Photoactivated disinfection in periodontal treatment: A randomized controlled clinical split-mouth trial

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Abstract

Background: Photoactivated disinfection (PAD) could support the periodontal treatment outcome. The effect of the light emitting diode (LED) as an innovative light source in PAD is under discussion. The aim of this study was to evaluate the clinical and microbiological effect of adjunctive PAD in the treatment of periodontitis with a red LED as light source.

Methods: Twenty patients with periodontitis completed this split-mouth study. The left and right side of the jaws were randomly assigned to either test or control group. After conservative periodontal treatment in both groups, the test group received two sessions of adjunctive PAD (red LED, 635 nm, photosensitive dye, 0.01% tolonium chloride), whereas the control group received no adjunctive PAD. The parameters of clinical periodontal examination—including probing depth (PD), clinical attachment level (CAL), bleeding on probing (BOP) and microbiological assays (PCR) were evaluated before and after treatment.

Results: After 3 months, both treatment groups showed significant improvements regarding BOP, PD, and CAL compared to baseline, with no significant difference between control and treatment group. The recolonization of Porphyromonas gingivalis and Treponema denticola was reduced after adjuvant treatment, but not significantly.

Conclusions: The positive effect of adjunctive PAD regarding clinical parameters was reported in recent trials. In this study and with the current settings, both treatment groups showed similar clinical results after initial periodontal treatment, without beneficial effect of adjunctive PAD.

KEYWORDS
periodontal disease, periodontal pocket, periodontitis, photochemotherapy, randomized controlled trial

1 | INTRODUCTION

Periodontitis is an inflammatory disease of the periodontal tissues and causes progressive attachment loss and destruction of the alveolar bone.1 The activation and extent of periodontal destruction is determined on the one hand by the quantity and virulence of the bacteria, on the other by the response of the host (immune status, genetics).2 Three forms of periodontitis can be distinguished, based on pathophysiology: necrotizing periodontitis, periodontitis as a direct manifestation of
systemic diseases and periodontitis.\(^3\) Conservative periodontal treatment offers a very efficient reduction in microbial count and also improves clinical parameters.\(^4\) However, it has been observed that periopathogenic microorganisms penetrate other cells outside the epithelial pocket and may linger there despite conservative periodontal treatment\(^5,6\) and hence evade the immune response of the host and the effect of antimicrobial substances. This may result in recolonization of the periodontal pockets with periopathogenic microorganisms, which may lead to recurrence and increased chronicity of the disease.\(^7\) Systemic antibiotics are often applied as an adjunct to conservative treatment, yet the increasing resistance rates of the pathogenic germs have caused concern worldwide.\(^8\)

Photoactivated disinfection (PAD) in periodontal treatment is based on the concept of photodynamic therapy (PDT). PDT is a treatment method that is associated with the use of a light source and a photosensitizer (PS).\(^9\) PDT was first successfully applied 100 years ago,\(^10\) but the development of antibiotics led to a stagnation in the use of PDT for infectious diseases. However, due to an increase in antibiotic resistance, this treatment method is re-emerging as a possible alternative for treating infection. We are currently not aware of evidence of the development of resistance of periopathogenic microorganisms to PDT.\(^11\) Key factors for the effect of PDT are the light dose applied, the absorption coefficient of the PS and the concentration of PS at the target location.\(^12\) The light source should emit light near to the absorption maximum of the PS. Since shorter wavelength light is richer in energy, but longer wavelength light can penetrate more deeply into the tissues, blue dyes are preferred which absorb light above 600 nm.\(^13\) The PS is a photosactive substance which specifically binds to cells and after absorption of light induces a chemical reaction that results in the release of radicals and singlet oxygen.\(^14\) The cytotoxic species react with subcellular organelles and macromolecules, which results in apoptosis and/or necrosis of the targeted cells, showing no impact on healthy cells.\(^15\) Cationic PS such as toluidine blue O (TBO), also referred to as tolonium chloride, is suitable for PDT in periodontology.\(^16\) Other PS in use is methylene blue and poly-L-lysine-chlorine\(\_\)n conjugates.\(^16\) The main difference between the various PS is the cell structure that they target. TBO reacts with lipopolysaccharides in the cell membrane of Gram-negative bacteria, even without light-activation.\(^17\) However, after activation at 630 nm it shows maximum absorption and good photodynamic properties suitable for eliminating bacteria.\(^18\) TBO can bind to Gram-positive as well as to Gram-negative microorganisms.\(^19\) Tolonium chloride is also used in histology to stain specimens,\(^20\) and in dermatology to distinguish benign from precancerous leukoplakia.\(^21\) The PS used in this study is a medical grade 12.7 \(\mu\)g/mL tolonium chloride solution, which is available in two viscosities by the manufacturer. PDT, with various PS, is more effective against Gram-positive than against Gram-negative microorganisms.\(^22\) The varying effectiveness of the treatment method lies in the structure of the cell walls of the microorganisms. Gram-positive microorganisms have a semi-permeable layer of murein (peptidoglycan) and lipoteichoic acid around the cytoplasmic membrane, through which the PS is able to diffuse.\(^23\) In contrast, Gram-negative microorganisms have an internal cytoplasmic membrane and an external membrane that can be divided by a murein layer and the periplasmic space.\(^24\) The external membrane offers efficient permeability protection and prevents the binding and penetration of many PSs.\(^24\) The PSs that are used in periodontology therefore have a positive charge and are effective against both Gram-negative and Gram-positive microorganisms in the context of PDT. The interaction of the PS with the bacteria occurs within a few minutes and this incubation time should elapse before the area is exposed to light.\(^16\)

Coherent and non-coherent light sources may be used for PAD.\(^25\) Lasers are coherent and light emitting diodes (LED) are non-coherent light sources. Due to their coherence, lasers enable the transfer of light through fiber applicators directly to the affected areas. Helium-neon (He-Ne) lasers and semiconductor lasers (diode lasers) have already been used successfully in photo-activated disinfection.\(^26\) Studies have already shown a significant reduction in bleeding values\(^27\) and in the bacterial count of periopathogens after adjuvant PDT compared with conservative treatment alone,\(^28\) as well as a greater reduction in pocket depth and clinical attachment gain.\(^29\) Compared with single treatment, repeated PDT treatment has produced better clinical\(^10\) and anti-inflammatory results.\(^31\) Treatment should always be preceded by scaling and root planing (SRP), since the antimicrobial effect is reduced by biofilm.\(^25\) Graham et al. (2009) observed in an in vitro study that PDT treatment potently and functionally inactivated tumor necrosis factor alpha (TNF-\(\alpha\)) and interleukin (IL)-1\(\beta\), when the cytokines were exposed to a 60-second PDT.\(^33\) This study showed that PDT deactivated pro-inflammatory cytokines, in order to inhibit the activation of E-selectin in endothelial cells. In addition, PDT appears to influence antigen-presenting cells such as macrophages and Langerhans cells, by reducing the ability of T-lymphocytes to activate, and weakening the inflammatory reaction.\(^34\) Tanaka et al. showed in an animal study that PDT can positively influence the host immune response.\(^35\) Histopathological investigations revealed a higher incidence of neutrophilic granulocytes in inflammatory areas in the test group of an arthritis model. A recent review reported positive effects of photobiomodulation on healing responses and the host immune system.\(^36\) PDT showed positive effects on cells in the periodontal ligament, such as inhibition of pro-inflammatory mediators, encouragement of cellular chemotaxis and promotion of local vasodilation and angiogenesis.\(^17\) PDT with lasers and LEDs does not result in an increase in tissue temperature, and is therefore tolerated by dental tissue. PDT is
potentially cytotoxic due to the effect of dyes on the body’s own cells, and the light dose selected. TBO has a cytotoxic action on gingival keratinocytes and fibroblasts, and these effects are dependent on the concentration. Up to a light exposure of 2 minutes and a concentration of toluidine blue of 2.5 or 5.0 μg/mL, no negative effects were observed in the study by Soukos et al. on the functioning of keratinocytes and fibroblasts. A previous study suggested that TBO can induce a significant increase in chromosomal defects; this was not confirmed in an animal study. There is no evidence of toxicity from mouthwashes or the direct topical application of 1% toluidine blue solution in humans. Therefore, the light dose and the concentration of dye should be carefully considered in order to prevent the cytotoxic effect on host cells. In recent years, due to the limitations of conventional treatment, PAD has been researched as a possible therapeutic approach to treat periodontal disease. Rising resistance rates of pathogenic microorganisms to systemic antibiotic treatment have raised concern internationally. The light of an LED is monochromatic and suitable as a non-laser light source for PAD. The radiation of the LED is less harmful to the eyes than laser irradiation and the device is less expensive. To the present date, only a few studies have investigated the effect of the red LED as an innovative light source in PAD, however with controversial results. The aim of this study was to investigate the effect of PAD using an LED as an adjuvant treatment to conservative periodontitis treatment for chronic periodontitis.

2 | MATERIALS AND METHODS

2.1 | Study population

The study protocol was approved by the Ethics Committee of the Medical University Vienna (EK Nr: 1860/2014). The study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013. Patients were recruited from February 2015 to March 2016 at the University Clinic of Dentistry, Medical University of Vienna, after initial periodontal screening. The inclusion criteria, with regard to the new classification of periodontitis, were as following: localized or generalized periodontitis of periodontal stage II, III, or IV with grades B or C, age of >35 years with probing depths >5 mm in at least one site in each quadrant, radiologically detectable alveolar bone loss in all quadrants and good general health. Exclusion criteria were current pregnancy, systemic or local antimicrobial treatment in the last 6 months, periodontal treatment in the last 6 months, the presence of an infectious disease, chronic pulmonary disease, cancer, or diabetes and other apparent oral infection, intake of immunosuppressive medication or immunodeficiency. All of the study participants signed an informed consent form after clarification about the study protocol and possible side effects of the adjunctive therapy. Figure 1 features a flow diagram of progress through the phases of this randomized clinical trial.

2.2 | Clinical examination and treatment

Recruitment of the patients took place at the University Clinic of Dentistry in Vienna during the patient’s first assessment and was performed by one of the examiners. Patients with a level of three or four Periodontal Screening Index (PSI) in all quadrants and radiologically detectable alveolar bone loss in all quadrants, who fulfilled the other study-related criteria and signed a written consent, were admitted to the study. The patients were numbered by the examiners according to date of inclusion in the study. Each patient received both treatments in a split-mouth design, treatment allocation was randomized in an equivalent way to tossing a coin after the initial periodontal examination, by a person other than the examiners. Twenty-two patients with periodontitis were admitted to the study and were subjected to clinical and microbiological tests. The initial examination by one calibrated examiner was followed by two to four debridement sessions with manual and ultrasonic instruments and ultrasonic instruments, and oral hygiene controls. After scaling and root planing in all quadrants, PAD was applied in one side of each study participant’s maxilla and mandible. This procedure was repeated in a subsequent session in the same order. The contralateral side remained untreated and served as a control. The PAD solution contained 12.7 μg/mL of toluidine chloride. The LED emitting at a wavelength of 635 nm was positioned on six sites around the teeth and applied with an optic fiber inside the periodontal pocket. The maximum output power from the end of the light guide was 750 mW. The activation time in the pocket was 60 second with maximum energy density of 14J/cm². The treatment parameters were chosen based on a previous study using an LED device that showed beneficial clinical effects, with a similar wavelength (628 nm; output power of 1000 mW; energy density 20 J/cm² for 10 second exposure time), photosensitizer dosage (0.1 mg/mL), and treatment time according to the manufacturer’s recommendation however in the present study with a repeated application of PAD. Treatment allocation was not revealed to the patient. The same experienced periodontist performed the adjuvant therapy. All patients were allocated a sequential code number (pseudonymized). The data to be evaluated were provided with this code only and stored on an Excel sheet on a PC with access limited to the Division of Conservative Dentistry and Periodontology, and subsequently evaluated. Only authorized persons have access to the original data. The general medical and dental history, as well as clinical and radiological investigations, was performed before treatment for all

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patients. The approximal-plaque-index (API)\(^5\) and the papillary bleeding index (PBI)\(^6\) were recorded to assess oral hygiene. A complete periodontal status was recorded by a calibrated examiner. Probing depth (PD) and clinical attachment level (CAL) were measured to the nearest millimeter using a calibrated standard probe.\(^7\) Presence of bleeding on probing (BOP), plaque, and suppuration were noted. The patients were offered individual oral hygiene instructions before treatment and at subsequent appointments. All patients were instructed on how to use an electric toothbrush and interdental brushes of various sizes or dental floss, depending on their individual needs. The patients received initial periodontal treatment with supra and subgingival debridement (SRP) using ultrasonic instruments,\(^1\) universal curets and Gracey curets.\(^2\) Initial treatment was completed over two to four debridement

\(^{1}\) Sonicflex 2003 KaVo, Biberach, Germany

\(^{2}\) HU-Friedy, Chicago, IL.
sessions. During initial treatment and before PAD, microbiological samples were taken; test and control side samples were pooled separately. During the final two treatment sessions, PAD was performed twice in one quadrant of the maxilla and mandibula (right or left). All treatment steps were performed in accordance with the manufacturer’s protocol.

Adjuvant treatment was carried out at six locations around all teeth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, and distolingual), and protective eyewear was provided to all participants. The PS was a 0.01% tolonium chloride solution, which is available in two different viscosities, depending on the depth of the pocket to be irradiated and the location.

Managing dry conditions and in case of appropriate hemostasis, the photosensitizer was applied to the pocket and left in place for 60 seconds. After the onset time the area was irradiated with light for 60 seconds per location according to the manufacturer. The pockets were than irrigated with physiological saline solution. During initial treatment and prior to PAD, microbiological samples were taken, and test and control side samples were pooled.

A re-evaluation took place within 12 weeks after the final cleaning session, by one calibrated examiner. Analysis of the data was performed by a blinded examiner.

2.3 | Examiner calibration

All clinical parameters were measured by a calibrated examiner. Duplicate measurements of pocket probing depth, clinical attachment level and bleeding on probing from teeth were obtained from three patients at two separate sessions seven days apart. The measurements were performed with a calibrated standard probe. Calibration was accepted if percentage agreement between measurements at baseline and after seven days was >90%.

2.4 | Microbiologic examination

Before subgingival debridement, samples of subgingival plaque were taken from one deepest periodontal pocket in all four quadrants. After removal of saliva and supragingival plaque, the sample sites were isolated with cotton rolls and paper points were applied to the deepest pockets for a minimum of 10 seconds. The paper points from the test and control side were pooled separately, and transported in a carrier medium to the microbiology laboratory for further processing. Analysis of plaque samples was performed in the Core Facility Oral Microbiology and Hygiene.† Eleven periodontopathogenic bacteria were investigated with a commercially available polymerase chain reaction (PCR) DNA probe test kit: A. actinomyctelemcomitans, P. gingivalis, T. forsythia, T. denticola, P. intermedia, P. micros, F. nucleatum, C. rectus, Eubacterium nodatum, E. corrodens, Capnocytophaga species, which is based on a DNA strip technique.

Multiple PCR was followed by reverse hybridization where amplicons bind specifically to their complementary probes, which had been commercially placed in strips. This test had a detection limit at \(10^3\) for A. actinomyctelemcomitans and \(10^4\) for the other bacteria.

2.5 | Calculation of sample size

Sample size calculations were performed based on a previous study.42 For a split mouth study, we assumed a standard deviation of 15% points for bleeding on probing in the difference in treatments, and calculated under simplifying assumptions, that 20 patients were needed to detect a clinically interesting difference of 10% points for bleeding on probing with 80% power at an \(\alpha\) level of 0.05.

2.6 | Statistical analysis

Graphical representations of the data are given as box plots and bar charts. For inferential statistics on CAL and PD, we assumed approximate (multivariate) normality and computed linear mixed models using potential confounders baseline, sex, age, smoker, API, and PBI as fixed covariates, treatment as fixed effect, and position nested in tooth, which itself is nested in Patient ID, as random effects. Note that this respects the dependence structure of the data. For the binomial variable BOP, this model was generalized to a logit mixed model.47

This analysis was repeated stratified by PD > 5. Since approximate normality assumptions would not hold for microbiological measurements which are on a log scale and only take few levels, this model was further extended to a (ordinal) cumulative logit-mixed model.49 All computations were done using R version 3.4.3.49 Graphics were created using ggplot2.50

3 | RESULTS

The mean age of patients was 46.20 ± 6.96 years and seven of the 20 patients were smokers. The number of treated sites within groups is shown in Table 1. At baseline, there was virtually no difference between treatment groups regarding PD and CAL. Both values were relatively similar in smokers and non-smokers as well. API was 58.83 ± 25.31 at baseline and was reduced to 31.32 ± 15.12 at the point of reevaluation (Figure 2A). PBI was recorded at baseline 27.86 ± 8.51 after treatment (Figure 2B). Note that a comparison of treatments with respect to PBI and

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<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline, n</th>
<th>Re-evaluation, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRP</td>
<td>1692</td>
<td>1661</td>
</tr>
<tr>
<td>SRP + PAD</td>
<td>1722</td>
<td>1680</td>
</tr>
</tbody>
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Baseline = beginning of treatment; re-evaluation, 12 weeks after treatment; SRP, scaling and root planing; PAD, photoactivated disinfection.

API was not assessed due to the split-mouth design of this study. There were no other statistical differences between treatment groups regarding clinical parameters ($P > 0.05$) (Figures 3 and 4). Both treatment groups showed a reduction in the pathogen concentration after basic therapy, but no significant difference between treatment groups. The microbiological analysis showed a slightly higher reduction in \( P. \) gingivalis and \( T. \) denticola after adjuvant PDT, which was not significant (Figure 5). No adverse effects or side effects have been reported during the trial and at regular follow-ups.

4 | DISCUSSION

The results of this study were not able to confirm additional benefit for PAD with the red LED. Both groups demonstrated a reduction in probing depth and an attachment gain, in addition to an improvement in bleeding values. The microbiological investigations showed reduced colonization of \( P. \) gingivalis, comparing with the control group, even though not statistical significant. A limitation of this split-mouth study design is the potential bias that should be considered through the carry-across effect. There has been discussion recently about the use of PAD as adjuvant treatment in periodontitis and peri-implantitis. Clinical study results to date have been very variable, so there are still no accurate recommendations regarding the practical application.51 Bonito et al. have shown that SRP does not eliminate all pathogenic bacteria.7 At most, SRP brings about a temporary reduction in microorganisms, but reinfection can occur in <2 weeks. Systemic and local antibiotics are often prescribed as adjuvant treatment; however, increasing resistance rates of periodontal pathogens are causing international concern and may also result in poorer treatment outcomes.52 Innovative treatment methods are therefore desirable to improve the clinical results of

![Figure 2](image)

**Figure 2**  A) Plaque percentages at baseline and reevaluation for both groups. Baseline = beginning of treatment; Reevaluation = 12 weeks after treatment; SRP = scaling and root planing; PAD = photoactivated disinfection; API = approximal plaque index; B) Papillary bleeding percentages at baseline and reevaluation for both groups. Baseline = beginning of treatment; Reevaluation = 12 weeks after treatment; SRP = scaling and root planing; PAD = photoactivated disinfection; PBI = papillary bleeding index

![Figure 3](image)

**Figure 3**  Bleeding on probing percentages for baseline and reevaluation in both treatment groups; BOP = bleeding on probing; SRP = scaling and root planing; PAD = photoactivated disinfection
conservative periodontal treatment. Studies have already revealed the in vitro\textsuperscript{23} and in vivo\textsuperscript{24} antibacterial efficiency of PDT in periodontics. Better clinical and anti-inflammatory results have been shown with repeated PDT treatments compared to single treatment.\textsuperscript{30,31} The antimicrobial effect is reduced by a biofilm\textsuperscript{32}; therefore, the adjunctive treatment should be applied after conservative therapy. Eighteen randomized clinical studies were compared in a systematic review and the potential additional effect of PDT on top of initial treatment was investigated.\textsuperscript{51} Eight of the 18 studies had statistically significantly better results in probing depth and clinical attachment level after adjuvant PDT compared to conservative treatment alone; however, the remaining 10 studies showed no significant differences in these parameters. An additional positive effect was observed in five studies regarding BOP. The results of a clinical study on patients with aggressive periodontitis showed that PDT demonstrates a greater antibacterial effect against \emph{A. actinomycetemcomitans}, compared to scaling and root planing with hand curets.\textsuperscript{53–55}

Braham et al. showed that PDT also eliminates \emph{P. gingivalis} and deactivates the pro-inflammatory cytokines TNF-\textalpha{} and IL-1\beta{}.\textsuperscript{33} Carvalho investigated the efficiency of PDT with a diode laser at a wavelength of 660 nm in 34 patients with residual pockets after initial treatment.\textsuperscript{56} The results showed no significant differences between the groups. Birang et al. (2015) were able to demonstrate only a short-term significant difference to the control group regarding clinical attachment loss.\textsuperscript{57} However, Polansky et al. were not able to observe any positive clinical or microbiological effect of PDT compared to conservative periodontal treatment alone.\textsuperscript{41} An important aspect of PAD is the lack of resistance of the bacteria to this type of therapy; in view of the increasing resistance to antibiotics, this type of adjuvant treatment could become more significant. There are limited clinical studies to date investigating the LED as a light source in PAD as adjuvant treatment for periodontitis. Mongardini et al. investigated the short-term effect of PAD using a red LED in a clinical split-mouth study on 30 patients.\textsuperscript{42} Patients with chronic periodontitis and at least two residual pockets after initial treatment were included in the study. One week after adjuvant PAD, the clinical and microbiological parameters were recorded. The results showed a significant reduction in BOP, PD and microbial count in the test group. However, Bassir et al. was unable to confirm an additional positive effect for PAD with LED.\textsuperscript{43} Sixteen patients with chronic periodontitis were included in the split-mouth study, and following initial treatment additionally treated over two sessions with LED (without photosensitizer), PAD or photosensitizer alone. Clinical parameters were recorded after 1 and 3 months; there were no significant differences between the groups. The results of the present study are in accordance with those of Bassir et al. Additionally, microbiological samples were taken and investigated. In combination with BoP, we calculated and compared the microbial assessment (log scale) similar to the microbial analysis of subgingival plaque samples in previous studies.\textsuperscript{43,58} The sample size calculations for this study were computed for BOP as the main analysis; a much larger number of samples would be required for the microbiological analysis in order to claim equivalence of treatments. Adequate residence time of the dye in pockets may be problematical as this should not <60 seconds, and access to deeper pockets for example may be hampered by residual concretions. A second critical factor is potential access of the light using current light applicators, as they are not very flexible and consideration must be given to the fact that the tip could cause harm, especially in deeper pockets. One option for improvement is depth markings on the light guide tip to

\textbf{FIGURE 4} \textsuperscript{A) Distribution of the values of periodontal pocket depth for baseline and reevaluation in both treatment groups; Baseline = beginning of treatment; Reevaluation = 12 weeks after treatment; SRP = scaling and root planing; PAD = photoactivated disinfection; PD = probing depth; B) Distribution of the values of clinical attachment level for baseline and reevaluation in both treatment groups; Baseline = beginning of treatment; Reevaluation = 12 weeks after treatment; SRP = scaling and root planing; PAD = photoactivated disinfection; CAL = clinical attachment level.
guide it during treatment of deeper pockets, without causing any bleeding. An alternative solution could be possibly to make tips of different lengths and angulation for different access areas in order for example to also adequately treat furcation areas. Based on the penetration depth of the light, transgingival radiation represents an interesting treatment option, which is to be researched further. There are currently no recommendations for the successful use of the red LED in PAD, and no unified treatment protocol. Furthermore, long-term clinical studies are necessary to investigate the effect of this method in order to be able to use it efficiently in the treatment of periodontitis.

5 | CONCLUSIONS

Previous studies showed the positive clinical outcomes of adjuvant PAD with a 0.53-mm mean gain in CAL, compared with SRP alone. Within limitations of this clinical trial, the adjunctive PAD with red LED at current settings did not result

**FIGURE 5** Percentages of detection levels in periodontal pockets of four major periodontopathic bacteria at baseline, after scaling and root planing, and after 12 weeks in both groups. Baseline = beginning of treatment; post treatment = after scaling and root planing; re-evaluation = 12 weeks after treatment; Aa = A. actinomycetemcomitans; Pg = P. gingivalis; Tf = T. forsythia; Td = T. denticola
in additional benefits regarding clinical parameters in chronic periodontitis, comparing to debridement alone. The microbial analysis indicate that the recolonization of P. gingivalis and T. denticola is reduced after adjunct treatment with the red LED, but a larger number of samples are needed to underline these results.

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