



BMJ Open Antimicrobial photodynamic therapy with erythrosine and blue light on dental biofilm bacteria: study protocol for randomised clinical trial

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ABSTRACT

Introduction The objective is to investigate the effect of antimicrobial photodynamic therapy (aPDT) mediated by erythrosine and a blue light-emitting diode (LED) in the reduction of bacteria in dental biofilm.

Methods and analysis This clinical trial will be conducted with 30 patients who have biofilm, but without the presence of periodontal pockets, and who are being treated at the Dental Clinic of Universidade Metropolitana de Santos. A split-mouth model will be used (n=30), with group 1 control (conventional treatment) and group 2 (conventional treatment and aPDT). The bicarbonate jet will be used to remove dental biofilm in both groups. The treatment will be carried out in one session. aPDT will be performed before cleaning/prophylaxis, only in group 2. Participants will rinse with the photosensitiser erythrosine (diluted to 1 mM) for 1 min of pre-irradiation time, so that the drug can stain all the bacterial biofilm. Then, the D-2000 LED (DMC) will be applied, emitting at a wavelength of $\lambda=470$ nm, radiant power of 1000 mW, irradiance of 0.532 W/cm² and radiant exposure of 63.8 J/cm². Irradiation will be performed until the biofilm of the cervical region is illuminated for 2 min/point (4 cm²). The microbiological examination will be performed from samples of supragingival biofilm collected from the gingival sulcus. Collection will be performed in each experimental site before irradiation, immediately after the irradiation procedure and after the prophylaxis. Colony-forming units will be counted and the data will be submitted for statistical analysis for comparison of pretreatment and post-treatment results and between groups (conventional X aPDT).

Ethics and dissemination This study has been approved by the Ethics Committee of Universidade Metropolitana de Santos under process number 66984123.0.0000.5509. Results will be published in peer-reviewed journals and will be presented at conferences.

Trial registration number NCT05805761.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ Procedures using antimicrobial photodynamic therapy have been developed for the decontamination of dental biofilm, but there are few controlled clinical trials in the literature confirming its efficacy.
- ⇒ Erythrosine is a dye that dentists use for bacterial plaque evidence. Therefore, it is accessible for them to perform the proposed technique.
- ⇒ The reddish colour of the dye allows its use with blue light-emitting diodes, devices that dentists already use in their offices.

INTRODUCTION

Dental biofilm is a three-dimensional structure of bacterial communities adhered to the tooth surface.¹ In a well-structured dental biofilm, multiple species of oral microorganisms are present. Once in the biofilm, microorganisms are less susceptible to antimicrobial substances, due to the formation of resistant cell subpopulations. There are infections, such as caries and periodontal disease, produced by the biofilm that occur due to the diversity of microbial species.² There are limitations associated with the conventional approaches to controlling dental plaque, such as lack of awareness of correct brushing techniques or difficulty manipulating a toothbrush. Increases in the incidence of antimicrobial drug-resistant bacteria show the need for the development of alternative approaches to control dental biofilm,³ such as antimicrobial photodynamic therapy (aPDT).

aPDT involves photoexcitation, which occurs when a photosensitiser (PS) dye is illuminated by a light of a matched wavelength, resulting in its activation and stimulation of a phototoxic response in the

presence of ambient oxygen.⁴ The main advantages of aPDT over the conventional antimicrobial therapies include the immediate onset of action, elimination of resistant microorganisms, local delivery of the PS and double selectivity (deleterious effect only on sites where both PS and light are delivered concomitantly).⁵ The use of aPDT for the decontamination of dental biofilm and periodontal pockets has been assessed *in vitro*,² in systematic reviews^{1,4} and in randomised clinical trials.^{3,6} Some studies have even addressed the issue of COVID-19 and how aPDT could aid in oral decontamination, including prior to dental procedure, diminishing the risks of cross-infection.^{7,8} However, most studies that tested aPDT in the dental biofilm use methylene blue as a PS, associated with a red-light source. While the use of this dye is well established in the literature, the use of red dyes with blue light sources is being more and more researched.

Red dyes such as curcumin, erythrosine and porphyrin are being used in studies as potential PSs.^{9–12} In the dentistry field, the biggest advantage in using red dyes lies in the fact that dentists already use blue light-emitting diodes (LEDs) in their offices, due to the photopolymerisation of composite resins.¹³ Since the use of red dyes allows the performance of aPDT with a blue-light source, it makes the therapy more accessible to dentists. Erythrosine has been used for aPDT against microorganisms such as *Streptococcus mutans*,^{14,15} *Lactobacillus casei* and *Candida albicans*.^{16,17} The use of this dye combined with LEDs has also been tested *in vitro*^{18,19} and in studies with mice.^{20,21} Moreover, erythrosine is also already present in dental offices, as it is used for dental plaque evidence.²² Consequently, the use of aPDT with erythrosine and blue LEDs could very easily be introduced in dental clinical practice.

Even though the technique of aPDT with erythrosine and LEDs has been addressed in the literature, PS concentrations and light parameters differ between studies, making it difficult to establish a protocol and reinforcing the need for clinical trials in this area. In addition, there is a need to test alternative ways for dental biofilm decontamination. Taking this into consideration, the objective of this study is to investigate the effect of aPDT mediated by erythrosine and blue LED in the reduction of bacteria in dental biofilm.

METHODS AND ANALYSIS

Study design

The present study is characterised as a protocol for a clinical, microbiological, randomised, parallel-group, superiority and split-mouth trial. It will be carried out at the Dental Clinic of Universidade Metropolitana de Santos (UNIMES). Recruitment will begin on 20 August 2023, and the anticipated ending date for the study is 20 December 2023. As it is a protocol for a clinical trial, this paper was written in accordance with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines. Figure 1 shows an activity diagram and it is the SPIRIT figure.

Participants

This clinical trial will be carried out with individuals who have biofilm, but without the presence of periodontal pockets, and who are being treated at the Dental Clinic of UNIMES or who are referred for treatment at this centre, where all clinical procedures will be carried out. As they will already be in treatment, it is believed that

TIMEPOINT**	Enrolment	Allocation				Close-out
	20 August 2023 – 20 November 2023	0	t ₁ Baseline	t ₂ Immediately after the dye application (G1) X irradiation (G2)	t ₃ Immediately after the prophylaxis	20 November 2023 – 20 December 2023
ENROLMENT:						
Eligibility screen	X					
Informed consent	X					
Allocation		X				
INTERVENTIONS:						
Conventional treatment (bicarbonate jet)			X	X	X	
Antimicrobial photodynamic therapy (aPDT)			X	X	X	
ASSESSMENTS:						
Microbiological Evaluation			X	X	X	
Statistical Analysis						X

Figure 1 Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) figure as recommended by 2013 SPIRIT statement.

this will improve recruitment and adherence to the trial. Research participants must read, understand and sign a written informed consent form, which was approved by the Human Research Ethics Committee of the university. The same researcher (who is a dentist) responsible for the procedures will be in charge of obtaining the consent form. The researcher will explain all the procedures to be carried out, the purpose of the trial, the risks and benefits of participating in the research, and the fact that the volunteers have complete freedom to choose to participate and/or continue in the research, being able to withdraw at any time, without prejudice. If the participant shows any discomfort, which is not expected, during the treatment session, it will be discontinued and the participant will be excluded from the study. However, they will still be followed up for safety assessment. Seeing as the trial will be conducted in one session, there is no relevant concomitant care to be permitted or prohibited during the trial, and there will be no follow-up sessions to be completed. No major harms are expected, but possible interurrences, such as tooth or gum pain, will be monitored and recorded. Any additional assistance participants may need will be provided.

Sample size calculation

The number of participants ($n=30$) was based on a previous study carried out by Bhat *et al.*²³ With the medians obtained in this study, the calculation was performed on the ClinCalc.com website, using a power of 95% (alpha 0.05), usually adopted in clinical/microbiological trials. The calculation showed that 30 participants would be needed per group.

$$k = \frac{n_2}{n_1} = 1$$

$$n_1 = \frac{(\sigma_1^2 + \sigma_2^2 / K) (\bar{z}_{1-\alpha/2} + \bar{z}_{1-\beta})^2}{\Delta^2}$$

$$n_1 = \frac{(0,94^2 + \frac{0,94^2}{1}) (1,96 + 1,64)^2}{0,87^2}$$

$$n_1 = 30$$

$$n_2 = K \times n_1 = 30$$

Inclusion criteria

- ▶ The participants must present with between 25% and 50% of biofilm index, to standardise the amount of biofilm to be decontaminated.
- ▶ Have at least 20 teeth present, without clinical probing depths greater than 3mm, because bigger measurements could indicate periodontal disease and different types of bacteria among participants. The same researcher will perform the measurement in all participants, in order to standardise force.
- ▶ Participants with a minimum age of 18 years and a maximum age of 30 years will be recruited, so an age range is respected in the study, providing a more homogeneous sample.

Exclusion criteria

- ▶ Smokers, due to higher risk of periodontal diseases and different microbiota.
- ▶ Participants with false pockets, caused by gingival overgrowth.
- ▶ Uncontrolled diabetes or hypertension, because prophylaxis is not a risk-free procedure, and these conditions should be treated prior to such procedures.
- ▶ Cancer or its treatment, because these patients may be immunocompromised.
- ▶ Pregnant women, seeing as this is usually not an essential procedure for them.
- ▶ Use of antibiotics in the last 6 months, which may change oral microbiota.
- ▶ Coagulation disorders (use of anticoagulants, presence of liver diseases, thrombocytopenia and immunosuppression) due to the risk of bleeding.
- ▶ Patients undergoing orthodontic treatment, seeing as appliances might also alter the microbiota or hygiene conditions.

Randomisation

Since the study is a split-mouth model, the 30 participants will receive different treatments, one on each side of the mouth. That is, the treatments assigned to groups 1 and 2 will be performed on the same participant, one on each hemiarch of the mouth. On the side referring to group 1 ($n=30$), prophylaxis with a bicarbonate jet will be performed, as a conventional treatment. On the side referring to group 2, aPDT will be performed before the bicarbonate jet prophylaxis. For the random distribution of groups 1 and 2 between the sides, randomisation will be performed by drawing lots, using the research randomiser program (<https://www.randomizer.org/>). The allocation sequence will be made by a different researcher, who will not be involved in the procedures.

Clinical procedures

The researchers responsible for the treatment application will enrol participants and assign participants to interventions. The study is not blind. Researchers will be previously trained to collect data and perform evaluations, according to the parameters described in procedures and outcome measures. Individuals will be previously examined for evaluation of periodontal clinical parameters, with a 15 mm North Carolina millimetre probe for measurements of: plaque index, bleeding on probing, probing depth, gingival recession and clinical attachment loss. All patients will receive a demonstration of oral hygiene techniques, with instructions on brushing technique and recommendation for daily use of dental floss.

The bicarbonate jet (Dabi Atlante, Ribeirão Preto, São Paulo, Brazil) will be used to remove the biofilm in both groups. It has an autoclavable tip and works with a pressurised mixture of air, water and sodium bicarbonate, conducted through ducts to the nozzle of the handpiece, where it forms a uniform jet used to remove

bacterial plaque, alba matter and dental stains. Dabi Atlante sodium bicarbonate sachet will be used, which has particles of the appropriate size for the device (extra fine granulation). The 'sodium bicarbonate jet' function does not have a power indication, as it works with stabilised air pressure (45 a 50 PSI). Treatment time will be of 20 s per tooth. The treatment will be carried out in one session. aPDT will be performed before each cleaning/prophylaxis, only in the side correspondent to group 2. Participants will swish with the PS erythrosine (diluted to 1 mM) for 1 min of pre-irradiation time, so that the drug can stain all the bacterial biofilm. The D-2000 LED (DMC) will be applied, emitting at a wavelength of $\lambda=470$ nm, radiant power of 1000 mW, irradiance of 0.532 W/cm² and radiant exposure of 63.8 J/cm². Irradiation will be performed until the biofilm of the cervical region is illuminated for 2 min/point. Each irradiation point will be approximately 0.4 cm². In order to avoid possible heat problems in the gingiva, a stop will be placed in the tip of the device, so that it will be kept at a distance of 2 cm from the tissue. All the vestibular surfaces of the aPDT side of the mouth will be irradiated.

The treatments between groups will be compared to check whether aPDT can promote biofilm contamination before its removal, possibly decreasing bacterial load in the teeth.

Outcome measures: microbiological evaluation

This is the only and primary outcome of the study. The microbiological examination will be carried out from biofilm samples collected from the gingival sulcus of the upper canines (teeth 13 and 23). Collection will be performed in each experimental site before irradiation, immediately after the irradiation and after the prophylaxis procedure. To collect the subgingival biofilm, the teeth will be isolated with cotton rolls, the supragingival biofilm will be removed with sterile gauze and the subgingival biofilm sample will be obtained by introducing a tip of sterile absorbent paper (#30) inside the periodontal pocket, being kept in position for 30 s. The tips will be removed and stored in properly identified sterile plastic microtubes; each tube containing 1 mL of sterile brain heart infusion (BHI) culture medium will be packed on ice and analysed immediately after collection.

The samples will be used to determine the CFUs (colony-forming units). Each tube with 1 mL BHI will be vortexed and will undergo serial dilution from 10⁻¹ to 10⁻⁵ times the original concentration. Aliquots of 10 μ L in five dilutions will be seeded in the form of streaks on the surface of blood agar in Petri dishes. The plates will be incubated at 37°C for a period of up to 72 hours under anaerobic conditions for evaluation of total recovered bacteria. After this period, the CFUs will be counted and the data will be submitted for statistical analysis.

Statistical analysis

The collected data will be tabulated (MS Office, Excel V.2010) and submitted for statistical analysis using the

SPSS statistical package (V.20; IBM Corporation), in computers to which only the authors will have access and only they will be able to edit the information, maintaining data accuracy and validation. Distribution will be determined by the Shapiro-Wilk test after logarithmic transformation of the mean values of the CFUs. In case data are parametric, Student's t-test will be used for intergroup (unpaired t-test) analysis and analysis of variance will be used for the intragroup analysis. If data are non-parametric, the Friedman test will be used for the intragroup analysis, and the Mann-Whitney test will be used for the intergroup analysis. As the treatment will be carried out in one session, no losses are expected.

Expected results

It is expected that there is a significant difference between the groups, with a greater microbial reduction in group 2, verifying the effectiveness of aPDT in biofilm decontamination.

Ethics and dissemination

The study will be conducted in accordance with the ethical standards in the Declaration of Helsinki (World Medical Association Declaration of Helsinki, 2008). The protocol was approved by the Ethics Committee of UNIMES (number 66984123.0.0000.5509, report number 5.956.128). Changes in the protocol will be reported to this same committee and altered in ClinicalTrials.gov. Patients will be informed of all the possible risks involved in the trial and the confidentiality of the data. The participants will be told that they can withdraw from the study at any time for any reason. The researchers will also be able to remove the participants from the study, if needed.

The data sets generated in the study will be available from the corresponding author at a reasonable request. Once the data are entered electronically, participants' identification details will no longer be attached to their data, as they will be reported only by numbers. The authors intend to publish the results in a peer-reviewed journal and present them in scientific events. The corresponding author can make the final decision to terminate the trial, if deemed necessary. This will only happen in case it becomes impossible to clinically attend the participants as, for example, in outbreaks of infectious diseases. Only the authors will have access to these interim results.

The authors themselves will coordinate and steer the trial. As this is not a study that involves significant safety concerns, risks or complexity, neither is it a multicentre study of long duration, no data monitoring committee was considered required. Monitoring will be carried out monthly by sponsor personnel or representatives at the university.

Patient and public involvement

Individuals deemed eligible will receive clarifications by the researchers regarding the objectives, procedures and study design. Participants will sign a written statement of informed consent, which will also be explained orally.

Patients will not take part in recruitment or conduct of the trial. After the analysis of the data, volunteers will be invited to a meeting and the results will be shared, in case they wish to attend it. Possible burdens of the interventions will be assessed by patients. The identity of all individuals will be preserved throughout all stages of the research.

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Contributors MLLG, APTS, LJM, ACRTH, EMS and SKB participated in the conception and design of the study. MLLG, JMAGS, SI, PBM and EPF will participate in the data collection and drafted the present protocol. LJM will perform statistical analysis. MLLG, TG, ACRTH and SKB critically reviewed the manuscript for intellectual content. SKB coordinated the study. All authors read and approved the final protocol.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

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