Journal of Innovative Optical Health Sciences
Vol. 4, No. 4 (2011) 437–446

DOI: 10.1142/S1793545811001745

World Scientific
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PHOTOTHERAPY OF GINGIVITIS: PILOT CLINICAL STUDY

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Accepted 18 July 2011

The goal of this work was to evaluate the safety and efficacy of the Red Light Emitted Toothbrush (R-LETB) emitting at wavelength of 663 nm with power density of 3.3 mW/cm^2 in combination with 0.1%-methylene blue (MB) solution for the reduction of plaque and treatment of gingivitis. A microbiological *in vitro* study and a pilot clinical study were conducted. The microbiological study has shown total suppression of pathogenic flora after a 3-min exposure to the dye solution followed by a 20-sec treatment with the R-LETB. For the clinical study, 37 subjects of both sexes with gingivitis were enrolled and randomly assigned to one of two groups. Subjects in the first (treatment) group were instructed to rinse their mouth with MB solution provided for 1 min and then brush the teeth with the R-LETB and standardized toothpaste. The second (control) group used only the toothpaste and a regular toothbrush. Subjects in both groups followed their respective procedures 2 times a day (morning and evening) for 30 days. Indices of plaque, gingival bleeding, and inflammation were evaluated at 14-day and 30-day timepoints. In the both groups, all indices improved in comparison with baseline. However, the treatment group demonstrated more pronounced improvement of the studied indices that was attributed to additional anti-microbial action of red light and MB on gum tissue. Thus, the use of R-LETB with MB appears to have a multifactor therapeutic action on oral pathological microflora: mechanical removal of the bacteria and suppressing action on microorganisms due to photodynamic reaction.

Keywords: Tooth brushing; methylene blue; photodynamic therapy; pathogenic bacteria.

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1. Introduction

Periodontal disease is an acute medical problem. According to WHO statistics, about 95% of adults and 80% of children worldwide suffer from some form of periodontal disease. Severe periodontal disease, which may result in tooth loss, is found in 5%−20% of middle-aged adults.[1] The main cause of the periodontal disease is vital activity of conditionally pathogenic microflora of oral cavity.[2,3] Currently, the main method of both prophylaxis and treatment of catarrhal gingivitis is a rigorous oral hygiene regime with the use of toothbrush and toothpaste.[4,5] However, more effective aids of individual hygiene are desirable.

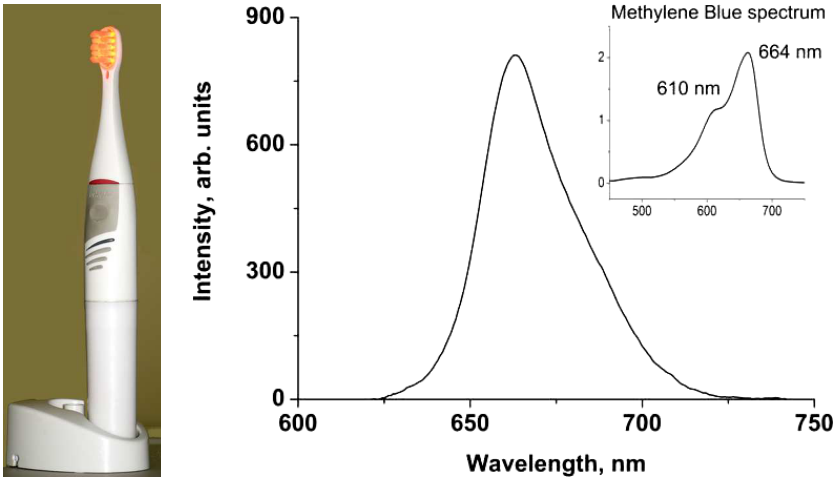
Antibiotic therapy is sometimes used to control periodontal disease.[6,7] However, medicamental therapy is insufficiently effective and not always justified. This is caused by the following factors: high frequency of allergic reactions, contra-indications and side effects to prescription drugs; resistance of microflora to the widely used antibiotics and antiseptics; and adverse effects on benign microflora of oral cavity.[8,9]

Antibacterial effects of light of different wavelengths used to illuminate mucosa have been reported in the literature.[10−13] Violet and blue portions of the spectrum are the most effective visible wavelengths for photoactivation of the major endogenous porphyrins of bacteria but have poor penetration depth into tissue.[14] Light of infrared wavelengths is absorbed by cellular water and may lead to overheating and even fragmentation of cellular structure.[12,13] The adverse effects associated with overheating include thermal damage of root surfaces and production of toxic by-products.[13]

The red light penetrates deeper but is less efficient in photoactivating of endogenous porphyrins. However, in combination with an exogenous sensitizer such as methylene blue (MB) red light may controllably produce free radicals for soft photodynamic therapy,[15−19] including gingival treatments. Antimicrobial efficacy of combined application of He-Ne laser radiation (wavelength 632.8 nm) and 0.1% MB solution to elective anaerobic component of total microflora of periodontal pockets has been reported in Ref. 20.

Absorption bands of MB solution are centered at the wavelengths of 610 and 664 nm (see Fig. 1), therefore MB can be efficiently excited by 663 nm emission of LEDs. This red light penetrates well into tissue, much deeper than shorter visible and UV wavelengths.[15] Red light may also have additional photobiomodulating effect through influencing release of proinflammatory cytokines from macrophages, which stimulate fibroblast proliferation and production of growth factors.[21] Use of MB is beneficial due to its low toxicity,[15] low cost, and availability.

We propose the combined use of low-intensity red LED toothbrush emitting at wavelength of 663 nm and 0.1% MB solution as a novel tool of individual oral hygiene. Brushing of teeth in combination with



(a) (b)

Fig. 1. R-LETB in a charger (a) and the emission spectrum of the R-LETB (b). Insert shows absorption spectrum of MB rinse solution.

phototherapy can provide a multifactor therapeutic action. The goal of this pilot study was to evaluate safety and efficacy of the treatment for reduction of tooth plaque and improvement of gingivitis.

2. Materials and Methods

2.1. *Light-emitting toothbrush*

For microbiological and clinical study, Red Light Emitting Toothbrushes (R-LETBs) with 10 optical bristle bundles and photon-recycling mirror were used. The central wavelength was 663 nm; the spectral width was about 30 nm. The power density of the R-LETBs was 3.3 mW/cm^2. The photograph and the emission spectrum of the R-LETB are presented in Fig. 1. R-LETBs were 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. (Burlington, MA, USA).

2.2. *Microbiological study*

Samples of subgingival plaque have been obtained clinically by standard dental applicator and stored in 1 ml of isotonic solution. About 0.1 ml of obtained substance has been diluted in 0.9 ml of sugar substrate. The 0.05 ml of the suspension has been seeded on Petri dish with sugar substrate and placed into thermostat with temperature 37°C for 24 h. Multisectional plate for irradiation of suspensions of cultures was used (volume of each cell was 0.2 ml). Three bacterial strains were studied: *Micrococcus*, *Stomatococcus,* and *Staphylococcus lugdensis*.

For the study of MB action on the pathogenic subgingival microflora, 0.1 ml of MB has been put into base substrate to obtain final concentrations of the dye at 0.001%, 0.01%, and 0.1%. Time of MB action was 3 min.

To find the appropriate staining and radiation parameters for photodynamic inactivation of pathogenic subgingival microflora using R-LETB irradiation of bacteria photosensitized by MB, three samples were used. The surfaces of three cover glasses have been covered by the bacterial suspension (0.05 ml on each glass). The glasses with suspension have been put into thermostat with temperature 37°C for 30 min. The first sample served as a control, the second one was used for mechanical treatment, and the third one was subjected to both mechanical and photodynamic treatments.

The first glass with dried suspension has been put into bath with 2 ml of isotonic solution. About 0.1 ml of the obtained substance has been then diluted in 0.9 ml of sugar substrate. The second sample has been treated by toothbrush with toothpaste (six circular motions). The glass with suspension has been put into bath with isotonic solution. The third glass with the dried suspension has been treated by 0.1%-MB solution during 3 min. The suspension has been treated by R-LETB and toothpaste (six circular motions). Then the glass with emulsion has been put into bath with isotonic solution.

After the treatments, 10X consecutive dilutions (from 10^{-2} to 10^{-7}) of all samples have been prepared. Test tubes with dilutions have been put into thermostat with temperature 37°C for 24 h.

2.3. *Clinical study*

A total of 37 subjects of both genders with catarrhal gingivitis were randomly divided into 2 groups. The first (treatment) group included 7 volunteers (3 females and 4 males) who were treated with R-LETB and MB; and the remaining 30 subjects (16 females and 14 males) formed the second (control) group, which used standard toothbrushes "Braun Oral-B" (Procter & Gamble, Cincinnati, OH, USA). The average age of volunteers was 21 years. The severity of gingivitis was moderate (35% of volunteers) or light (65%). People were excluded if they had diseases of internal organs or orthodontic pathology. In total, 320 persons were screened for the study; frequency of catarrhal gingivitis was found to be about 9%. All subjects gave their informed consent for participation in the study.

This study was designed as a split-mouth, single-blind, randomized, prospective clinical study. Prior to the start of the study, all subjects received the R-LETB or the "Braun Oral-B" toothbrush according to their assigned group and standard toothpaste "Blend-a-Med cavity protection mineral action" (Procter & Gamble, Cincinnati, OH, USA). All subjects from the first group were instructed how to use the R-LETB. Instructions were given verbally and followed by a demonstration.

For both groups, the method of the brushing was similar. Subjects were instructed to brush two times a day: in the morning and in the evening, with toothpaste "Blend-a-Med cavity protection mineral action" for 3 min. The first group of the volunteers used the paste with the prior rinsing of oral cavity for one minute by 0.1% MB solution. All clinical examinations were performed by the same examiner using the same dental units and operating lamp. At the time of examination, the examiner was unaware of the subject's group assignment. Records of earlier examinations were not available to the examiner at the time of re-examination. Treatment effects were determined using the comparison of the patient's scores from each follow-up visit to the baseline scores, which were also documented using Nikon Coolpix 990 (Japan) digital camera.

Clinical evaluation of changes in gingivitis compared with the baseline was visually assessed using original augmented Approximate Hygiene Index (AHI)[22] and standard indices complying with the American Dental Association Acceptance Program Guidelines[23]: Gingival Bleeding Index^{ADA} (GBI),[24] and Gingivitis Index PMA.[25] Gingiva papilla is the main part of a gum where inflammation sets in, which then extends on gingiva marginalis and gingiva alveolaris. Besides, plaque retention in an interdental space promotes the development of approximal caries. Used hygienic indices allow quantitative valuation of tooth plaque on both facial and lingual surfaces of teeth[26,27] as well as qualitative evaluation of the presence of plaque on a contact surface.[28]

We have used augmented AHI[22] based on the method of evaluation of Turesky modification of the Quigley-Hein plaque index,[27] but instead of evaluation of facial and lingual surface, medial and distal tooth surfaces were evaluated. Tooth staining with 1% rosein solution was used to visualize the plaque. To evaluate AHI, a score of 0 to 5 was assigned to each medial and distal surface of all the teeth except third molars, as follows: 0, no plaque; 1, separate flecks of plaque at the cervical margin of the tooth; 2, a thin continuous band of plaque (up to one mm) at the cervical margin of the tooth; 3, a band of plaque wider than one mm but covering less than one-third of the crown of the tooth; 4, plaque covering at least one-third but less than two-thirds of the crown of the tooth; 5, plaque covering two-thirds or more of the crown of the tooth. An index for the entire mouth was determined by dividing the total cumulative score by the number of surfaces examined:

$$Index = \frac{Total\ score}{the\ number\ of\ surfaces\ examined}. \quad (1)$$

Evaluation of gingival bleeding degree by the Gingival Bleeding Index (GBI) suggested by Cowell *et al.*[24] was carried out in area of 12, 16, 24, 32, 36, and 44 teeth on both vestibular and oral surfaces by button or special dulled probe. The tip of the probe was pressed against the wall of the sulcus and passed slowly from medial to distal side of the tooth. The state of the gingival bleeding was graded on a scale from 0 to 3: 0, gingival bleeding was absent; 1, gingival bleeding appeared not earlier than in 30 sec; 2, gingival bleeding appeared right away or during 30 sec; and 3, gingival bleeding appeared during food intake or tooth brushing.

To evaluate the Gingival Index PMA, the gingiva was divided in three regions: papilla interdentalis (P), gingiva marginalis (M), and gingiva alveolaris (A). A Shiller–Pisarev probe was used for gum staining (solution of 1 g crystal iodine, 2 g potassium iodide, and 40 ml distillate water). Gum color changes depending on the inflammatory state: for a healthy gum, mucosa is straw-yellow colored, at chronic inflammation due to glycogen store it is brown colored. By intensity of staining stages of inflammation were differentiated: negative, straw-yellow colored; light-positive, light-brown colored; positive, brown colored; Inflammation of the papilla was evaluated as Grade 1, inflammation of marginal gingiva as Grade 2, and inflammation of alveolar gingiva was evaluated as Grade 3. All highest grades for each tooth were summarized[25]:

$$PMA = \frac{[(\Sigma\ Grades) \times 100\%]}{(3 \times number\ of\ teeth)}. \quad (2)$$

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

Subjects completed a final self-assessment questionnaire based on the overall product effectiveness. Subjects were asked to compare the state of their teeth's and gingiva's health at the end of the treatment period to that at the baseline.

3. Results and Discussion

3.1. *Treatments of dental plaque samples*

Figure 2 shows action of MB solution on microflora of tooth plaque. It can be clearly seen that 3-min staining of the samples with different solutions of MB did not influence by itself (in darkness) the number of microbial colonies. The control number of the colonies was 7.6×10^7. After staining by MB solutions with concentrations of 0.001%, 0.01%, and 0.1%, the average number of the colonies was about 6.4×10^7, 8.2×10^7, and 7.6×10^7, respectively. Thus, the staining alone at the used concentration of MB did not suppress the microflora of plaques.

Average values of bacterial seeding rate of tooth plaques for different treatments are shown in Fig. 3. The first column corresponds to the control group without any treatment. The result of mechanical action by the standard toothbrush alone is presented by the second column. The third column shows combined effect of MB exposure in 3 min, mechanical action and red light irradiation in 15−20 sec on dental plaque bacteria. The mechanical treatment decreased the number of the colonies up to 91.2% in comparison with the control sample due to the mechanical removal of the bacteria. However, the staining and irradiation with simultaneous mechanical action decreased the total number of colonies up to 99.9%. Thus, practically total suppression of the pathological flora was observed.

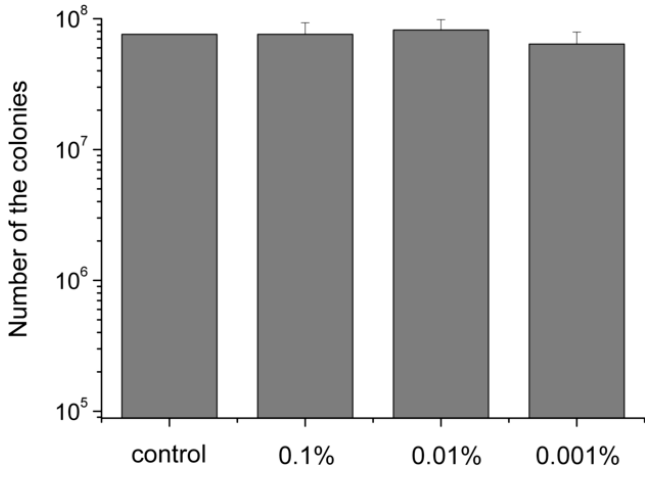


Fig. 2. Average value of *in vitro* measured bacterial colony forming rate (number of the colonies) of bacteria taken from tooth plaque after exposure to MB solution. The first column corresponds to control sample (without staining); the second, third and fourth columns to 3-min duration of 0.1%, 0.01%, and 0.001% MB solution action, respectively.

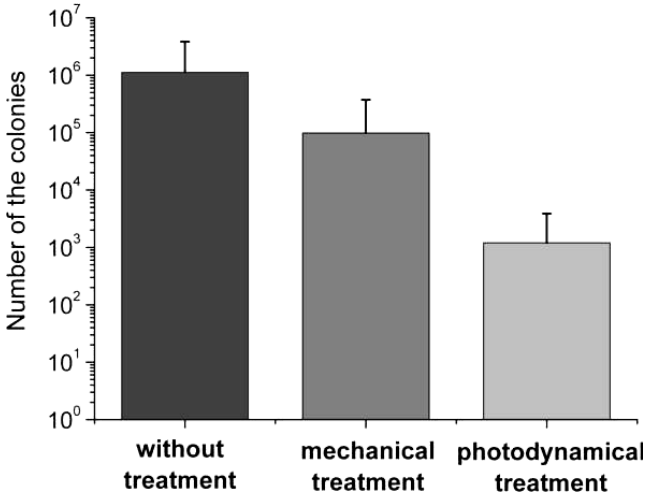


Fig. 3. Average value of *in vitro* measured bacterial colony forming rate (number of the colonies) of bacteria taken from tooth plaque: without treatment (control), after mechanical and photodynamic treatments.

It was previously reported in the literature that MB with red light had a high photobactericidal activity against *S. aureus*, *S. epidermidis*, *S. pyogenes*,[16,17,29] *P. aeruginosa*,[30] and other pathogens. Proteins and lipids forming the cell membranes are easy to be photooxidized. This process leads to the loss or change of the functions of photodamaged biological molecules, which causes morphological changes in the cell. Thus, MB sensitizes damage of the membrane under the action of red light in the vicinity of the MB molecule localization. The damage of the membrane may cause the photosensitizer molecule to move away from the place of its localization, its diffusion inside and redistribution within the cell, and the damage of secondary targets such as mitochondria and the Golgi complex of the cell.[31,32]

The use of R-LETB combined with MB resulted in multifactor therapeutic action on oral pathological microflora: besides mechanical removal of the bacteria as in ordinary tooth-brushing procedure, it has shown additional suppression action on microorganisms due to photodynamic reaction.

3.2. *Treatments of subjects*

No adverse side effects were observed. Figure 4 presents the dynamics of the average value of plaque

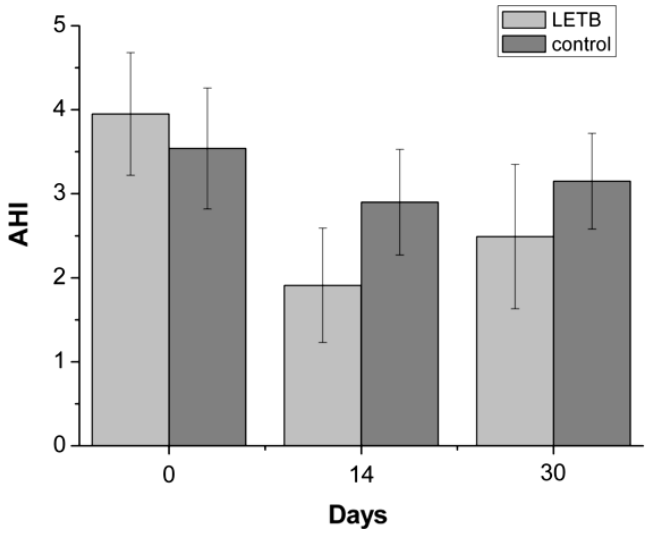


Fig. 4. Dynamics of AHI (Approximate Hygiene Index). Light grey and dark grey columns correspond to the results of tooth brushing by R-LETB with methylene blue prior rinsing and standard toothbrush, respectively. Bars show standard deviation.

index AHI (Approximate Hygiene Index). The index characterizes the area of the tooth covering by plaques. It is seen well that due to treatment the index decreased for both groups of the subjects. The most significant reduction was observed on the 14th day of the treatment (in about 2 times for the tested group and 1.2 times for the control group). At the 30 days timepoint, the decrease of the index values was only 1.6 times for the tested group and 1.1 times for the control group. The improvement tooth status (I) was calculated with the formula:

$$I = \frac{\left(\begin{array}{c}\textit{Baseline index value} \\ -\ \textit{Current index value}\end{array}\right)}{\textit{Baseline index value}}, \qquad (3)$$

where *Current index values* were scored for the 14th and the 30th days.

The improvement of the tooth status for the patients from the tested group on the 14th and 30th days in comparison with the baseline was 35% and 16% for AHI, respectively. For comparison, the improvement of the patients from the control group was 18% and 11% only. The dynamics of the plaque index behavior in both groups can be explained by "learning effect." It has been discussed earlier[33] that professional instructions regarding correct toothbrushing procedure promoted better results in plaque removal. Thus, in the course of the present study, a decrease in the plaque index in the control group was also observed. By the end of the study the values of the index started to go up, i.e., the tooth plaque increased. Apparently, it was the result of the partial loss of mechanical properties of the brushes. When the subjects used their tooth brushes for a month (to the end of the study), they became softer, bristle bundles of the brushes disoriented and caused less gingival abrasion as a result of brushing. Besides, it is possible that in the beginning of the study the subjects brushed their teeth carefully and fulfilled all requirements of the investigator, but by the end of the treatment period they reverted to their usual manner of tooth brushing. Nevertheless, in spite of the partial increase of the plaque for the tested group, the tooth status in that group was better than that of the control group (even though standard toothbrushes kept their high quality during whole study to a higher degree than R-LETB). It also should be noted that R-LETB construction does not allow one to provide the flexibility and good mechanical contact with oral cavity tissues at brushing as standard toothbrush did.

Gingival bleeding index has shown more significant differences in two studied groups (Fig. 5). The average value of the GBI in the tested group decreased more than 2 times in 14 days and more than 3 times in 30 days, whereas in the control group the decrease was about 1.6 and 1.5 times, respectively, at 14 and 30 days timepoints. Thus, after 14 days the improvement of bleeding index

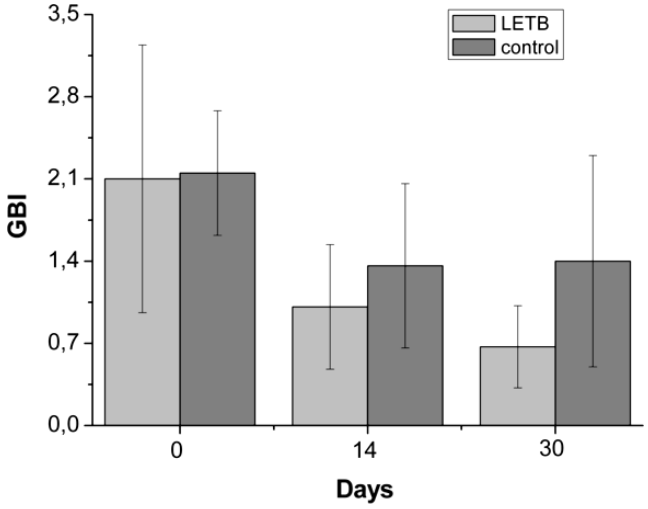


Fig. 5. Dynamics of GBI (Gingival Bleeding Index). Light grey and dark grey columns correspond to the results of tooth brushing by R-LETB with methylene blue prior rinsing and standard toothbrush, respectively. Bars show standard deviation.

evaluated with the help of the formula (3) was 52% in the tested group and 37% in the control one. After a month the improvement of GBI increased up to 68%, and decreased to 35% in comparison with baseline in the tested and control groups, respectively.

This correlates with dynamics of gingival inflammation development presented in Fig. 6. The average value of the index PMA in the tested group decreased more than 4 times in 14 days and 6.6 times in 30 days, whereas in the control group the decrease was 2 and 3.4 times respectively in 14 and 30 days. As a result of the brushing by the R-LETB, the improvement of gum inflammation was 76% in 14 days and 84% in 30 days of the treatment. Control group has shown improvement by 52% and 70% in 14 and 30 days, respectively.

Inflammatory periodontal disease is caused by dental plaque, which is a biofilm. Bacterial endotoxins, cytotoxins, and other pathogenic substances are released from the biofilm and diffuse into the adjacent soft tissues where they elicit an inflammatory response that results in tissue disruption and degradation.[34–36] Therefore, removal of plaque and plaque-derived products has been a key to the treatment of periodontal disease.[36,37] Result of the proper tooth brushing was the decreasing inflammation in both groups of the volunteers. Since the bleeding is caused by gingival inflammation, the decreasing of inflammation process leads to significant improvement of the status of gums. However, in the 30th day of the study, the differences in the GBI between the patients from the first and the second groups increased. It can be related to the additional anti-inflammatory action of red light and MB on gum tissue. It was found that bleeding of gums on probing is the most sensitive method to detect differences in the development of gingivitis between experimental groups.[38] Our study has shown that effectiveness of photodynamic therapy of gingivitis can be evaluated by both GBI and PMA indices.

Figure 7 illustrates the improvement of the status of tooth plaque and gingival inflammation during the combined treatment by R-LETB and MB. AHI allows for evaluating the size of tooth plaque. It can be seen that at the baseline plaque covered the main part of tooth, and the color of the plaques is saturated brown. During the treatment,

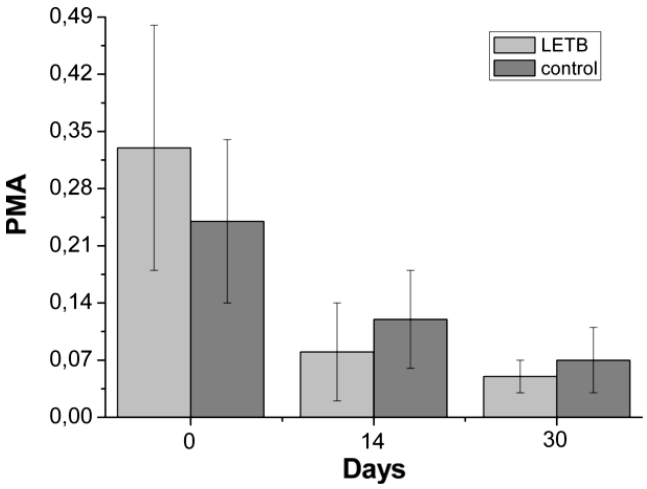
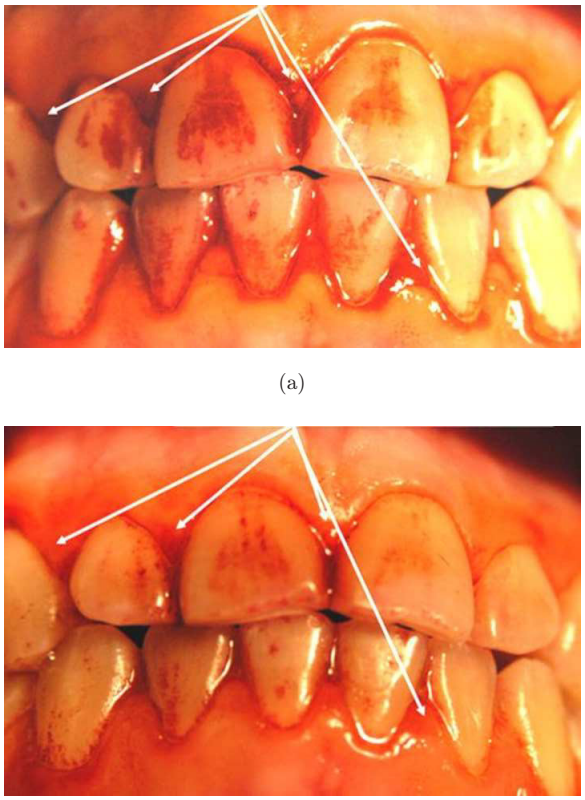


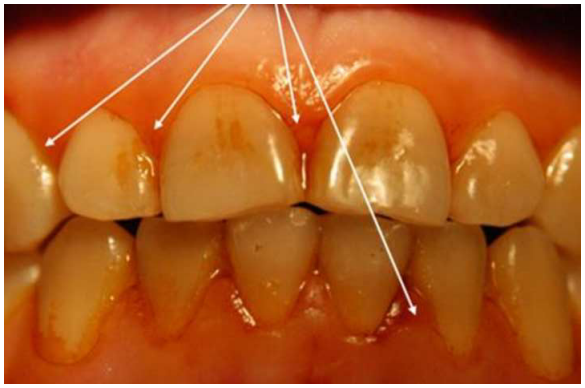
Fig. 6. Dynamics of Gingival Index PMA (P, papilla interdentalis; M, gingiva marginalis; A, gingiva alveolaris). Light grey and dark grey columns correspond to the results of tooth brushing by R-LETB with methylene blue prior rinsing and standard toothbrush, respectively. Bars show standard deviation.



(a)

(b)

Fig. 7. Images of a subject's teeth before the treatment by R-LETB: (a) 14 days into the study, (b) 30 days into the study, (c) arrows mark areas of gum stained by Shiller–Pisarev probe. Intensity of brown color shows degree of inflammation process.



(c)

Fig. 7. (*Continued*)

the size of plaque decreased significantly, and the color became lighter. Gingival Index PMA also showed improvement of gingival inflammation. Intensity of the staining decreased, and the color changed from brown to yellow. Arrows mark areas where inflammation process in gums was registered.

Successful using of light and photosensitizers for bacteria suppression in periodontal therapy was described by a number of authors.[39−42] However, the presented methods required carrying out treatments in dental clinics by medical personnel. The use of R-LETB can significantly simplify the procedure and allow carrying out phototherapy by patients themselves at home. Besides, low power of light irradiation eliminates discomfort caused by heating of tooth and gums. In the questionnaires, 85.7% of volunteers said that they brushed their teeth with pleasure, it was interesting and gave good results. Then 14.28% of volunteers further commented on improvement of color of their teeth (became more white). Thus, combined use of R-LETB and MB is an effective method of dental therapy of tooth plaque and gingivitis.

4. Conclusion

The present pilot clinical study was aimed at the evaluation of safety and efficacy of R-LETB in combination with MB with the use of indices characterizing the status of the teeth and gingiva. In both the control and experimental groups, the improvement of gingivitis characterized by tooth plaque, gingival bleeding, and inflammation indices was found. It was caused by an improvement in oral hygiene for both groups. However, for experimental group, R-LETB treatment efficiency was better. The study also has shown that the R-LETB treatment becomes more effective when using the brush for a longer period of time.

As results of this study suggest, the R-LETB technology can be significantly improved if mechanical cleaning properties (brush flexibility and quality of bristles) are made at least as good as those of regular toothbrush. The R-LETB technology should be tested for treatment of other oral conditions such as caries, tooth yellowing, halitosis and etc.

Acknowledgments

The authors are grateful to Palomar Medical Technologies, Inc. (Burlington, MA, USA) for funding this work. The research described in this paper was also supported by grant No. 224014 PHOTONICS4LIFE of FP7-ICT-2007-2 (2008−2013) and Governmental contracts 02.740.11.0484 and 02.740.11.0879.

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