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No Benefit of an Adjunctive Phototherapy Protocol in Treatment of Periodontitis: a Split-Mouth Randomised Controlled Trial

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Data availability statement:

Data subject to third party restrictions. The data that support the findings of this study are available from Colgate-Palmolive (Europe). Restrictions apply to the availability of these data, which were used under license for this study. Data are available from the authors with the permission of Colgate-Palmolive (Europe).

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ABSTRACT

Aim: To assess the efficacy of a commercially-available adjunctive phototherapy protocol ('Perio-1') in treatment of periodontitis.

Materials and Methods: In an examiner-blind, randomised, controlled, split-mouth, multi-centre study, 60 periodontitis patients received root surface debridement (RSD) in sextants either alone (control sextants) or with the adjunctive phototherapy protocol (test sextants). Re-evaluation was performed at 6, 12 and 24 weeks.

Results: No statistically significant differences in mean (± standard deviation) clinical attachment level (CAL) change from baseline to week 24 were observed between test (-1.00±1.16 mm) and control sextants (-0.87±0.79 mm) at sites with probing pocket depths (PPDs) ≥ 5 mm ("deep sites") at baseline (p=0.212). Comparisons between test and control sextants for all other parameters (CAL change at all sites, PPD change at deep sites/all sites, bleeding on probing, plaque scores), and for all change intervals, failed to identify any statistically significant differences.

Conclusions: The phototherapy protocol did not provide any additional clinical benefits over those achieved by RSD alone. (German Clinical Trials Register DRKS00011229).

(164 words)

Keywords: periodontitis; periodontal debridement; fluorescence; photobiomodulation therapy.

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CLINICAL RELEVANCE

Scientific rationale for the study

An adjunctive phototherapy protocol ('Perio-1') is available for treatment of periodontitis. However, there is a lack of evidence regarding the clinical efficacy of the protocol.

Principal findings

Sextants treated with root surface debridement (RSD) plus the adjunctive phototherapy protocol did not demonstrate better clinical outcomes compared to sextants treated with RSD alone.

Practical implications

The findings of this research do not support use of the phototherapy protocol in the treatment of periodontitis. It is important that properly designed studies that investigate mechanisms of action and clinical efficacy are performed prior to new technologies being tested or used in patient care.

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INTRODUCTION

Periodontitis is a chronic inflammatory disease that results from the complex interplay between the host immune-inflammatory response and the long-term presence of a dysbiotic bacterial biofilm in the subgingival environment. Biofilm control is a key component of therapy, and is routinely achieved via professionally delivered non-surgical therapy that includes root surface debridement (RSD) to remove and reduce biofilm and calculus, together with patient education to improve oral hygiene. Whereas the clinical improvements that can be achieved by RSD are well recognised (Cobb, 2002), it is also known that there are limitations in the effectiveness of the procedure, particularly in deeper pockets. Bacteria may persist within anatomical niches or within the soft tissues, leading to recolonization of the pocket following therapy (Uzel et al., 2011). Accordingly, efforts have been expended to develop local adjuncts to mechanical debridement that may further reduce the bacterial challenge, with the aim to improve outcomes of treatment.

An important group of such adjunctive therapies includes those activated by light (i.e. phototherapies), including antimicrobial photodynamic therapy (aPDT) and photobiomodulation (PBM). In periodontal therapy, aPDT involves application of a photosensitising agent into the subgingival environment followed by its activation by laser light of specific wavelength, resulting in the formation of reactive oxygen species that enhance bacterial killing (Chambrone et al., 2018). Systematic reviews on the use of aPDT in periodontal therapy that were conducted as part of the 2019 European Workshop on Periodontology, and which informed the EFP S3-level evidence-based guidelines (Sanz et al., 2020), did not support the use of aPDT at wavelengths of either 660–670 nm or 800–900 nm in patients with periodontitis during either step I-II of therapy (Salvi et al., 2020) or supportive periodontal care (Trombelli et al., 2020). PBM refers to phototherapy in which low energy light is used to elicit a biological response. This has been investigated and employed in several branches of medical practice, and has been previously delivered using either laser or light-emitting diode (LED) light emission. Several possible mechanisms may be involved in therapeutic gains from these therapies, and such gains may be greater in tissues with high turnover, inflammation, oxidative stress or tissue damage (Hamblin, 2017, Hamblin, 2018, Passarella and Karu, 2014). PBM (alone or combined with photodynamic therapy) has been reported to be effective in management of oral mucositis for patients undergoing cancer treatment (Pires Marques et al., 2020) as well as for pain relief following endodontic treatment (Nunes et al., 2020).

Recently, a new phototherapy product for use in periodontal treatment has become commercially available that has built on developments in dermatology (Antoniou et al 2016). This methodology is based on delivery of light absorbing molecules (chromophores), which subsequently are able to act to convert incident light energy such as that from a local light source into emitted fluorescence. This, in turn, is purported to act to modify cellular activity, or at certain wavelengths, to have possible antimicrobial activity. The penetration and activating ability of these agents can potentially be adjusted by selection of differing fluorescence agents, related to fluorescence emission wavelength. Preliminary observations on the combined use of

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RSD with the phototherapy product showed a clinical benefit in terms of attachment gain and probing depth reduction (Kamma et al., 2015). However, clinical efficacy in a larger-scale randomised controlled trial (RCT) has not been reported.

Therefore, we conducted a clinical study that aimed to assess the efficacy of the phototherapy product (as based on manufacturer's recommendations and protocol) when used as an adjunct to RSD in the treatment of periodontitis. The null hypothesis was that there would be no significant differences in change in the primary efficacy variable between sextants treated with the adjunctive phototherapy compared to sextants treated by RSD alone.

MATERIALS AND METHODS

Study outline

This was an examiner-blind, randomised, controlled, split-mouth, multi-centre study conducted at three university research centres: School of Dental Sciences at Newcastle University (UK), Faculty of Dentistry Oral and Craniofacial Sciences of King's College London (UK), and University of Ferrara (Italy). The clinical phases of the study ran from September 2016 (first patient recruited 09.09.2016) to June 2017 (last visit of last patient 30.06.2017). The aim was to assess the effectiveness and safety of the phototherapy protocol when used as an adjunct to RSD in patients with periodontitis. The study was conducted in compliance with the requirements of directive 93/42/EEC, and was registered on the German Clinical Trials Register (https://www.drks.de/drks_web/ ref. DRKS00011229). Ethical approval was obtained from ethics committees in the UK (London Camden and Kings Cross NHS Research Ethics Committee, ref. 16/LO/1259) and in Italy (Comitato Etico Unico della Provincia di Ferrara, ref. CE 160587) prior to commencement. The study was sponsored by Colgate-Palmolive (Europe) and monitoring and statistical analyses were performed by a contract research organisation (OPIS Europe).

Study participants

Participants were recruited from hospital clinics at the three study centres. Participants were diagnosed with moderate-to-severe chronic periodontitis according to the classification system in use at the time (Armitage, 1999), corresponding to periodontitis stage 3 or 4, grade B according to the new classification (Tonetti et al., 2018). Inclusion criteria were: (i) signed informed consent, (ii) male or female, aged 18-70 years inclusive, (iii) good general health (e.g. free from systemic diseases such as diabetes, arthritis, HIV, genetic disorders, malignancy, as determined by medical history), (iv) periodontitis with a minimum of two posterior sextants with probing pocket depths (PPDs) 5 mm or greater. Posterior sextants were defined as the dentition from the distal of the canine to the distal of the second molar, and containing at least two teeth in contact. Exclusion criteria were: (i) orthodontic appliances, (ii) tumours or other significant pathology of the oral cavity, (iii) PPDs of ≥10 mm in test or control sextants, (iv) caries lesions requiring immediate care, (v) participation in any other clinical study within the last 30 days, (vi) pregnant or lactating women (by

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medical history), (vii) history of allergies to tooth whitening products, H_2O_2, personal care consumer products or their ingredients, (viii) use of locally applied or systemic antibacterial agents or dental prophylaxis in the last 30 days, (ix) use of drugs or products known to induce photosensitivity reactions, (x) any medical condition with known photosensitivity, (xii) skin hypersensitivity.

Test protocol

The test protocol was based on a CE-marked medical device ('Perio-1', Klox Technologies, Quebec, Canada) comprising a two-phase photo-converter gel containing an active ingredient chromophore (specific absorption to blue-green light), producing O_2 and singlet oxygen when photo-activated with light at 430 nm. According to the manufacturer's instructions, the gel was mixed then applied and photo-activated twice: immediately before RSD and then repeated following RSD. The method of application was the same both times, and the gel was applied to all sites in the test sextant. Specifically, the gel was dispensed to the depth of the pockets using a syringe until the pockets were filled and slightly overflowing. It was then photo-activated by inserting a clear plastic probe-like tip attached to the 10 mm diameter tip of a standard dental curing light (Bluephase LED light, Ivoclar Vivadent) to the depth of the pockets at each tooth in the sextant. This multi-LED light source emits a broadband spectrum of 385-515 nm, with a main peak at 465 nm and a secondary peak at 405 nm. The light was illuminated for 30 seconds buccally, and then 30 seconds lingually/palatally at each tooth, keeping the tip slowly moving so that it occupied approximately 10 seconds at each periodontal site. The power density of the LED light is estimated to be 1,200 mW/cm^2 at a distance of 3-5 mm from the light source with a radiant fluence (or dose) during a single 10 second exposure of 0.3W/cm^2 or 3J/cm^2 per site. Any visible gel at the end of photo-activation was removed with gauze, and it was not washed off or washed out of the pockets.

Randomisation, blinding and allocation concealment

The study sextants were two posterior sextants that were allocated randomly to one of the two treatments (test sextant and control sextant, i.e. split mouth design). Stratification was undertaken based on the number of deep sites at baseline (PPD ≥5 mm) to ensure similar extent of disease in test and control sextants. Randomisation was undertaken centrally through an electronic system integrated into the electronic case report form (e-CRF) and only occurred when all inclusion/exclusion criteria had been verified and the patient was attending for the treatment visit, to ensure allocation concealment. The study was performed under examiner-blind conditions. Assessing examiners (i.e. clinicians who examined patients and recorded periodontal indices), study monitors, study coordinators, data managers, and statisticians, were unaware of the treatment that was administered in the study sextants. Knowledge of the randomization list was restricted to individuals involved in the application of the study product only. All investigators, treating clinicians and clinical examiners from the three study centres undertook a 2-day joint training exercise in the administration of the phototherapy protocol and the clinical evaluations prior to study commencement. Those clinicians who performed the periodontal treatment and phototherapy procedure took no part in any other study procedures or evaluations.

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Study procedures and outcome measures

The study protocol is detailed in Figure 1. At visit 1 (baseline), written informed consent was obtained, and demographic and medical history data were collected. Randomisation of sextants occurred at visit 2, immediately prior to RSD. The control sextant was treated by RSD using a combination of ultrasonic and hand instruments under local anaesthesia as indicated clinically. Following this, the test sextant was treated in the same manner, except with the application and photo-activation of the gel immediately before and immediately after RSD. Thus, the sequence of treatment was: (i) control sextant RSD, (ii) test sextant, first gel application and photo-activation, (iii) test sextant RSD, (iv) test sextant, second gel application and photo-activation.

All participants were provided with a standard toothpaste (Colgate Great Regular Flavour fluoride toothpaste containing sodium monofluorophosphate 0.79%, 0.1% w/v fluoride) for use during the study. Participants were asked to continue to use their regular manual or powered toothbrush, and to use interproximal cleaning devices as indicated, but were asked to refrain from using any other adjunctive oral home care products (e.g. mouthrinses).

Participants completed a pain diary to record any pain in study sextants (test/control) following the treatment, how long it lasted following recovery of sensation (after anaesthesia had dissipated), and its severity on a 100mm visual analogue scale (VAS, limits marked ‘no pain’ and ‘unbearable pain’). The clinician who provided the treatment at visit 2 indicated the sextants to be considered in the pain diary (e.g. upper right, lower left, but did not specify where gel had been applied), and this was returned by post to the study centre within 24 hours and received by staff who took no part in any clinical assessments to maintain blinding.

General health status, oral soft tissue status, and details of any concomitant therapy or adverse events were recorded at each visit. Periodontal parameters were recorded at 6 sites per tooth at all visits except visit 2. Plaque score was determined following disclosing (Butler Gum Red-Cote) as absent (0) or present (1). PPDs and recession were recorded using a manual UNC-15 periodontal probe and bleeding on probing (BOP) was recorded as absent (0) or present (1) following probing at each site. Clinical attachment level (CAL) was calculated from PPD and recession. If RSD was also required in regions other than the test and control sextants, this was provided between 1 and 7 days following visit 2. At visits 3 and 4, if supportive periodontal therapy or reinforcement of oral hygiene was required, this was provided following the collection of study data. Following visit 5 (end of study), participants were entered into routine recall for periodontal maintenance or scheduled for any further interventions as indicated clinically. Participants who withdrew from the study prematurely underwent the final examination at the time of study termination.

Statistical analyses

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The primary efficacy variable was change in mean CAL at deep sites (sites with PPD ≥5 mm at baseline) from baseline to 24 weeks. A sample size of 50 participants was determined to be sufficient to detect a clinically relevant difference of 1 mm in the primary efficacy variable, assuming a standard deviation (SD) of 2 mm in both groups, using a two-tailed paired test of the difference between treatment means, significance level α=0.05 and 90% power (Kamma et al., 2015). To allow for drop-outs, the recruitment target was 60 participants.

The unit of analysis was the study sextant, i.e. test sextants treated with RSD plus adjunctive phototherapy, and control sextants treated with RSD only. Analysis was performed comparing test sextants vs. control sextants. The primary efficacy variable (mean CAL change at deep sites) was analyzed by analysis of covariance (ANCOVA), with subject (random factor), treatment (fixed factor), study centre (fixed factor), number of deep sites at baseline (≤4 deep sites or >4 deep sites, fixed effect) and centre-by-treatment interaction (fixed factor) as terms, and baseline mean CAL as covariate in the model. Analysis of the primary efficacy variable (and also mean CAL change at all sites) was conducted for the intent-to-treat (ITT) population using a last observation carried forward (LOCF) approach. The secondary efficacy variables were analysed using the same ANCOVA model, and included changes in mean plaque scores, PPDs (at deep sites and all sites) and %BOP. Analysis of the secondary efficacy endpoints was conducted for the ITT population but did not use a LOCF approach. Normality testing was performed using the Shapiro Wilk test, and in the case of non-normal distribution of the data, the ANCOVA model was applied on rank transformed data. Safety variables were the number of adverse events (AEs) and serious adverse events (SAEs) occurring. Systemic AEs were analysed at the patient level, while oral cavity AEs were analysed at the sextant level by treatment group.

Two analysis sets are reported: an ITT population and a safety population. The ITT population (n=60) consisted of each randomised participant in the study who received the study treatment, and from whom at least one post-treatment measurement was available in both study sextants. The safety population consisted of all participants who received the study treatment (n=69).

RESULTS

A total of 81 potential participants were screened, and 69 were randomised to study treatment (safety population). Of these, 68 participants completed the study, with one participant discontinuing the study early due to a SAE. Nine of the 69 randomised participants were excluded from the ITT population because of using systemic antibiotics during the study. Accordingly, the ITT population consisted of 60 participants (Newcastle: n=24; London: n=19; Ferrara: n=17). Figure 2 presents the flow chart of the study.

The mean ± SD age of the ITT population was 51.4±8.4 years, 29 (48.3%) were female, and the racial distribution was white Caucasian: 50 (83%); Black or African American: 2 (3.3%); Asian: 5 (8.3%) and other: 3 (5.0%). 4 participants (8.3%) were cigarette smokers, smoking an average of 11.8 ± 5.4 per day, and one

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smoked cigars (3 per day). Regarding use of oral hygiene products, 25 (41.7%) used a manual toothbrush and 35 (58.3%) used a powered toothbrush. 56 participants (93.3%) reported routine use of interdental cleaning aids; of these, dental floss was used by 25.0%, interdental brushes by 92.9% and woodsticks by 5.4%.

Table 1 presents mean CAL data at deep sites and all sites over the course of the study, and Table 2 presents mean CAL change data at deep sites and all sites from visit 1 (baseline) to visit 5 (week 24), using the LOCF approach. With regards to the primary efficacy variable, although mean CAL values were significantly lower at both deep sites and all sites in text and control sextants at visit 5 compared to baseline, there were no statistically significant differences in CAL change between test and control sextants from baseline to week 24 (Table 2, deep sites p=0.212; all sites p=0.255). A subsequent sensitivity analysis performed by means of a mixed-effect ANCOVA model fitted according to the conventional LOCF approach without adjustment for number of deep sites at baseline provided further confirmation of group homogeneity, with no statistically significant differences identified between test and control sextants (p=0.223). An additional per-protocol analysis (which excluded data from any participants who experienced protocol deviations) also failed to identify statistically significant differences between test and control sextants over the course of the study (data not shown). Furthermore, no statistically significant differences in CAL change (at deep sites or all sites) between test and control sextants were identified at the individual treatment centres (data not shown).

Table 3 presents mean CAL and PPD data (for deep sites and all sites) as well as %BOP and plaque, and Table 4 presents change data from visit 1 (baseline) to visit 3 (week 6), visit 4 (week 12) and visit 5 (week 24), not using the LOCF approach. For mean CAL (deep sites and all sites), PPD (deep sites and all sites), and %BOP (all sites), statistically significant reductions were observed from baseline to all post-treatment time points (all $p<0.0001$). However, comparisons between test and control sextants for all parameters and for all change intervals failed to identify any significant differences between test and control (all $p>0.05$). Furthermore, no significant changes in plaque scores were detected between baseline and any post-treatment time point in either test or control sextants, or between test and control sextants at any change interval. Sensitivity analyses performed without adjustment for number of deep sites at baseline yielded similar outcomes, with no significant differences between test and control sextants identified for any assessed variable (all $p>0.05$). Furthermore, no significant differences (centre effects) in the secondary efficacy variables were identified when analyzing data from the individual treatment centres (data not shown). We also evaluated the number of residual deep sites (PPD ≥5 mm) at visit 5, but found no evidence of differences between groups; in test sextants, the mean number of deep sites reduced from 9.2 ± 4.9 (visit 1) to 5.1 ± 4.5 (visit 5), compared to 9.7 ± 5.0 (visit 1) to 5.3 ± 4.3 (visit 5) in control sextants.

Analysis of the pain diaries completed after visit 2 revealed that 19 participants reported pain/discomfort in test sextants, which lasted for 11.1 ± 9.2 hours, compared to 15 participants who reported pain/discomfort

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in control sextants, which lasted for 9.1 ± 9.7 hours. Mean VAS scores for severity of pain/discomfort were 28.2 ± 23.7 mm in test sextants and 26.5 ± 30.3 mm in control sextants. No significant differences between test and control sextants were evident for either duration or severity of pain/discomfort following treatment.

Analysis of safety outcomes (safety population, n=69) revealed one SAE (myocardial infarction, not considered related to study procedures or study participation) recorded in one participant who exited the study between visits 3 and 4. The most frequently recorded systemic adverse event was influenza/respiratory tract infections, reported by 7 participants. The most frequently reported oral cavity-related adverse events were procedural pain (e.g. as a result of RSD or other study procedures such as probing, test sextants, n=40; control sextants, n=37), and sensitivity/toothache (test sextants, n=4; control sextants, n=5).

DISCUSSION

In our study, using a split-mouth RCT design, we aimed to evaluate the efficacy of a new commercially-available phototherapy that had shown initial promise in the management of periodontitis, as reported in a EuroPerio abstract (Kamma et al., 2015). Our findings demonstrated no clinical benefit of the adjunctive treatment, whether considering either the primary efficacy endpoint (mean CAL change at deep sites from baseline to week 24), or the secondary efficacy endpoints (mean CAL change at all sites, mean PPD change at deep sites and all sites, mean change in %BOP and plaque scores). We could not identify statistically significant differences between test and control sextants for any clinical parameter, or across any change interval from baseline, whether considering the ITT or per protocol analysis.

Post-treatment CAL and PPD changes in test and control sextants were consistent with reports from the literature given the baseline PPD values (Cobb, 2002). Furthermore, %BOP scores also reduced following treatment, with levels at week 24 (27-30%) reasonably consistent with those that might be expected following an initial course of non-surgical therapy (Claffey et al., 2004). However, plaque scores remained consistently high throughout the study, with no clear evidence of reductions following treatment and were well above typical target values that might be considered in periodontal therapy. The reason for this finding is not clear, particularly given that the (clinically relevant) PPD and BOP reductions were consistent with what would be expected following non-surgical treatment. We consider that the method of plaque scoring (using disclosing solution) in combination with a low threshold for examiners to indicate the presence of plaque at a site may have contributed to the high recorded plaque scores. We did not identify any evidence of a centre effect in relation to the plaque scores, and consider that a plaque index system (e.g. the Silness and Loe Plaque Index) may have provided better sensitivity for detecting changes in plaque levels (Silness and Loe, 1964).

Our primary outcome variable was change in mean CAL. However, we also recognize the current guidance that threshold changes (e.g. CAL gain $\geqslant$ 2 mm or $\geqslant$ 3 mm) are preferable to use as they are more

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clinically relevant (Loos and Needleman, 2020). These authors also stated that the target outcome for periodontal treatment should be resolution of inflammation as manifested by PPD and BOP reductions, and that the achievement of shallow pockets ($\leqslant$ 4 mm) that do not bleed on probing confers the highest chance of periodontal stability. Indeed, a key factor for clinicians when evaluating the outcome of therapy is to assess the number of deep sites (e.g. PPDs $\geqslant$ 5 mm) that remain. Accordingly, we evaluated this outcome, and identified that there was no difference observed between test and control sextants, which demonstrated similar numbers of deep residual sites at visit five (5.1 ± 4.5 and 5.3 ± 4.3 in the test and control sextants, respectively).

Our study has some limitations. Whereas every precaution was taken to ensure examiner blinding, we were not able to blind participants, as this would have involved the use of a placebo gel (which we did not have access to), and/or sham light activation. On the other hand, a positive aspect of our study was the multi-centre approach with a reasonably large participant population. We utilized a split-mouth design, principally in an attempt to reduce the potential for any differences between subjects to influence group comparisons that might have occurred if we had randomized at the patient level. However, we recognize the limitation of this approach in that treatment in the test sextants may potentially carry across to control sextants (Hujoel and DeRouen, 1992), and careful consideration to this matter should be given in future studies with regards to the use of split-mouth versus parallel-group designs.

According to the manufacturer's instructions and protocol for this commercially available product, the adjunctive phototherapy treatment was applied both before and after debridement of the root surfaces as it was supposed to facilitate instrumentation (Kamma et al., 2015). However, this EuroPerio abstract does not provide any detail on what is meant by treatment being facilitated or how this was measured. Our data demonstrated no efficacy of the adjunctive protocol over RSD in the treatment of periodontitis and accordingly we cannot recommend its use following the specified protocol. Although the intimate mechanism of biostimulation has been proven in other medical fields (Hamblin, 2017, Hamblin, 2018, Passarella and Karu, 2014), the scientific background for periodontal application of the technology that was tested in this research is, to the best of the author's knowledge, unknown, and we are unaware of any publications in the scientific literature that confirm the mechanism of action of the chromophore gel (i.e. fluorescence biostimulation) in the subgingival environment. Many PBM protocols rely on the application of specific light wavelengths, and this may have a major impact on tissue responses. According to the manufacturer's instructions for this adjunctive therapy, we used a dental curing light emitting a broad spectrum of 385 nm - 515 nm, and delivered the light to the depth of the pocket by using a clear plastic probe-like tip. A major issue is the amount of energy that could be converted by the chromophores. In the previously published data on this product (EuroPerio abstract), significant clinical improvements were obtained following application of light at wavelengths 532 nm and 430 nm (Kamma et al., 2015). In our study, activation of the chromophores was considered to require light at wavelength 430 nm, but not 532 nm, and the wavelengths with energy transfer maxima of the dental curing light (i.e., 405 nm and 465 nm)

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did not correspond to those needed for chromophore activation. In addition, the known power density of the LED light (that was referred to a distance of 3-5 mm between the light source and the target) may have suffered from a substantial energy loss when transferred by the working tip to the periodontal pocket. Thus, it may be that only a fraction of the energy was used, preventing adequate power density at the tip within the periodontal pocket with the most suitable wavelength distributions to cause chromophore activation, if indeed chromophore activation was occurring.

These aspects raise the question on why the findings of this study should be published. A protocol has been tested in an appropriately designed RCT. Ethical approval was granted for the study, and the product is licensed (with a CE mark in Europe and a Medical Device License in Canada) and commercially-available. Patients and clinicians may become aware of the product and wish to know whether or not its use would be of benefit. Based on the evidence from our study, we cannot support the use of this product (according to the protocol followed), in the treatment of periodontitis. Given that patients have been exposed to an intervention, we consider it important from an ethical perspective that the findings are published so that the clinical community can become aware of the outcomes, and make more informed decisions on whether to use the product according to the protocol suggested by the manufacturer. However, we recognise the fundamental concern that despite a multicentre RCT being conducted, little, if any, biological effect might be expected given that it is not known whether the treatment protocol employed was able to generate the desired outcome (fluorescence biostimulation) in the context of periodontitis treatment. It can be speculated that a different outcome might be seen with a more powerful light source, or if different specific wavelengths of light were delivered to the chromophore *in situ*. The EuroPerio abstract (Kamma et al., 2015) reports only clinical data, and we are unaware of any publications in the scientific literature that document the mechanism of action of the tested combination of chromophore gel and light source in the subgingival environment.

Concerns regarding specific technologies used as adjunctive periodontal therapies have recently been raised in the context of aPDT. Following publication of the systematic review and meta-analysis on the use of aPDT in non-surgical periodontal treatment (Salvi et al., 2020), a subsequent letter to the editor raised concerns about some of the included studies, specifically that they utilised laser wavelengths that did not correspond to the absorption peak of the photosensitizer employed in the study (Damante, 2021). Given that, in order for a photodynamic reaction to occur, the absorption peak of the photosensitizer should be in the same range as the laser wavelength, the author makes the observation that in studies where this was not the case, then the desired photodynamic reaction would be unlikely to occur, and thus there would be little or no clinical benefit. This can create problems in interpretation of data from multiple studies (e.g. in the context of a meta-analysis) because the systematic review process does not necessarily evaluate all the technical details of included studies (Salvi et al., 2021). Clearly, it is important that future investigations of phototherapy protocols should use light wavelengths that match the absorption peak of the photosensitiser employed (Chapple & Jepsen, 2021).

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Accordingly, we consider it important that commercially-driven protocols of new products are carefully evaluated prior to committing to conduct larger scale multi-centre clinical trials. Preliminary studies should provide evidence not only on clinical outcomes, but also mechanisms of action. It is clear that appropriately designed RCTs are essential for reducing the potential bias in the evaluation of new products. The absence of clinical efficacy in our study might have been expected, given the lack of published scientific data regarding any biological effect in the subgingival environment of the combination of chromophore gel and application of light that was used.

CONCLUSION

Within the limitations of our research, we found that the tested adjunctive phototherapy protocol did not provide any additional clinical benefits over those achieved by RSD alone in terms of CAL gain, or PPD and BOP reductions. This may have resulted from a lack of biological effect of the tested protocol, and it is important that future studies must be founded on published data that not only demonstrate clinical effect of new products in pilot studies, but also provide robust evidence of mechanism of action.

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Table 1 **Mean CAL values in test and control sextants at each time point**

	Visit 1 (baseline)		Visit 3 (week 6)		Visit 4 (week 12)		Visit 5 (week 24)	
	Test sextants (n=60)	Control sextants (n=60)	Test sextants (n=60)	Control sextants (n=60)	Test sextants (n=60)	Control sextants (n=60)	Test sextants (n=60)	Control sextants (n=60)
CAL deep sites (mm)	6.61 ± 1.00	6.40 ± 0.95	5.69 ± 1.61	5.43 ± 1.23	5.67 ± 1.68	5.49 ± 1.28	5.61 ± 1.68	5.52 ± 1.28
CAL all sites (mm)	4.53 ± 1.22	4.51 ± 1.12	4.22 ± 1.12	4.22 ± 1.07	4.23 ± 1.13	4.32 ± 1.13	4.23 ± 1.13	4.33 ± 1.14

Data presented as mean ± standard deviation for the intent-to-treat (ITT) population according to the last observation carried forward (LOCF) approach. CAL, clinical attachment level.

Table 2 **Mean change data for CAL in test and control sextants from visit 1 (baseline) to visit 5 (week 24)**

	Change: visit 1 to visit 5		
	Test sextants (n=60)	Control sextants (n=60)	p value (T vs. C)†
CAL change deep sites (mm)	-1.00 ± 1.16*	-0.87 ± 0.79*	0.212
CAL change all sites (mm)	-0.31 ± 0.71*	-0.18 ± 0.69*	0.255

Data presented as mean ± standard deviation for the intent-to-treat (ITT) population according to the last observation carried forward (LOCF) approach. Negative values for CAL change indicate reduction from visit 1 to visit 5 (i.e. CAL gain, clinical improvement). CAL, clinical attachment level.

† p value for mixed-effect ANCOVA model for comparison of change data between test (T) versus control (C) sextants.

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* statistically significant reduction from baseline within test or control sextants, $p<0.0001$.

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Table 3 **Mean CAL, PPD, BOP and plaque values in test and control sextants at each time point**

	Visit 1 (baseline)		Visit 3 (week 6)		Visit 4 (week 12)		Visit 5 (week 24)	
	Test sextants (n=60)	Control sextants (n=60)	Test sextants (n=60)	Control sextants (n=60)	Test sextants (n=59)	Control sextants (n=59)	Test sextants (n=59)	Control sextants (n=59)
CAL deep sites (mm)	6.61 ± 1.00	6.40 ± 0.95	5.69 ± 1.61	5.43 ± 1.23	5.70 ± 1.68	5.52 ± 1.27	5.59 ± 1.69	5.43 ± 1.20
CAL all sites (mm)	4.53 ± 1.22	4.51 ± 1.12	4.22 ± 1.12	4.22 ± 1.07	4.25 ± 1.13	4.35 ± 1.11	4.22 ± 1.14	4.30 ± 1.14
PPD deep sites (mm)	5.94 ± 0.60	5.82 ± 0.56	4.57 ± 1.02	4.51 ± 0.96	4.62 ± 1.02	4.54 ± 0.90	4.47 ± 1.08	4.41 ± 0.81
PPD all sites (mm)	3.89 ± 0.94	3.91 ± 0.84	3.29 ± 0.73	3.34 ± 0.72	3.27 ± 0.76	3.39 ± 0.73	3.22 ± 0.76	3.31 ± 0.76
BOP all sites (%)	51.6 ± 27.3	47.7 ± 27.9	32.0 ± 23.0	31.4 ± 22.3	30.7 ± 22.3	31.3 ± 21.9	29.6 ± 23.3	27.5 ± 23.0
Plaque all sites (%)	63.9 ± 26.2	62.6 ± 24.8	62.6 ± 23.8	64.6 ± 21.6	62.2 ± 21.6	60.0 ± 23.4	61.7 ± 22.4	57.2 ± 23.8

Data presented as mean ± standard deviation for the intent-to-treat (ITT) population, not using the last observation carried forward (LOCF) approach.

CAL, clinical attachment level; PPD, probing pocket depth; BOP, bleeding on probing.

Table 4 **Mean change data for CAL, PPD, BOP and plaque in test and control sextants from visit 1 to visits 3, 4 and 5**

	Change: visit 1 to visit 3			Change: visit 1 to visit 4			Change: visit 1 to visit 5		
	Test sextants (n=60)	Control sextants (n=60)	p value (T vs. C)†	Test sextants (n=59)	Control sextants (n=59)	p value (T vs. C)†	Test sextants (n=59)	Control sextants (n=59)	p value (T vs. C)†
CAL change deep sites (mm)	-0.92 ± 1.12*	-0.97 ± 0.87*	0.991	-0.93 ± 1.22*	-0.89 ± 0.80*	0.406	-1.01 ± 1.17*	-0.91 ± 0.76*	0.230

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CAL change all sites (mm)	-0.31 ± 0.60*	-0.30 ± 0.59*	0.965	-0.31 ± 0.65*	-0.19 ± 0.61*	0.139	-0.32 ± 0.72*	-0.21 ± 0.65*	0.334
PPD change deep sites (mm)	-1.37 ± 0.86*	-1.31 ± 0.82*	0.764	-1.33 ± 0.89*	-1.28 ± 0.83*	0.967	-1.46 ± 0.93*	-1.37 ± 0.69*	0.858
PPD change all sites (mm)	-0.60 ± 0.55*	-0.56 ± 0.57*	0.619	-0.63 ± 0.57*	-0.53 ± 0.59*	0.195	-0.69 ± 0.56*	-0.61 ± 0.56*	0.481
BOP change all sites (%)	-19.6 ± 20.4*	-16.3 ± 19.6*	0.733	-21.3 ± 22.7*	-17.0 ± 19.0*	0.527	-22.3 ± 21.5*	-20.7 ± 17.9*	0.657
Plaque change all sites (%)	-1.24 ± 18.0	1.97 ± 19.5	0.437	-1.61 ± 21.5	-2.52 ± 20.8	0.451	-2.81 ± 20.1	-5.6 ± 23.2	0.188

Data presented as mean ± standard deviation for the intent-to-treat (ITT) population, not using the last observation carried forward (LOCF) approach. Negative values indicate reductions (i.e. clinical improvement) from visit 1 to subsequent time points (i.e. CAL gain, PPD reduction and BOP reduction). CAL, clinical attachment level; PPD, probing pocket depth; BOP, bleeding on probing.

† p value for mixed-effect ANCOVA model for comparison of change data between test (T) versus control (C) sextants.

* statistically significant reduction from baseline within test or control sextants, $p<0.0001$.

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FIGURE LEGENDS

Figure 1: Study visit protocol.
V, visit number; RSD, root surface debridement.

Figure 2: Study flow chart.
ITT, intent-to-treat; LOCF, last observation carried forward; RSD, root surface debridement.

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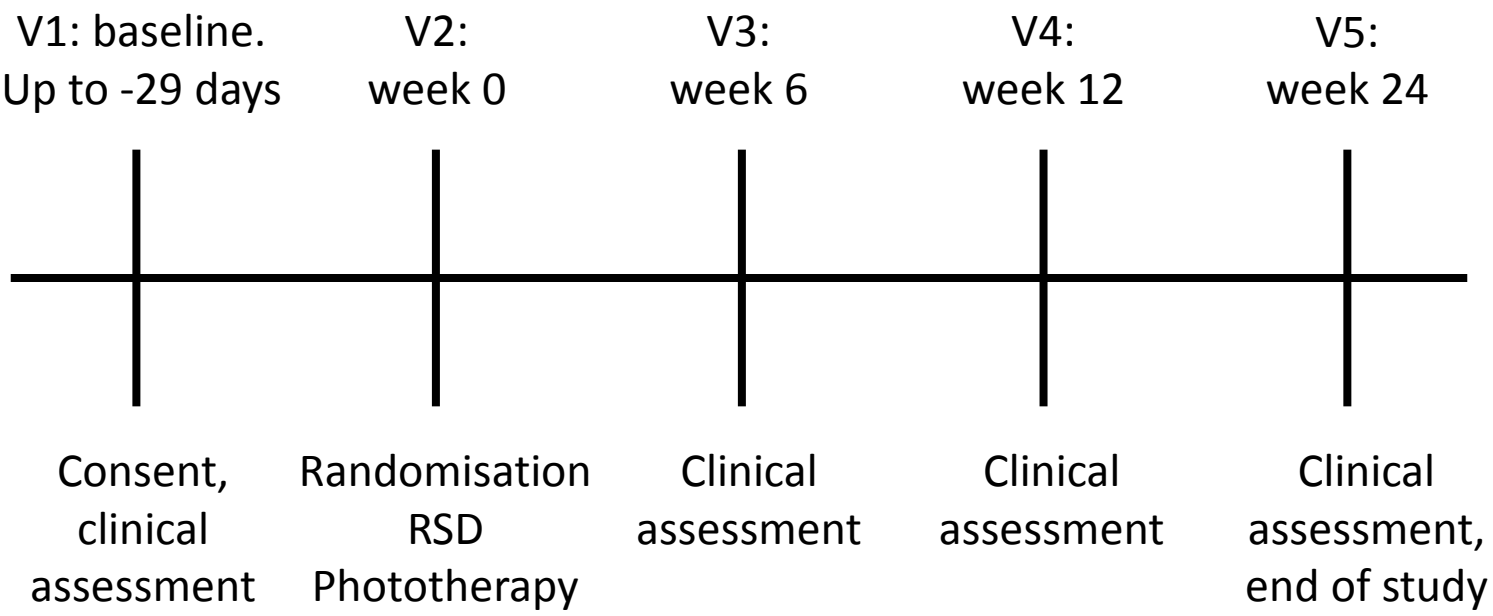

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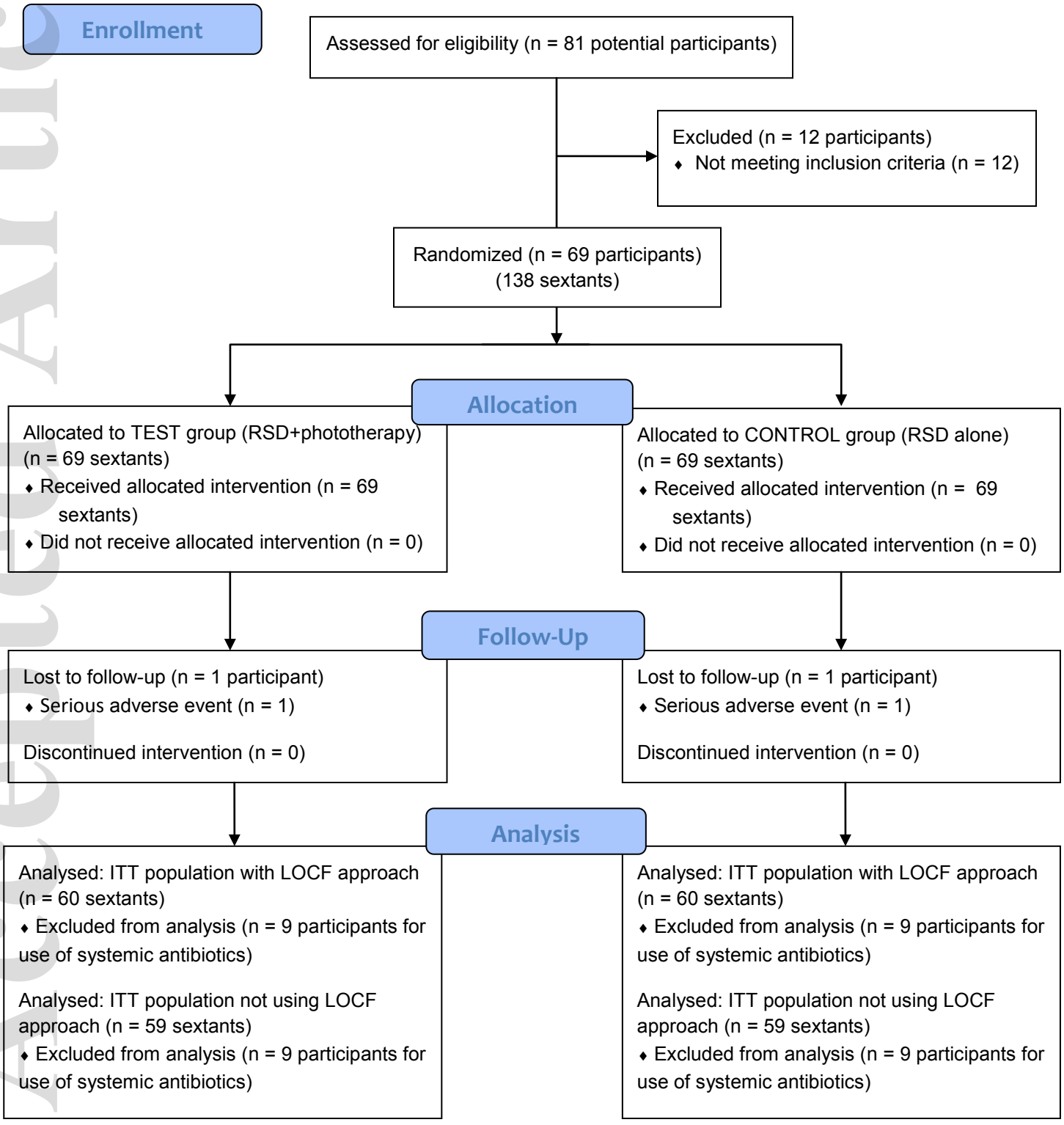

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