Clinical and microbiologic outcomes of adjunctive antimicrobial photodynamic therapy in the non-surgical and surgical treatment of teeth with periodontal disease

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Protocol Title:	Clinical and microbiologic outcomes of adjunctive antimicrobial photodynamic therapy in the non-surgical and surgical treatment of teeth with periodontal disease
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Population:	20 patients, M/F, 18 years or older, no systemic conditions, UT Health School of Dentistry at Houston
Number of Sites:	Single site / UT Health School of Dentistry at Houston
Study Duration:	3 years
Subject Duration:	4 months

General Information

From the UT Health School of Dentistry, 20 patients having 2 teeth with bone loss in two different quadrants per patient will be recruited for this study. An initial exam will be done, and if the patient meets the inclusion criteria, the perio maintenance/scaling and root planing (SRP) will be done. During the non-surgical treatment, in one quadrant, only SRP will be done, and in the other quadrant, SRP with laser will be done. A periodontal re-evaluation will be done in 4-6 weeks following the non-surgical treatment, and then the patient will be brought back for osseous surgery. Three months after the osseous surgery, the pockets will be measured again. All measurements will be taken at baseline, at the periodontal re-evaluation, and 3 months after the laser therapy.

Background Information

Diode laser (660nm) will be used in conjunction with methylene blue photosensitizing dye. Antimicrobial photodynamic therapy uses low level laser light and aims to destroy pathogens around the tooth and can also act as a photobiomodulator reducing inflammation and stimulating cellular proliferation (Mizutani et al. 2016). Antimicrobial photodynamic therapy works through an interaction between the photosensitizer (methylene blue or toluidine blue), red light (625–740 nm), and oxygen. The methylene blue dye is taken up by the bacteria and the visible red light excites the dye. Excited dye transfers energy to tissue oxygen forming a singlet oxygen molecule. Singlet oxygen is highly reactive and will affect the bacterial cell by damaging the cell membrane and cell wall. The bactericidal effect is through DNA damage and more importantly damage to the cytoplasmic membrane (Takasaki et al. 2009). To activate the photosensitizer, the laser light can be delivered via a fiber placed into the pocket or transmitted through the mucosa. The photosensitizer, such as methylene blue, undergoes a strong cationic charge,

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which can bind to the outermost membrane of gram-negative bacteria and can penetrate bacterial cells. This creates selectivity of the dye to affect bacterial cells rather than human tissue cells (Takasaki et al. 2009). Also, the singlet oxygen molecule has a short lifetime and limited migration, so the reaction and cellular damage is contained within a limited space (Takasaki et al. 2009).

Managing periodontal disease with adjuncts like antiseptics and systemic antibiotics has a few disadvantages. Systemic antibiotic usage can produce antimicrobial resistance. Conventional mechanical debridement of the tooth surface is limited by the contaminated microstructure of the tooth surface. Antimicrobial photodynamic therapy may resolve some of difficulties of conventional antimicrobial and mechanical therapy and can work as an adjunctive to conventional mechanical treatment. The advantages of aPDT are its wide antimicrobial activity, can be applied multiple times without creating antibiotics resistance, and easier topical delivery reaching the tooth surface. Studies have shown that aPDT can be considered a safe and efficient technique in addition to mechanical debridement for reducing the pocket depth in chronic periodontitis patients (Derikvand et al 2020).

Null hypothesis: Antimicrobial photodynamic therapy (aPDT) has no statistically significant advantage over traditional non-surgical mechanical debridement in improving clinical outcomes (# bleeding sites (BOP), probing depth reduction (PPD), reduction in levels of pathologic microbes or pro-inflammatory cytokines) in the treatment of teeth with periodontitis.

Objectives

Primary Objectives: This study aims to compare clinical outcomes (change in BOP and PPD) after mechanical debridement of at sites exhibiting plaque induced inflammation with or without adjunctive aPDT and to also compare both these diseased sites with that of a healthy site. These measurements will be measured at baseline, at the re-evaluation appointment, and 3 months after the laser therapy.

Secondary Objectives: In addition to the clinical outcomes, plaque samples and gingival crevicular fluid will be collected to assess the microbiologic profile before and after treatment with or without aPDT and to compare the profile with the healthy site. These measurements will be measured at baseline, at the re-evaluation appointment, and 3 months after the laser therapy.

Study Design

This is randomized clinical control study designed to assess improved clinical and microbiological outcomes with the use of laser antimicrobial photodynamic therapy (aPDT) as an adjunct to the gold standard of mechanical debridement in the treatment of periodontal diseases. Twenty patients will be recruited. Clinical measurements including bleeding on probing, pocket depth, clinical attachment loss, recession, plaque, and gingival crevicular fluid (GCF) will be measured at baseline, at the perio reevaluation, and at 3 months after the laser therapy. The 2017 World Shop classification will be used to diagnosis periodontitis. Only diseased teeth will be included and only one tooth in two different quadrants per patient (split mouth design) will be studied. The hypothesis is that the periodontitis sites treated with mechanical debridement and adjunctive aPDT will have greater reduction in bleeding and pocket depth reduction than those sites treated with mechanical debridement alone. The potential for

greater healing and greater antibacterial activity on the tooth surface will benefit patients who have periodontal disease. The results can be applied in the managing and in the armamentarium for treatment of periodontal disease.

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There will be six appointments including the baseline appointment, non-surgical treatment, the periodontal re-evaluation, osseous surgery for both quadrants, and 3-month post-ops. Some patients might need more post-op appointments than others, and those appointments are not related to the study. The details of each visit will be mentioned in 6.3. Study Visits and Procedures.

Assessment of Efficacy: All eligible patients needing non-surgical treatment of periodontal disease will receive the appropriate treatment of mechanical debridement. The patients in the adjunctive aPDT group may have improved healing as observed through surrogate clinical measures of inflammation (BOP, PPD) and greater microbiologic reduction.

Assessment of Safety: 1) Study related risks: Initial mild pain, swelling and bleeding related to nonsurgical mechanical debridement of teeth. Potential for antimicrobial methylene blue dye to reversibly stain gingiva around tooth site.

2) Protection against risk: All efforts will be made to minimize risks to all and every participant. Laser safety protocols will be followed including laser safety mechanisms, labels and signage, safety zone implementation, proper eye protection for patient and clinician.

Study Population

We will recruit 20 patients, with split mouth design, that can detect a large effect size with 1 degree of freedom, alpha of 0.05 at 80% power.

The patients who need non-surgical and possible surgical treatment of periodontitis will be recruited in the study. These patients should be systemically healthy to receive this procedure.

Twenty subjects will be recruited amongst patients attending the Clinic for Graduate Periodontics, Graduate Prosthodontics, or the UT Dentists, who have at least two teeth with pockets depth of >4mm from periodontitis that requires non-surgical or surgical treatment. All subjects will be ≥18-year-old and systemically healthy or with controlled common systemic conditions, such as hypertension, that will not affect wound healing.

Criteria for subjects included in the study:

1. One pocket on each side of the mouth (split design)

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2. Single rooted tooth
3. Pocket depths measured greater than 4mm with bleeding on probing
4. Horizontal bone loss
5. No furcation involvement

Patients will be excluded if they are current heavy smokers (>10 cigarettes/day), have uncontrolled diabetes (HbA1c ≥ 6.5%) or other uncontrolled systemic diseases that may comprise healing, such as Vitamin C deficiency, any neutrophil deficiencies, immunodeficiency syndromes, or leukemia. Patients will also be excluded if they take antibiotics within 3 months before the procedure, or have vertical bone defects that requires surgical regenerative treatment. Previous smokers who stop smoking more than one year are eligible. Pregnancy status of subjects who are women with childbearing potential will be orally confirmed at the screening. The pregnant subjects will be excluded from the study.

A subject may be discontinued from participation in the study for any of the following reasons:

1. Withdrawal of consent
2. Subject noncompliance with the protocol, as determined by the investigator
3. Any event or condition that would make continued participation in the study not in the best interest of the subject, as determined by the investigator
4. Pregnancy
5. Development of any medical condition that might affect the treatment and clinical outcomes, as determined by the investigator.
6. Initiation of any treatment or exposure that might affect the healing of therapy, as determined by the investigator.
7. Investigator discretion

Subjects who withdraw from the study can be replaced. However, to complete the study within the time allocated, the center will not enroll subjects after 24 months from enrollment initiation.

Study Procedures

Study Visits and Procedures: Therapy group allocation (diseased test or diseased control) will be performed by the investigators before treatment is rendered based on computer-generated randomized (R Statistical Software). Three examiners will be used to take clinical measurements (presence/absence of Plaque, BOP, PPD, GI, CAL) at baseline, at the re-evaluation and 3 months after laser therapy. Patients in the control group will be blinded by performing mechanical debridement plus a “sham” aPDT (saline with non-light emitting laser). In addition to the diseased test and diseased control sites, plaque and GCF samples will be obtained from a healthy tooth site in order to have microbial data collection for the comparison with the diseased sites. This comparison will determine if with therapy, the diseased sites have developed a microbial composition towards health compared to the microbial composition of the diseased sites at baseline.

The investigator performing the treatment cannot be blinded because they will know if the dye has been applied and if the laser has been activated. Patient will be blinded because the control group will use a "sham" procedure including the use of saline and non-light emitting laser. The patients will be informed of benefits of all the procedures and realize that a minimum they are receiving the gold standard of periodontal treatment, mechanical debridement.

For the split mouth design, two quadrants in each participant will be randomized into (A) Diseased test side: receiving traditional non-surgical mechanical debridement with adjunctive use of aPDT at the tooth. (B) Diseased control side: traditional non-surgical mechanical debridement alone with "sham" aPDT with saline and non-light emitting laser on the tooth. Clinical measurements and plaque samples will be taken at the baseline appointment, the re-evaluation appointment and at the 3-month follow up after osseous resective surgical procedure. Full mouth prophy or periodontal maintenance will be completed with ultrasonics, hand instruments, and prophy paste. Oral hygiene instructions will be reviewed with all patients including techniques to clean around teeth at each appointment. Mechanical debridement of tooth surfaces will be completed with scalers and ultrasonics removing supragingival and subgingival plaque. Antimicrobial photodynamic therapy will be done at tooth sites by applying a photosensitizing dye methylene blue (0.1mg/ml) with a disposable syringe from the bottom of pocket in a coronal direction. After **5 minutes** in situ, the surrounding gingival tissues will be irradiated at four sites (mesio-buccal, disto-buccal, mesio-lingual, and disto-lingual) around the tooth using a diode laser with a wavelength of **660nm**, providing an energy density of **10 J/site, 100mW power,** time equal to 60 seconds and area equal to 0.12cm^2**.** After irradiation, the site will be thoroughly rinsed with saline. Only pockets >4mm will be treated with the diode laser.

Clinical Measurements: Tooth sites will be evaluated at baseline, periodontal re-evaluation in 4-6 weeks, and 3 months after laser therapy. Improvement in sites resulting from a reduction in inflammation will be analyzed based on pocket depth, clinical attachment loss, bleeding on probing, and the presence of plaque. For clinical measurements, six sites around the tooth will be measured: mesial buccal, mid buccal, distal buccal, mesial lingual, mid lingual, and distal lingual. Bleeding on probing will be evaluated by gently sweeping the periodontal probe just within the gingival sulcus of the tooth and the presence or absence of bleeding will be recorded. The presence or absence of plaque will then be evaluated at six sites around the tooth surface. Plaque sampling will be performed prior to the remaining clinical measurements at the baseline, periodontal re-evaluation, and 3 months after laser therapy using a curette within the gingival sulcus of the inflamed site. Periodontal pocket depth is measured from the free gingival margin to the base of the pocket, with a UNC periodontal probe with 1mm measurement units. Clinical attachment loss is measured by subtracting the pocket depth from distance of the free gingival margin.

Microbiologic Collection and Analysis: Plaque samples will be taken from the deepest probing site of each diseased test and diseased control tooth at baseline, at the re-evaluation, and 3 months after laser therapy. If more than one sites presented similar probing values, the most anterior site would be chosen for ease of obtaining an appropriate sample. Plaque and GCF samples will also be taken from a tooth, which is not periodontally involved and is considered "healthy". Health will be defined according to the 2017 World Shop classification where there is pocket depth of 1-3mm, no BOP, and no bone loss. The sample sites will first be isolated by cotton rolls and supragingival and marginal plaque will be removed

before subgingival biofilm samples collected using sterile scalers. The collected samples will be immediately placed in separate sterile Eppendorf tubes containing 0.15 ml TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.6). Samples will be stored at −80 °C until further analysis. DNA will be purified using the QIAmp DNA Mini Kit (Qiagen, Hilden, Germany) or Qiagen DNA MiniAmp kit (Qiagen, Valencia, CA, USA), according to the manufacturer's protocol. 16S rRNA gene V4 amplification and sequencing will be completed. 16S rRNA gene data analysis will also be completed for the plaque samples.

GCF Collection and Analysis: GCF will be collected from the sulcus around the target tooth using paper strips (PerioPaper, Oraflow). With proper isolation using cotton rolls in the buccal and lingual aspects of the study site, the area will be dried for 5 seconds with compressed air. The paper strip will be gently introduced into the mucosal crevice around the tooth for 30 seconds per site in four sites (mesial, distal, facial/buccal, lingual/palatal). The strips will then be removed from the crevice, and the volume of fluid collected in each strip measured using a micromoisture metering device (Peritron, Oraflow). After confirming the adequateness of the volume, the paper strips from each tooth will be transferred into labeled tubes and stored at -80°C for later use. For analysis, the paper strips will be analyzed using multiplexed fluorescent bead-based immunoassay. Assessments will be made in triplicate, and for the statistical analysis, median of the replicates will be used.

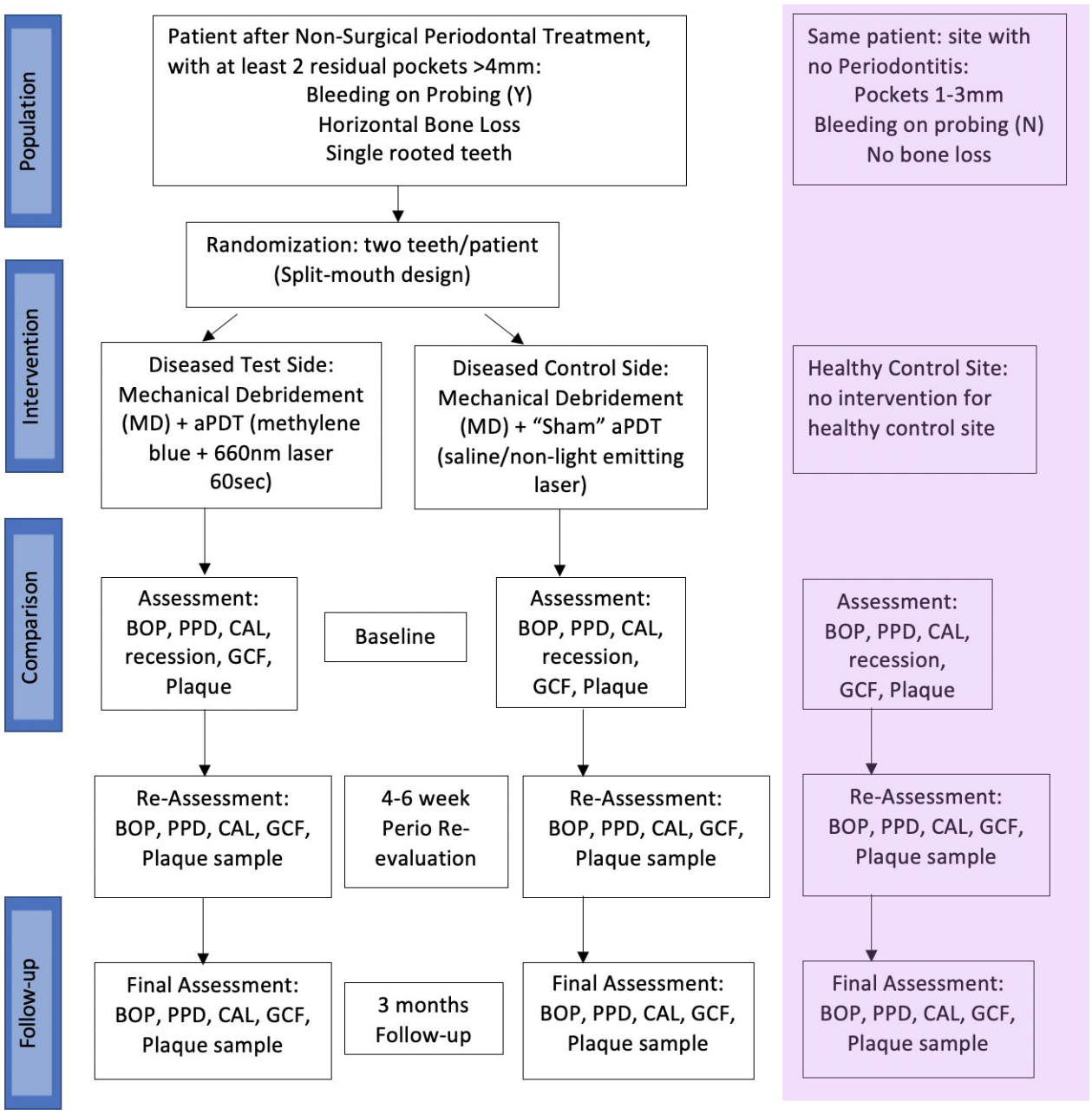
Post Study Follow-up and Procedures: The 3 month follow up after osseous surgery will be the subject's last visit for this clinical research. The patient will have clinical measurements taken as previously mentioned. The subjects will continue having routine maintenance appointments to follow up the outcomes related to the surgery and periodontal health in the periodontics clinic of UTSD. If the patients have symptoms or complications, the necessary treatments, will be performed.

Discontinuation Visit and Procedures: Subjects are free to withdraw from participation in the study at any time upon request. A subject may be discontinued from participation in the study for any of the following reasons:

1. Withdrawal of consent
2. Subject noncompliance with the protocol, as determined by the investigator
3. Any event or condition that would make continued participation in the study not in the best interest of the subject, as determined by the investigator
4. Pregnancy
5. Development of any medical condition that might affect the treatment and clinical outcomes, as determined by the investigator.
6. Initiation of any treatment or exposure that might affect the outcomes of therapy, as determined by the investigator.
7. Investigator discretion

Any subject with a serious adverse event, such as life-threating diseases, hospitalization, that is ongoing at the time of discontinuation will be followed until the event returns to baseline, resolves, or stabilizes. If the serious adverse event does not meet these outcomes within 30 days after discontinuation or after

study completion, the subject will be referred to an appropriate practitioner for continued care. If the study is discontinued, subjects will be referred back to the qualified clinicians for necessary dental care.



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Data and Safety Monitoring

All unanticipated problems will be reported in this study. The Committee for the Protection of Human Subjects (CPHS) considers unanticipated problems to be any incident, experience, or outcome that meets all of the following criteria:

- Is unexpected in terms of nature, severity, or frequency given a) the research procedures that are described in the IRB-approved research protocol and informed consent, and b) the characteristics of the subject population being studied;
- Is related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Places subjects or others at a greater risk for physical, psychological, economic, or social harm than was previously known or recognized.
- An incident, experience, or outcome that meets the 3 criteria above will generally warrant consideration of substantive changes in order to protect the safety, welfare, or rights of subjects or others. Examples of corrective actions or substantive changes that might need to be considered in response to an unanticipated problem include the following:
- Changes to the research protocol initiated by the investigator prior to obtaining IRB approval to eliminate apparent immediate hazards to subjects
- Modification of inclusion or exclusion criteria to mitigate newly identified risks
- Implementation of additional procedures for monitoring subjects
- Suspension of enrollment of new subjects
- Suspension of research procedures in currently enrolled subjects

For this study, a severe adverse event (SAE) is defined as an unanticipated problem occurring during the study that fulfils 1 or more of the following criteria:

1. Results in death
2. Is immediately life-threatening
3. Requires inpatient hospitalization or prolongation of existing hospitalization
4. Results in persistent or significant disability or incapacity
5. Is a congenital abnormality or birth defect
6. Is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above

Hospitalization for elective procedures or surgeries will not be considered SAEs, nor will inpatient hospitalizations for convenience.

Pregnancy in women with childbearing potential should not be reported as an SAE, but if pregnancy occurs, it must be reported in accordance with the procedures described in Section 6.2. Pregnancy will not be regarded as an SAE unless there is suspicion that a study intervention may have interfered with

the effectiveness of a contraceptive medication and the event meets the criteria for an unanticipated problem. If the pregnancy results in an outcome other than a normal birth or elective abortion of a healthy fetus, it will be reported as an SAE.

Collecting, Recording, and Reporting of Adverse Events: Examination and close follow-up of parameters capturing subjects' oral health will be collected on case report forms (CRFs). These will be completed at every study visit, and data will be compiled into a pre-specified format and reviewed monthly by the PI for safety oversight. Serious adverse events (as defined in Section 9.1) will be collected from the time of enrollment until the last clinic visit and will be recorded in the electronic health records (EHR) system. At each study visit, the clinician or investigator will inquire about the occurrence of SAEs since the last assessment. The investigator will review all source documentation related to study procedures for evidence of SAEs. Events will be followed for outcome information until they return to baseline or stabilize, or until 30 days after study completion or subject discontinuation. Subjects who have an SAE that is ongoing 30 days after study completion or discontinuation will be referred to an appropriate practitioner for continued care. Upon learning that a subject has experienced an SAE, the investigator must report the event to CPHS within 24 hours after becoming aware of the event.

On a monthly basis, the following events will be reported to every PI:

- Number of subjects experience severe complications and number of subjects enrolled. Severe complications include severe pain, continuous bleeding and severe swelling that needs prescription to control.
- Duration of observation of subjects experiencing severe complications and duration of observation of subjects enrolled.
- Any tooth loss, abscess, or other adverse oral health development requiring therapy or other intervention and the etiology (as captured in the dental history) - Every PI will review the monthly reports for any safety signals.

Safety Monitoring Plan: The purposes of the clinical monitoring activities are to ensure that the rights of human subjects are protected, the study is implemented in accordance with the protocol, and the integrity of study data is maintained. All subjects will be monitored for postoperative healing and tissue response at a regular interval while the entire oral health will be maintained throughout the study period.

Statistics

Determination of Sample Size: We will recruit 20 patients that can detect a large effect size with 1 degree of freedom, alpha of 0.05 at 80% power between the two treatments.

Statistical and Analytical Plans: We will present means and standard deviations for completeness of the report. The statistical significance level to test the primary endpoint was set at $p<0.05$.

Safety will be evaluated by tabulations of adverse events and will be presented with descriptive statistics at baseline and follow-up visits each month. Adverse events will be classified as severe complications

and summarized for baseline and follow-up visits. All information pertaining to adverse events noted during the study will be listed by subject, detailing verbatim given by the investigator, preferred term, date of onset, date of resolution, severity, and relationship to procedure. The onset of adverse events will also be shown relative (in number of days) to the day of performing the surgery.

Statistical Analysis Plan: We will analyze these data sets using generalized linear mixed effects models with the appropriate error structure (e.g., binomial, poison) and specifying treatment (control mechanical debridement vs mechanical debridement + aPDT) as a fixed factor and repeated measures of individuals at baseline, 4-6 weeks after nonsurgical therapy for the re-evaluation appointment, and 3 months after laser therapy as a random factor. All analyses will be performed in R Statistical Software (R Core Team 2017)

Ethics

Study protocol and consent forms will be approved by the Institutional Review Board at the University of Texas Health Science Center at Houston. The clinicians treating patients at UTSD will be told the information of this clinical research. The potential subjects will be identified in the clinic of Department of Periodontics, Prosthodontics, or UT Dentists for initial screening. The principal investigator will confirm the eligibility of these patients.

All patients will sign the consent forms and will be informed of the details of study procedures as well as potential complications. After informed consent is obtained, the non-surgical periodontal therapy will be scheduled as the first visit.

IRB Review: The protocol, informed consent form(s), and all advertising and subject materials will be submitted to the IRB for review and approval. Approval of both the protocol and informed consent form must be obtained before the enrollment of any subject. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented in the clinic.

Informed Consent: Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of study participation will be provided to the subjects (and their families if indicated). A consent form describing in detail the study interventions, procedures, and risks will be given to the subject. Consent forms will be IRB-approved, and the subject will be asked to read and review the document. The investigator or designee will explain the research study to the subject and answer any questions that may arise. The subject will sign the informed consent document prior to any study-related assessments or procedures. Subjects will be given the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. They may withdraw consent at any time throughout the course of the clinical research. A copy of the signed informed consent document will be given to subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their clinical care will not be adversely affected if they decline to participate in this study.

The consent process will be documented in the clinical or research record. This documentation will include the following:

- A notation of the date that the consent was obtained
- A statement that the consent was obtained prior to the initiation of study procedures
- A statement that the subject had adequate time to review the consent and that all questions were answered prior to initiation of study procedures
- A notation confirming that a copy of the signed consent was given to the subject

Confidentiality of Data and Patient Records: The subject's name will appear only on the consent form and clinical record, both of which will be kept separate from collected study data. All subject files will be kept confidential and placed in a double-locked office. A unique coded study number will be assigned to

each subject for data collection. The number will not contain any personal information (e.g., dates, age) to further ensure protection.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study, or the data will be released to any unauthorized third party without prior written approval of the PI. No subject names will be used in publications or presentations.

Data handling and record keeping

Patients will be assigned identifying codes that will be linked to all collected study data, stored in secured database by PI. All the electronic files will be encrypted and are stored in primary investigator's external drive, that will be locked in the PI's office cabinet. The following individuals/ institutions will have access to the records: the Principal Investigator and coinvestigators, and the University of Texas Health Science Center at Houston, including the Institutional Review Board. Absolute confidentiality cannot be guaranteed because of potential need to share this information with the above parties. The aggregate results of this study, with preservation of patient confidentiality, may be used for teaching, meeting presentation or publishing purpose. Records will be maintained for at least 6 years from the starting date of each subject

Quality control and assurance

Data and measurements will be checked by two separate investigators as well as analyzed statistically to ensure that the data obtained is accurate, complete and reliable.

All data will also be independently evaluated by Dr. Holland, who is not directly involved in the study.

Publication Plan

Following completion of the study, the investigator is expected to publish the results of this research in peer-reviewed journal in dentistry which would be available for dental subscribers. In addition, these data will be presented at the 2023 American Academy of Periodontology annual meeting.

ATTACHMENTS

1. Schematic of Study Design
2. Study Schedule
3. Consent Document
4. Case Report Form

5. Linking Log

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