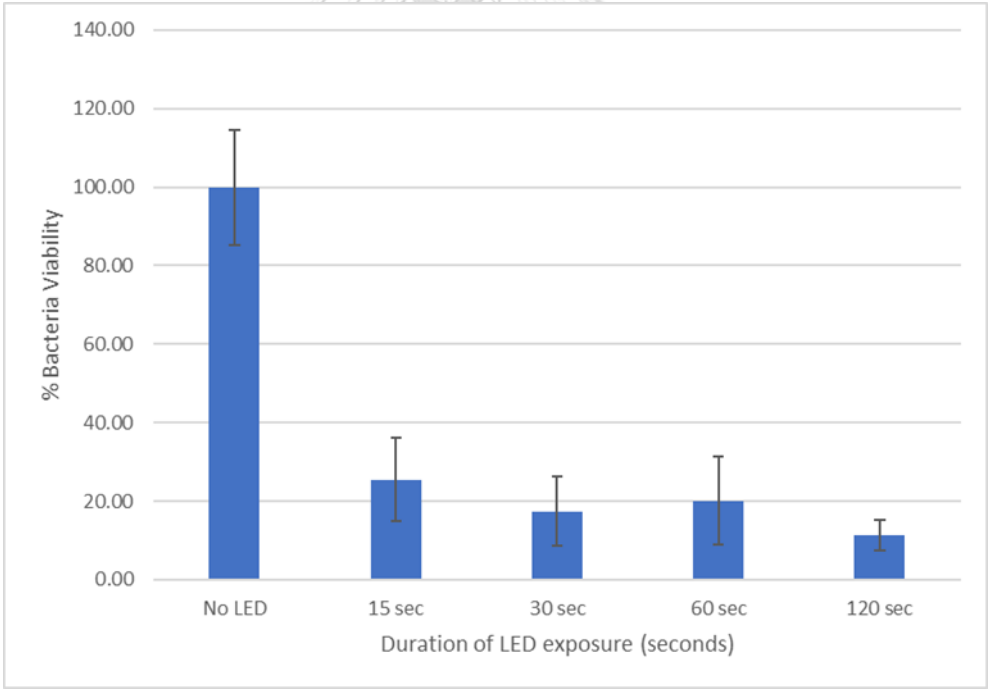

Figure 6 The comparison of percentage of bacterial viability

CHAPTER 5 DISCUSSION

This study was analyzed as a parallel-group randomized controlled trial with a 28-day brushing period to compare the effectiveness of the LED toothbrush to the manual toothbrush in terms of reducing dental plaque and gingival inflammation. The randomized design minimizes the biased error for the specific intervention. However, there are concerns regarding the use of parallel-group design because it includes within-subject confounding factors such as age, gender, and personal oral hygiene. We recruited the patients who undergoing fixed orthodontic appliances for more than 1 month because it allowed time for the patients to adapt their brushing skills with the fixed orthodontic appliance, so the outcomes were not influenced by the time-dependent acquisition of brushing skills. Also, we recruited the participants who had less than 1 mm of the remaining crowding. The reason is that the difficulties of brushing by tooth malalignment would be fairly eliminated for all participants. The patients performed their assigned brushing methods at home to illustrate the normal condition. The accuracy of the brushing method and compliance were confirmed by questionnaires.

Our clinical results suggested that neither manual nor LED toothbrushes significantly reduced dental plaque and gingivitis (within-group comparisons). Between-group comparisons showed no significant difference in all indices. However, the LED toothbrush significantly reduced dental plaque at the gingival portion on the bracket side. Although there was no statistical significance, most results indicated the tendency of dental plaque and gingivitis reduction as we can see from the negative mean difference between after and before brushing by each method. Due to the limitation of time and the pandemic of COVID-19, we could not collect the data as we had planned. Comparing to the available data, a study by Genina et al found that the blue LED toothbrushes with 412 nm wavelength significantly reduce dental plaque, gingival bleeding, and inflammation more than the manual toothbrushes after using for one month(18). Resemble our study they used parallel-group randomized study design but

the subjects were 30 persons each group and tested in non-orthodontic patients. Another similar study design by Kwon et al(61) presented that there was no significant difference in plaque index at 3 weeks and 6 weeks while 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. This study used the advanced version called Electric 3-color LED toothbrush which is composed of 2 blue lights, 1 red light, and the other 1 white light. Again, this study was done in non-orthodontic patients. Overall, the LED toothbrush is likely to improve gingival health, but there was still no other study in orthodontic patients to conclude these issues, so it is better to collect more data.

The *in vitro* results revealed that the LED light from the LED toothbrush could significantly reduce the *S.mutans* viability in biofilm after 15 seconds exposure. The duration of 120 seconds had the lowest percentage of bacteria viability.

The concept of the bactericidal effect of light is that the bacteria contains photosensitizer, the agent that can absorb the light(46). 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(47). Blue light has been proved to have the bactericidal effect(17). The activity of blue light against *S.mutans* has been shown in previous *in vitro* studies(14, 17, 68). Chebath-Taub et al reported that blue light could reduce *S. mutans* biofilm re-formation(14). Relating to our study, our result supported that the visible blue light from the LED toothbrush could reduce the viability of *S.mutans* in biofilm. However, the light in our study had to pass through the silicon bristles which were 9 mm long. Thus, it is important to test whether the LED light in the toothbrush could still have an antibacterial effect. The results suggested that the light from the LED toothbrushes can significantly reduce *S. mutans* viability in biofilm after a 120-second exposure.

The exposure time is one of the most important factors for the bactericidal effect of the LED toothbrush. Previous *in vitro* studies(14, 17, 68) set the exposure duration from 1 to 10 minutes. However, 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 15 seconds. However, the LED toothbrush in our study was applied to the biofilm formed in 35x10 mm culture plate, which the size is similar to 2-3 teeth. According to the recommendation of the American Dental Association, toothbrushing should be performed for 2 minutes, twice a day(43). Thus, if an LED toothbrush was applied for 2 minutes, it may help to reduce bacterial viability in the oral cavity.

Overall, our clinical and *in vitro* study added the evidence of the effectiveness of the LED toothbrush. However, further clinical studies with larger participants would be beneficial for more solid evidence especially in fixed orthodontic patients.

Conclusion

LED toothbrush was not more effective in reducing dental plaque and gingival inflammation than the manual toothbrush in fixed orthodontic patients. 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 15 seconds.

APPENDICE

Appendix A

A.1 The individual mean Plaque Index Score (PI) of bracket and non-bracket sides before and after brushing by each method.

Patient No.	Bracket		Non-bracket	
	Pre	Post	Pre	Post
A01	0.89	1.14	1.67	1.50
A02	0.94	0.64	1.50	0.67
A03	1.06	1.39	1.33	1.50
A04	0.78	0.75	1.50	1.33
A05	1.08	0.95	1.17	0.83
A06	1.28	1.50	1.67	1.50
A07	0.67	0.92	1.00	1.50
A08	1.30	0.39	1.33	1.00
B01	1.00	0.95	1.83	1.17
B02	0.94	0.61	1.33	1.00
B03	0.89	0.81	1.67	1.83
B04	1.11	0.72	1.67	1.50
B05	1.33	1.78	1.50	1.67
B06	1.86	1.22	2.17	1.00
B07	1.20	0.22	1.00	0.17

A.2 The individual mean Plaque Index Score (PI) of a different tooth surface area around the bracket on the bracket side before and after brushing by each method.

Patient No.	Proximal		Gingival		Incisal	
	Pre	Post	Pre	Post	Pre	Post
A01	1.17	1.42	1.17	1.00	0.33	1.00
A02	1.33	1.25	1.17	0.67	0.33	0.00
A03	1.17	1.67	1.50	2.00	0.50	0.50
A04	0.83	1.08	1.33	1.17	0.17	0.00
A05	1.25	1.17	1.17	1.00	0.83	0.67
A06	1.67	2.00	1.50	1.33	0.67	1.17
A07	1.00	1.42	0.83	1.00	0.17	0.33
A08	1.58	0.83	1.33	0.33	1.00	0.00
B01	1.17	1.17	1.33	1.00	0.50	0.67
B02	1.33	1.00	1.00	0.67	0.50	0.17
B03	1.00	1.08	1.50	1.17	0.17	0.17
B04	1.67	1.33	1.17	0.83	0.50	0.00
B05	1.67	2.33	1.50	1.83	0.83	1.17
B06	2.25	1.33	2.17	1.33	1.17	1.00
B07	1.42	0.50	1.17	0.17	1.00	0.00

A.3 The individual mean Gingival Index Score (GI) of bracket and non-bracket sides before and after brushing by each method.

Patient No.	Bracket		Non-bracket	
	Pre	Post	Pre	Post
A01	1.50	1.17	1.67	1.50
A02	1.17	1.17	1.50	1.00
A03	1.67	1.67	1.33	1.50
A04	1.17	1.17	1.50	1.50
A05	1.17	1.17	1.17	1.17
A06	1.33	1.33	1.67	1.33
A07	1.17	1.33	1.00	1.33
A08	1.50	1.00	1.33	1.00
B01	1.33	1.00	1.83	1.17
B02	1.17	1.00	1.33	1.17
B03	1.50	1.17	1.67	1.67
B04	1.17	1.00	1.67	1.50
B05	1.33	1.50	1.33	1.50
B06	1.83	1.17	1.83	1.17
B07	1.00	1.17	1.17	1.00

A.4 The geometric mean of the numbers of *S.mutans* and the calculation to percentage of bacterial viability.

Experiment 1	CFU/ml ($x10^9$)	Mean	%viability	Mean % viability
Control 1	8.48		51.08	
Control 2	24.71	16.60	148.86	99.97
LED 120sec_1	3.17		19.10	
LED 120sec_2	4.15	3.66	25.00	22.05
LED 60sec_1	12.49		75.24	
LED 60sec_2	1.78	7.14	10.72	42.98
LED 30sec_1	0.87		5.24	
LED 30sec_2	9.77	5.32	58.86	32.05
LED 15sec_1	12.85		77.41	
LED 15sec_2	2.34	7.60	14.10	45.75

Experiment 2	CFU/ml ($x10^9$)	Mean	%viability	Mean % viability
Control 1	8.79		80.20	
Control 2	13.12	10.96	119.71	99.95
LED 120sec_1	0.06		0.55	
LED 120sec_2	0.23	0.15	2.10	1.32
LED 60sec_1	0.53		4.84	
LED 60sec_2	0.46	0.50	4.20	4.52
LED 30sec_1	0.46		4.20	
LED 30sec_2	0.38	0.42	3.47	3.83
LED 15sec_1	2.01		18.34	
LED 15sec_2	1.13	1.57	10.31	14.32

Experiment 3	CFU/ml ($x10^9$)	Mean	%viability	Mean % viability
Control 1	2.31	1.91	120.94	100.00
Control 2	1.51		79.06	
LED 120sec_1	0.16	0.20	8.38	10.47
LED 120sec_2	0.24		12.57	
LED 60sec_1	0.16	0.24	8.38	12.57
LED 60sec_2	0.32		16.75	
LED 30sec_1	0.46	0.31	24.08	16.23
LED 30sec_2	0.16		8.38	
LED 15sec_1	0.2	0.32	10.47	16.49
LED 15sec_2	0.43		22.51	

Appendix B

แบบประเมินความร่วมมือระหว่างเข้าร่วมโครงการ

1. เมื่อเข้าร่วมโครงการท่านได้ใช้แปรงสีฟันตามที่ได้รับมอบหมายทุกครั้งหรือไม่

☐ ใช้ทุกครั้ง

☐ ใช้เป็นส่วนมาก

☐ ใช้เป็นบางครั้ง

☐ ไม่ได้ใช้เลย

2. ท่านใช้ไหมขัดฟันและ/หรือแปรงซอกฟันหรือไม่

☐ ใช้ทุกวัน

☐ ใช้เป็นส่วนมาก

☐ ใช้เป็นบางครั้ง

☐ ไม่เลย

2. ท่านแปรงฟันวันละกี่ครั้ง

☐ 1 ครั้ง

☐ 2 ครั้ง

☐ มากกว่า 2 ครั้ง

3. ท่านใช้ระยะเวลาในการแปรงฟัน

☐ ประมาณ 30 วินาที

☐ ประมาณ 1 นาที

☐ ประมาณ 2 นาที

☐ มากกว่า 2 นาที

4. ท่านแปรงฟันด้วยวิธีการตามที่ทันตแพทย์แนะนำหรือไม่ (วางแปรง 45 องศาระหว่างขอบเหงือกและคอฟัน ขยับสั้นๆในแนวราบ 10 ครั้งต่อตำแหน่ง และทำความสะอาดด้านบนและด้านล่างของเครื่องมือจัดฟัน)

☐ ทุกครั้ง

☐ เป็นส่วนมาก

☐ บางครั้ง

☐ ไม่เลย

5. หลังจากแปรงฟันด้วยแปรงสีฟันที่ได้รับมอบหมาย ท่านรู้สึกว่าฟันของท่านมีความสะอาดในระดับใด

☐ สะอาดมาก

☐ สะอาดปานกลาง

☐ สะอาดน้อย

6. โดยภาพรวมท่านมีความพึงพอใจต่อแปรงสีฟันที่ได้รับมอบหมายในช่วง 1 เดือนที่ผ่านมาในระดับใด

☐ มาก

☐ ปานกลาง

☐ น้อย

7. ท่านใช้น้ำยาบ้วนปากในระหว่างเข้าร่วมโครงการหรือไม่

☐ ใช่

☐ ไม่ใช่

8. ท่านมีการใช้ยาปฏิชีวนะในระหว่างเข้าร่วมโครงการหรือไม่

☐ ใช่

☐ ไม่ใช่

9. ท่านได้รับการขูดหินปูนและ/หรือเกลารากฟัน ในระหว่างเข้าร่วมโครงการหรือไม่

☐ ใช่

☐ ไม่ใช่

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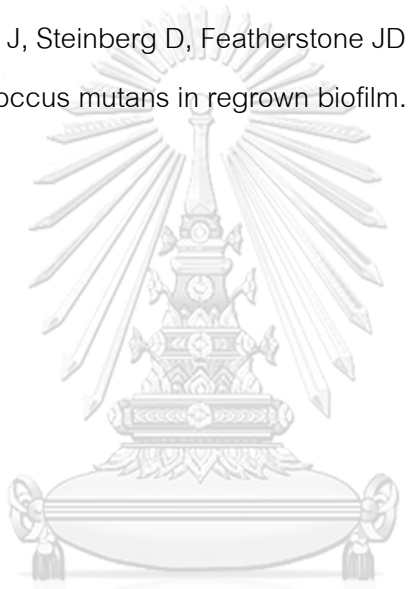

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