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Abstract. The efficacy of blue light-emitting toothbrushes (B-LETBs) (405 to 420 nm, power density 2 mW/cm²) for reduction of dental plaques and gingival inflammation has been evaluated. Microbiological study has shown the multifactor therapeutic action of the B-LETBs on oral pathological microflora: in addition to partial mechanical removal of bacteria, photodynamic action suppresses them up to 97.5%. In the pilot clinical studies, subjects with mild to moderate gingivitis have been randomly divided into two groups: a treatment group that used the B-LETBs and a control group that used standard toothbrushes. Indices of plaque, gingival bleeding, and inflammation have been evaluated. A significant improvement of all dental indices in comparison with the baseline (by 59%, 66%, and 82% for plaque, gingival bleeding, and inflammation, respectively) has been found. The treatment group has demonstrated up to 50% improvement relative to the control group. We have proposed the B-LETBs to serve for prevention of gingivitis or as an alternative to conventional antibiotic treatment of this disease due to their effectiveness and the absence of drug side effects and bacterial resistance. © 2015 Society of Photo-Optical Instrumentation Engineers (SPIE) [DOI: [10.1117/1.JBO.20.12.128004](https://doi.org/10.1117/1.JBO.20.12.128004)]

Keywords: phototherapy; tooth brushing; gingivitis; dental plaque; dental devices; home care.

Paper 150640R received Sep. 29, 2015; accepted for publication Nov. 19, 2015; published online Dec. 29, 2015.

1 Introduction

The 2014 Nobel Prize in physics was awarded to Isamu Akasaki and Hiroshi Amano (Japan) and Shuji Nakamura (United States) “for the invention of efficient blue light-emitting diodes which has enabled bright and energy-saving white light sources.”¹ The development of light-emitting diodes (LEDs) has advanced them to a stage where their use in phototherapy is possible. LEDs offer several advantages for clinical and laboratory use. The most important one is a wide choice of emission wavelengths from ultraviolet A to near infrared with a narrow bandwidth of 5 to 10 nm. In addition, LEDs are long-life inexpensive light sources with uniquely high efficiency.² Moreover, they can be arranged in different geometric combinations to compensate for difficult anatomic areas^{3–5} that can be useful and effective to use in dentistry for the treatment and prophylaxis of gingivitis.

Gingivitis is an inflammation of the gums caused by plaque and bacteria accumulation. According to the data of WHO, the majority of children have signs of gingivitis and a severe periodontal disease, which may result in tooth loss. This is also found in 15% to 20% of middle-aged adults.⁶ Dental plaque consists of a mixed bacterial flora, sometimes with desquamated epithelial cells and migrated polymorphonuclear leukocytes.^{7,8} Bacteria in plaque around the teeth release enzymes (collagenases) that can damage and erode the gum tissues. The infected gums swell, bleed easily, recede, and result in loosening of the teeth.

A treatment for dental active therapy usually includes debridement of tooth surfaces to remove supra- and subgingival plaque and dental calculus, and application of antimicrobial and antiplaque agents or devices.⁹ For instance, a key procedure for treatment of periodontal disease is plaque reduction or elimination by mechanical/chemical means.^{8–12} For many cases, mechanical removal of plaque and plaque-derived products^{10,11} leads to disease resolution. However, many clinical trials indicate that self-administered plaque control alone, without periodic professional reinforcement, is inconsistent in providing a long-term inhibition of gingivitis.^{8,12,13}

Antibacterial therapy is also considered as a very important component of complex treatment.^{8,9} Medicamental antibacterial therapy currently available for periodontic disease is sufficiently effective and adequate. However, there are many patients who cannot take the treatment. This is caused by the following factors: high frequency of allergic reactions, contra-indications and side effects of the prescription of drugs, adverse effect on oral microbiocenosis, and others. In addition, bacteria growing in biofilms exhibits resistance mechanisms.¹⁴ Therefore, recently, nonmedicamental methods of the treatment are being intensively developed.^{13,15–18}

Some periodontal pathogens are chromogenic bacteria, which are accumulating porphyrins.⁸ The amounts of endogenous porphyrin in oral black-pigmented bacteria from dental plaque samples have been evaluated in Ref. 19 as 267 ng/mg (*Prevotella*

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intermedia), 47 ng/mg (*Prevotella nigrescens*), 41 ng/mg (*Prevotella melaninogenica*), and 2.2 ng/mg (*Porphyromonas gingivalis*). Lipovsky et al.^{20,21} and Mohl et al.²² have reported the presence of endogenous porphyrins in *Staphylococcus aureus*. The UV-visible absorption spectrum of porphyrins exhibits an intense peak at around 405 to 415 nm.²³ As a result, excitation of porphyrin molecules by blue light causes energy transfer from its triplet state to molecular oxygen to produce the excited-state singlet oxygen, which can then oxidize and destroy various biological molecules such as lipids, proteins, and nucleic acids.²⁴ Inactivation of oral bacteria by visible light has been reported elsewhere.^{13,15,19,25–29} The killing efficiency of 405-nm LED light for *Propionibacterium acnes* and *Staphylococcus epidermidis* at constant doses of 35, 70, and 144 J/cm² with five different irradiation times from 30 to 240 min has been studied in Ref. 30. In addition, a significant increase in the mitotic rate of normal cells has been determined when illuminated with $\lambda = 410$ nm with a maximum at ~ 6 J/cm².³¹

Designing of the LED light source in a form of toothbrush allows a combination of mechanical/chemical means of plaque elimination with photodynamic antibacterial therapy. We have hypothesized that it can exert a synergic effect on the treatment impact. Thus, the goal of our pilot clinical study is an evaluation of the efficacy of the treatment of gingivitis with low intensive blue light-emitting toothbrushes (B-LETBs) based on photodynamic therapy (PDT) and biostimulation principles of recovery of inflammatory diseases.

2 Materials and Methods

2.1 Light Sources

B-LETBs have been designed and manufactured by the Laser Center of St. Petersburg State University of Information Technologies, Mechanics, and Optics (St. Petersburg, Russia) in cooperation with Palomar Medical Technologies Inc. The tested B-LETBs are prototypes of a standard mechanical toothbrush with a blue LED.^{32,33} The central wavelength of the B-LETB is 412 nm with a spectral width of about 25 nm. The photographs of the B-LETB and toothbrush head are presented in Figs. 1(a)–1(c). The scheme of the toothbrush head is shown in Fig. 1. An LED-matrix is placed on a Cu-plate which is cooled by water from a heat exchanger in the brush handle. The B-LETB contains 10 light transparent bristle bundles. LED emits light through and between the bristles. The Cu-plate surface surrounding the LED-matrix is coated with a reflecting thin layer of silver with a reflectance of 85% to 86% at the emission wavelength range that returns light from the tooth surface (a so-called photon-recycling mirror).

The B-LETB initial mean power density measured with a standard power meter (IMO-2N, Etalon, Russia) for all brushes (60 pcs.) used in the clinical study was 2.3 ± 0.2 mW/cm². With increasing time of the LETB use, we found some degradation of the bristle hardness as well as decay of the light power density that was, however, not less than 2 mW/cm² at the end of study for each device.

2.2 Microbiological Study

There is a wide spectrum of bacteria found in the pockets between the teeth and gums.³⁴ The prevalence rate of *Staphylococcus* species is found to be 73% in dental plaque and

Fig. 1 Photographs of a blue light-emitting toothbrush: (a) general view, toothbrush head in (b) OFF and (c) ON, and (d) regimes and scheme of toothbrush head.

84% in saliva.³⁵ In this work, a microbiological test was done with the aim of comparison of mechanical and photochemical cleaning facilities of the low-intensity B-LETB. Samples of sub-gingival plaques were obtained clinically by a standard dental applicator. Bacterial suspension was prepared as was described in our earlier paper.¹³ The surfaces of three cover glasses were covered by the suspension (0.05 ml of the suspension on each glass). The glasses with suspension were put into a thermostat with a temperature of 37°C for 30 min. The first sample served as a control, the second was treated mechanically with six circular motions and chemically with commercial toothbrush and toothpaste, and the third one was treated by the B-LETB (six circular motions) (PDT and mechanically) and a toothpaste (chemically).

After the treatment, the glasses were put into a bath with 2 ml of isotonic buffer solution. Then 10-fold consecutive dilutions (from 10^{-2} to 10^{-7}) of all samples were prepared. Test tubes with these solutions were put into the thermostat with a temperature of 37°C for 24 h. Bacterial growth suppression efficiency was estimated by counting the cell colony-forming units (CFU).

2.3 Subject Selection

Subject selection for the clinical study was done according to the American Dental Association Acceptance Program Guidelines.³⁴ The clinical trial was carried out in the Dental Clinic of Saratov State Medical University (Russia). Experiments were performed in accordance with the ethical standards of the Declaration of Helsinki.³⁵ Clinical protocols have been approved by the Ethic Committee of Saratov State Medical

University. The volunteers gave their written informed consent prior to participation in the study.

Before the clinical study, an investigator examined the subject's oral cavity to confirm the eligibility for the study. Selection criteria were the following: the subject had gingivitis, the subject had read and signed a written informed consent form, and the subject was a healthy volunteer and was free of any systemic diseases other than gingivitis that would interfere with the light exposure results or increase the risk of adverse reactions. Exclusion criteria were the following: the subject was on systemic antibiotics within the treatment period, the subject had severe concurrent diseases, tobacco smoking, and the subject was not able to comply with the study requirements. A total of 60 subjects from 17- to 39-years old of both genders with mild (73% of volunteers) to moderate (27% of volunteers) gingivitis were enrolled for testing of the B-LETBs. Assessment of disease severity was carried out with a Shiller–Pisarev probe: gingival mucosa was anointed with Shiller solution (1 g of crystalline iodide, 2 g of potassium iodide, and 40 ml of distilled water). Coloration varied, depending on the intensity of the inflammation. Gingivitis index PMA (P, papilla interdentalis; M, gingiva marginalis; and A, gingiva alveolaris)³⁶ characterizing inflammatory state of gingiva by color was used: for a healthy gum, mucosa was straw-yellow colored, at chronic inflammation due to glycogen store it was brown colored. Inflammation of papilla, marginal gingival, and alveolar gingiva was evaluated as 1, 2, and 3, respectively. All highest grades for each tooth were summarized. PMA was evaluated with the following equation:^{13,36}

$$\text{PMA} = [(\sum \text{Grades}) \times 100\%]/(3 \times \text{number of teeth}). \quad (1)$$

The value of the index up to 30% corresponded to gingivitis of a mild degree, 30% to 60% to gingivitis of a moderate degree, and more than 60% to gingivitis of a severe degree.

The condition of gingival mucosa was evaluated from the following parameters (as “Yes” or “No”): anemic, atrophic, hyperemic, hydropic, bleed at probing, cyanotic, ulcerous, hypertrophied, changed fibrously, and exfoliated from cervix of the tooth. The conditions of teeth and tooth plaques were also evaluated. Based on the investigation, both the diagnosis and severity of the disease were determined by a professional dentist.

2.4 Study Design

Selected subjects were randomly divided into two groups of 30 persons each. Group I (B-LETB treated) included the volunteers (17 females and 13 males), who were treated by the B-LETB, and group II (control) included the volunteers (16 females and 14 males), who used a standard Braun oral-B manual toothbrush (Procter & Gamble). In both groups, the same toothpaste “Blend-a-Med cavity protection mineral action” (Procter & Gamble) was used.

The duration of the treatment study was 4 weeks. The subjects from the first group were instructed how to use the B-LETBs, and all subjects were instructed regarding the right procedure of the tooth brushing.

For both groups, the method of the brushing was similar. The time of the full-mouth brushing was 2 min. It had to be carried out two times per day: in the morning and in the evening following meals.

The study of the toothbrushes was designed as a single-blind [the examiner did not know which group (experimental or control) the patient belonged to], randomized, prospective clinical study.

2.5 Method of Evaluation

Three visits were made by each volunteer to score the state of their oral cavity: baseline (before B-LETB use), 2 week period, and after a month of use. Effects of the treatment were evaluated using the comparison of the patient's scores from each follow-up visit to the baseline scores, and the average scores of the treatment and the control groups.³⁴

Clinical evaluation of gingivitis severity was visually assessed using an original augmented approximate hygiene index (AHI)¹³ and standard indices complying with the American Dental Association Acceptance Program Guidelines:^{34,37} gingival index of Löe–Silness^{ADA} (LSI);^{38,39} gingival bleeding index^{ADA} (GBI);⁴⁰ gingivitis index PMA;³⁶ and Turesky modification of the Quigley–Hein plaque index^{ADA} (TI).⁴¹ All indices were measured by a single examiner to exclude examiner bias.

To evaluate TI, a score of 0 (no plaque) to 5 (plaque covering two-thirds or more of the crown of the tooth) was assigned to each facial and lingual nonrestored surface of all the teeth except the third molars.⁴¹

Augmented AHI¹³ was based on the method of evaluation of TI, but instead of using the investigation of the facial and lingual surfaces, medial and distal tooth surfaces were taken into account. TI and AHI for the entire mouth were determined by dividing the total cumulative score by the number of surfaces examined.^{13,41}

The measurement of the state of oral hygiene LSI was based on recording both soft debris and mineralized deposits on a tooth within a gingival *sulcus*.^{38,39} Each of the four areas of a tooth (buccal, lingual, medial, and distal) was given a score from 0 (no plaque) to 3 (abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin). The scores from the four areas of the tooth were added and divided by four in order to give the plaque index for the tooth.

Evaluation of GBI was carried out on both vestibular and oral surfaces of a tooth by a special dulled probe.^{13,40} To evaluate the gingival bleeding degree, a score from 0 (gingival bleeding was absent) to 3 (gingival bleeding appeared during food intake or tooth brushing) was assigned. Both indices LSI and GBI for the patient were obtained by summing the indices for six teeth and dividing by six.

Each index was determined for each individual, and the average index was determined for the group. The improvement of tooth status in the both groups was calculated by dividing the difference between the baseline index value and the current index value by the baseline index value

$$\text{Impr}_1 = [(\text{Baseline} - \text{Current})/\text{Baseline}] \times 100\%, \quad (2)$$

where the current index values were scored on the 15th and the 30th days. The percentage improvement of the tooth state of the patients from the first group (treatment) in comparison with that of the patients from the second group (control) was calculated by dividing the difference between the control and B-LETB treatment scores by the control scores on the 15th and the 30th days

$$\text{Impr}_2 = [(Control - Treatment)/Control] \times 100\%. \quad (3)$$

Brushes were compared using the independent *t*-test (the statistical method for comparing two unrelated groups on the same conditions). At the level of significance $p < 0.05$, differences between average values of the baseline and current indices and between average values of indices for the treatment and control groups were accepted as statistically significant.

3 Results and Discussion

Figure 2 shows the result of different treatments of bacteria from the dental plaque. The first column corresponds to the control sample without any treatment. The second column presents the result of mechanical/chemical treatment. The third column shows a combined effect of mechanical/chemical treatment and blue irradiation with the low-intensity B-LETB on bacteria from the dental plaque. The average number of CFU without treatment has been evaluated as 3.9×10^6 . A significant reduction in the average number of CFU down to 1.96×10^5 (95%) in comparison with the control sample has been observed for the mechanical/chemical treatment. The mechanical/chemical + PDT treatment using the B-LETB has decreased the average number of the colonies down to 9.76×10^4 (97.5%). It can be supposed that with application of the B-LETB by the volunteers, a suppression of the pathological flora that are sensitive to blue light action has been observed.

Thus, the use of the B-LETB has resulted in multifactor therapeutic action on oral pathological microflora: in addition to mechanical removal of the bacteria as in ordinary tooth-brushing procedure, it has shown additional suppression action on microorganisms due to photodynamic action.

Before the experiment, we have evaluated average values of the indices and standard deviations in both groups. The results are presented in Table 1. At the level of significance, p equal or less than 0.05, the difference of initial values of the indices between the treatment and control groups can be considered to be statistically significant. However, as follows from Table 1, the value p for the studied groups is greater than 0.05, therefore, the differences can be accepted as statistically insignificant, i.e., values of the indices before experiment are homogeneous enough.

Figures 3(a)–3(e) present a temporal evolution of the studied indices normalized to their initial values for the both treated and control volunteer groups used the B-LETBs. Three of them (TI,

Table 1 Initial average values of clinical indices and standard deviations.

Index	Treatment group	Control group	p
TI	2.85 ± 0.52	2.78 ± 0.62	>0.5
AHI	3.4 ± 0.85	3.54 ± 0.72	>0.2
LSI	1.24 ± 0.47	1.46 ± 0.91	>0.2
GBI	1.98 ± 0.72	2.15 ± 0.53	>0.2
PMA	0.25 ± 0.1	0.24 ± 0.1	>0.5

AHI, and LSI) characterize the degree of the covering the tooth by plaque.

In Figs. 3(a)–3(c), it is well seen that the indices have decreased for both groups of the subjects; a significant reduction is observed on the 15th day of the treatment. The average improvement of the studied indices relative to the baseline for all subjects calculated by Eq. (2) is shown in Table 2 in the second and the third columns; the second column relates to the improvement of the indices in the treatment group, and the third one in the control group. It is seen that the corresponding values in these columns are close to each other. The fourth column shows the improvement of the indices from the treatment group in relation to the control group calculated by Eq. (3). Statistical analysis has showed that the differences in the indices after the treatment by the B-LETBs and standard toothbrushes are not significant by the 15th day. The second column of Table 3 shows the levels of significance obtained by comparison of these groups. All of them are greater than 0.05, thus differences between the treatment and control groups are statistically insignificant.

For the group treated by the B-LETBs, reduction of plaque size has continued during the month of examination; at the same time, the control group has demonstrated partial recovery of plaque [see Figs. 3(a)–3(c) and Table 2, columns 5 and 6]. Results show that on the 30th day of the treatment, differences between the indices in the treatment and control groups have increased (Table 2, columns 4 and 7). In Table 3, the third column corresponds to the level of significance of differences between the studied groups by the 30th day. All values are less than 0.05, which confirms the good cleaning properties of the B-LETBs.

Bacterial endotoxins, cytotoxins, and other pathogenic substances are released from plaques and diffuse into the adjacent soft tissues where they elicit an inflammatory response that results in tissue disruption and degradation.⁴² Therefore, removal of plaque and plaque-derived products, according to many authors, has been a key procedure for treatment of periodontal disease.^{8–12}

Our results have confirmed that improvement of gums is observed in both studied groups during the first half of the observation period. It can be explained by the “learning effect.” The dentist’s instructions relating to the guidelines of tooth brushing provide better results in plaque removal. The suggestion was made that in the beginning of the trials, the volunteers were brushing their teeth carefully and fulfilled all requirements of the investigator, but by the end of the trials, they reverted to their usual manner of brushing. Therefore, during the subsequent 2 weeks, the increase of the plaque indices in the control

Fig. 2 Average value of *in vitro* measured number of bacterial colonies (CFU) taken from tooth plaque: without treatment (control), after a standard toothbrush and a toothpaste, and the B-LETB with the standard toothpaste.

Fig. 3 Dynamics of some dental indices for evaluation of gingivitis severity: (a) Turesky modification of the Quigley–Hein plaque index^{ADA}, (b) approximate hygiene index, (c) gingival index of Löe–Silness^{ADA}, (d) gingival bleeding index^{ADA}, (e) and gingival index PMA. Shaded and solid columns correspond to the results of tooth brushing by the B-LETB and the standard toothbrush, respectively. Bars show standard deviation.

groups was observed. At the same time, the plaque indices (TI, AHI, and LSI) continued to fall down gradually in the groups with B-LETB testing and have shown a statistically significant difference between plaque removal actions of the B-LETBs and standard brushes. The difference between the two groups in all indices has increased in favor of the B-LETB group between 2 and 4 weeks. We can expect this trend to be more pronounced with an increase of the use of the B-LETB up to several months.

Figure 3(d) shows the kinetics of GBI for the two studied groups; and Table 1 shows the percentage of improvement of the gingival bleeding. Figure 3(e) and Table 2 demonstrate the temporal evolution of gingival inflammation. For these indices, statistically significant differences between the groups have also been observed only after a month (see Table 3).

From the PMA kinetics, it follows that proper tooth brushing decreases the inflammation in both groups of the volunteers. Kinetics of the GBI and LSI correlates with that of the gingival

Table 2 Results of average improvement of the studied indices relative to the baseline for the subjects from different groups and the B-LETB treated group relative to the control group.

Index	15 days			30 days		
	Impr ₁ ^a (%)	Impr ₁ ^b (%)	Impr ₂ (%)	Impr ₁ ^a (%)	Impr ₁ ^b (%)	Impr ₂ (%)
TI	29	25	6	28	18	13
AHI	21	18	5	25	11	18
LSI	58	51	16	59	43	32
GBI	48	37	23	66	35	50
PMA	53	52	5	82	70	48

^aImprovement of the indices for the B-LETB-treated group relative to the baseline.^bImprovement of the indices for the control group relative to the baseline.**Table 3** Level of significance of differences between average values of the clinical indices in the B-LETB-treated group and the control group (level of significance $p < 0.05$ corresponds to a significant difference between the groups).

Index	p	
	15 days	30 days
TI	0.300	0.040
AHI	0.480	0.002
LSI	0.300	0.030
GBI	0.100	0.003
PMA	0.750	0.005

inflammation development; since the bleeding is caused by gingival inflammation, a decrease of the inflammation process leads to a reduction in the GBI and LSI. Our results have shown that the action of blue light in the same time interval gives an additional reduction in these indices, possibly due to photodynamic suppression of bacteria growth and tissue biostimulation effects.³¹

Blue light can be an alternative to a conventional antibiotic treatment due to the absence of drug side effects and bacterial resistance. Blue light is effective for phototherapy since exposure to blue light induces photoexcitation of bacterial porphyrins, singlet oxygen production, and subsequent bacterial destruction.⁴³ The potential use of blue light sources (405, 415, 407 to 420 nm) for phototherapy of lesions caused by growth of pathological bacteria is discussed in the literature.^{2,16–22,25–30,44}

4 Conclusion

The present pilot clinical study has been aimed at the evaluation of the effectiveness of the B-LETBs by the use of standard dental indices characterizing the status of teeth and gingiva. A microbiological study has demonstrated the suppression of pathological microorganisms by B-LETB irradiation (up to 97.5%). In both control and treated groups of volunteers,

improvement of all indices compared to the baseline has been found. It can be explained by an improvement in oral hygiene for both groups due to careful and correct tooth brushing. However, for the B-LETB group, the efficiency of tooth brushing has been higher (25% to 82% in treatment group versus 11% to 70% in control group); differences between the control and treated groups are statistically significant. The study has also shown that the B-LETB-treatment has a great potential to be much more effective for use at a longer time interval. The use of the LETB can significantly simplify the procedure of the treatment of gingivitis and allows carrying out phototherapy by patients themselves at home. Thus, replacing a standard toothbrush with the B-LETB can be a very promising solution for treatment of gingivitis and prevention of periodontitis. Due to low cost of LED technology, we expect such a product to be affordable for most patients from children to the elder population.

Acknowledgments

We are thankful to G.B. Altshuler for useful discussions, A.V. Lepilin for the help in microbiological experiments, and S.V. Eremina for the help in paper translation to English. The research was supported by the Government of the Russian Federation (Grant 14.Z50.31.0004 to support scientific research projects implemented under the supervision of leading scientists).

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