Clinical and microbiologic outcomes of adjunctive antimicrobial photodynamic therapy in the non-surgical treatment of peri-implant disease

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Clinical and microbiologic outcomes of adjunctive antimicrobial photodynamic therapy in the non-surgical treatment of peri-implant disease

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1. INTRODUCTION

1.1 General Introduction

Dental implants have become an important and popular treatment option to replace absent or lost teeth. It is expected that implant prevalence could reach as high as 23% of US adult population by 2026 based on current NHANES data trends (Elani et al. 2018). The survival rate of dental implants is as high as 96.8% for 5 years and 92.8% for 10 years (Jung et al. 2007). Over time, the reported survival rates for all types of implant restorations have continued to increase, however, the incidences of esthetic, biologic, and technical complications are still high (Pjetursson et al. 2014). If untreated, complications associated with implant dentistry may result in progressive inflammation, bone loss, and ultimately, implant failure. Peri-implant mucositis is defined as inflammation in the peri-implant mucosa without peri-implant bone loss (Heitz-Mayfeild & Salvi, 2018). Clinical signs of inflammation of the peri-implant mucosa include bleeding on probing, erythema, swelling, and suppuration (Heitz-Mayfeild & Salvi, 2018). Peri-implantitis is defined as a pathological condition around implant tissues with inflammation and progressive loss of supporting bone around the implant (Schwarz et al. 2018). Clinically, peri-implantitis includes the presence of inflammation, radiographic bone loss, and increasing probing depth (Renvert et al. 2018). The etiology of peri-implant mucositis and peri-implantitis is through the development and accumulation of a bacteria biofilm around the implant. Peri-implant disease etiology is similar to the etiology of gingivitis and periodontitis. Disease is caused by the host response to a bacterial biofilm with a shift in bacterial species and type as the disease progresses. Biofilm-induced peri-implant mucositis is reversible by the removal of plaque from around the implant (Salvi et al. 2011). It is important to treat peri-mucositis professionally through mechanical removal of bacteria biofilm, preventing disease progression to peri-implantitis and prevent implant loss (Schwarz et al, 2015). The gold standard of care in the treatment of inflammation of implants is the mechanical removal of plaque with the use of curettes and ultrasonics. Conventional mechanical debridement of the implant surface is limited by the contaminated microstructure of the implant surface. Bacteria might be protected by residing in micro-irregularities of the titanium implant surface. Therefore, antimicrobial adjuncts have been advocated to increase the range of effective treatment in reducing inflammation around implants over the usual standard of mechanical debridement (Figuero et al. 2014). Options of adjuncts in the treatment of peri-implant inflammation include systemic antibiotics, local antibiotics, and antiseptics. Laser therapy such as antimicrobial photodynamic therapy (aPDT) can be used as an adjunct in the treatment of peri-implant mucositis and peri-implantitis. Antimicrobial photodynamic therapy uses low level laser light and aims to destroy pathogens around the implant and can also act as a photobiomodulator reducing inflammation and stimulating cellular proliferation (Mizutani et al. 2016). The advantages of aPDT are its wide antimicrobial activity, can be applied multiple times without creating antibiotic resistance, and easier topical delivery reaching the micro-irregularities of the implant surface.

1.2. Rationale and justification for the Study

a. Rationale for the Study Purpose

So far, there is limited evidence supporting the clinical impact of aPDT in the treatment of peri-implant diseases. The main goal of treating inflammation around implants is to reduce the bacterial biofilm. Mechanical debridement can disrupt this biofilm but cannot eliminate bacteria within the micro irregularities of the titanium implant surface. It is important to assess the use of aPDT as an adjunct to

mechanical debridement. Adjunctive use of aPDT may overcome the disadvantages of systemic and local antibiotic adjuncts and to overcome limitations of mechanical debridement of implant surfaces. A limited number of articles have evaluated the efficacy of aPDT with the use of laser (Diode Laser) and photosensitizing dye (Methylene blue). Bassetti *et al.* (2013) compared the use of locally delivered antibiotics and antimicrobial photodynamic therapy in 40 patients who had initial peri-implantitis. All implants were treated with mechanical debridement then were randomly treated with either local antibiotics or aPDT. Both groups had a decrease in BOP, PD, bacteria, and proinflammatory cytokines. This study showed that aPDT can be just as effective as locally applied antibiotics without challenges and risks of antibiotic use. Kerimin *et al.* (2016) in a randomized split mouth clinical trial showed that the aPDT test groups had statistically greater reduction in gingival inflammation, BOP, and probing depth than those implants treated with mechanical debridement alone. Few clinical trials have thoroughly evaluated aPDT as an adjunct to mechanical debridement of implants with peri-mucositis and peri-implantitis (Lin *et al.* 2017).

b. Rationale for Materials Selected

Mechanical debridement with curettes of implant surfaces have been shown to reduce and reverse peri-implant inflammation. Antimicrobial photodynamic therapy, used with a diode laser, 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. The 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). The photosensitizer, such as methylene blue, undergoes a strong cationic charge, 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).

c. Rationale for Study Population

The study will recruit subjects who have an implant diagnosed with either peri-mucositis or peri-implantitis based on the 2017 World Workshop periodontal classification (Renvert et al. 2018). The gold standard to treatment inflammation around implants is mechanical debridement. All patients recruited are in need of traditional mechanical debridement.

d. Rationale for Study Design

It is a randomized double blinded clinical control study. The randomized controlled trail (RCT) is usually considered the gold standard for a clinical trial and it is able to provide the highest level of evidence within all the study designs. Two treatments (test and control) are randomly assigned to a single implant for each patient. Both the examiners and patient will be blinded to which treatment was completed. The investigator completing the treatment will not be blinded. This design removes examiner and patient bias and placebo effects.

2. HYPOTHESIS AND OBJECTIVES

2.1 Hypothesis

Null hypothesis: Antibiotic 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), or reduction in levels of pathologic microbes) in the treatment of implants with peri-implant inflammatory disease.

2.2 Primary Outcome Measures

This study aims to compare clinical outcomes (change in BOP and PPD) after mechanical debridement of implant surfaces at sites exhibiting plaque induced inflammation with or without adjunctive aPDT. Bleeding on probing and periodontal probing depths will be assessed clinically through a periodontal probing exam at baseline, week 6, and week 12 follow up. Probing depth reduction endpoint value of 1mm (Bassetti et al. 2014, Morrison et al. 1980). Bleeding on probing endpoint value of 50-60% reduction of sites with BOP (Bassetti et al. 2014). Demographic and implant related variables will be analysed using multivariate analysis for correlation with peri-implant mucositis and peri-implantitis.

2.3 Secondary Outcome Measures

In addition to the clinical outcomes, plaque samples and peri-implant sulcular fluid (PISF) will be taken to assess the microbiologic and immunologic profile before and after treatment with or without aPDT. Subgingival biofilm samples will be collected using sterile titanium implant scalers and will be assessed at baseline and week 12 follow up. PISF will be collected from the tested implant site after clinical examination. The levels of interleukin (IL)-1α, IL-1β, IL-6, IL-8, IL-10, IL-12, IL-7A, tumor necrosis factor (TNF)-α, C-reactive protein, osteoprotegerin, leptin, and adiponectin will be determined using multiplex proteomic immunoassays.

2.4 Potential Risks and Benefits:

a. End Point – Efficacy

All eligible patients needing non-surgical treatment of peri-implant disease will receive the appropriate treatment of mechanical debridement. The mechanical removal of plaque around the implants is the gold standard of care in the treatment of implant inflammation. The patients in the adjunctive aPDT group may have improved healing as observed through surrogate clinical measures of inflammation (BOP, PPD), greater microbiologic reduction, and levels of key biomarkers in PISF that resembles peri-implant tissue health (Recker 2015).

b. End Points – Safety

1) Study related risks:
Initial mild pain, swelling, and bleeding related to non-surgical implant surface mechanical debridement. Potential for antimicrobial methylene blue dye to reversibly stain gingiva around implant site. Collecting PISF will not result in any additional side effects. Patient will not be provided medications for study

related pain. If patient reports pain after treatment will recommend over the counter analgesics such as ibuprofen or acetaminophen.

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.

3. STUDY POPULATION

3.1 List the number of subjects to be enrolled

We will recruit 74 patients in total, and with a dropout rate of 15%, we will have a sample size of 64. With 64 patients, 32 in each group, we can detect a large effect size with 1 degree of freedom, alpha of 0.05 at 80% power.

3.2 Criteria for Recruitment

The patients who need non-surgical treatment of implants that are suffering peri-implant diseases will be recruited in the study. These patients should be systemically healthy to receive this procedure.

3.3 Inclusion Criteria

Sixty-four subjects will be recruited amongst patients attending the Clinic for Graduate Periodontics, Graduate Prosthodontics, or the UTDentists, who have at least one implant with peri-implant inflammation that requires non-surgical treatment. Peri-implant diseases included are peri-implant mucositis and peri-implantitis. 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 diagnosis of peri-implant mucositis or peri-implantitis:

1. Red, swollen gingival tissues surrounding the implant
2. Presence of bleeding and/or suppuration on gentle probing around the implant
3. Increased probing depth compared to probing depth after restoration of the implant (greater than 2mm increase in probing depth)
4. May or may not have progressive bone loss in relation to radiographic bone levels assessed either 1 year following restoration of the implant OR ≥3mm of radiographic bone loss from the implant platform

3.4 Exclusion Criteria

Patients will be excluded if they are current heavy smokers (>10 cigarettes/day), have diabetes or other systemic diseases that may comprise healing, take antibiotics within 3 months before the procedure. Patients who stop smoking more than one year are eligible. Periodontal literature indicates risk factors for peri-implantitis (and reduced wound healing) as a history of periodontal disease, uncontrolled diabetes, and smokers (Schwarz et al. 2017).

3.5 Withdrawal Criteria

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

3.6 Subject Replacement

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.

4. TRIAL SCHEDULE

There will be three appointments including the baseline appointment and two follow-up appointments (6 weeks and 12 weeks) after therapy. The details of each visit will be mentioned in 6.3. Study Visits and Procedures.

5. STUDY DESIGN

5.1 Summary of Study Design

This is randomized double blinded 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 peri-implant diseases. Seventy-four patients will be recruited. Clinical measurements including bleeding on probing, pocket depth, plaque, and clinical attachment will be measured at baseline, 6 weeks, and 12 weeks after therapy. The 2017 World Shop classification will be used to diagnosis implants as peri-implant mucositis or peri-implantitis (as stated above in section 3.3). Only diseased implants will be included and only one implant per patient will be studied. The hypothesis is that inflamed implant 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 implant surface will benefit patients who have peri-implant diseases. The results can be applied in the managing and in the armamentarium for treatment of both peri-implantitis and peri-implant mucositis.

6. METHODS AND ASSESSMENTS

6.1 Randomization and Blinding

Therapy group allocation (test or control) will be performed by the investigators before treatment is rendered based on computer-generated randomized (R Statistical Software). Two blinded examiners will be used to take clinical measurements (presence/absence of Plaque, BOP, PPD, CAL) at baseline, and then six and twelve weeks after treatment. Patients in the control group will be blinded by performing mechanical debridement plus a "sham" aPDT (saline with non-light emitting laser).

6.2 Contraception and Pregnancy Testing

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.

6.3 Study Visits and Procedures

a. Screening Visits and Procedures

Study protocol and consent forms will be approved by the Institutional Review Board at the University of Texas Health Science Center at Houston. The trial will be registered with ClinicalTrials.gov. The clinicians treating patients at UTSD will be told the information of this clinical trial. The potential subjects will be identified in the clinic of Department of Periodontics, Prosthodontics, or UTDentists 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 implant therapy will be scheduled as the first visit.

Patient's Demographic Data Collection

Upon enrollment into the study and patient's consent form signed, patient's demographic data from patient's electronic health record (EHR) in the UT School of Dentistry will be collected. This information will include, but not limited to, patient's de-identifiable chart number, age, gender, race, medical history, dental conditions, and all pertinent dental implant related data. Radiographic information regarding the implant(s) included in the study will also be collected prior to treatment.

b. Study Visits and Procedures

Patients will be randomly divided into 2 treatment groups. (A) Test group: patients will undergo traditional non-surgical mechanical debridement with adjunctive use of aPDT at implant(s). (B) Control group: patients will undergo traditional non-surgical mechanical debridement alone with "sham" aPDT with saline and non-light emitting laser at implant(s). Clinical measurements and PISF will be collected at the baseline appointment, and at follow up 12 weeks after treatment. For PISF collection, the volume of fluid collected will be measured using Periotron then stored in -80° C freezer. Plaque samples will be taken at baseline and at the 12-week appointment. 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 implants. Mechanical debridement of implant surfaces will be completed with titanium scalers and ultrasonics removing supragingival and subgingival plaque. Antimicrobial photodynamic therapy will be done at implant sites by applying a photosensitizing dye methylene blue (0.1 mg/ml) with a disposable syringe from the bottom of pocket in a coronal direction. The methylene blue dye will be diluted from 1.0% to 0.1% before use. The dye will be applied topically confined to the epithelialized space surrounding the implant fixture and will not be internalized. After 5 minutes in situ, the surrounding gingival tissues will be irradiated at six sites around the implant using a diode laser with a wavelength of 660nm, providing an energy density of 10 J/site, 100mW power, time equal to 100 seconds**.** After irradiation, the site will be thoroughly rinsed with saline. Only pockets ≥4mm will be treated with the diode laser as this is the threshold pocket for diagnosing peri-implant inflammation per the 2017 World Work Workshop (Heitz-Mayfeild & Salvi, 2018).

Clinical Measurements

Implant sites will be evaluated at baseline, six weeks, and twelve weeks after treatment (2 follow-up visits in total). 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 implant 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 implant 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 implant surface. Plaque sampling will be performed prior to the remaining clinical measurements at the baseline/treatment appointment and 12 weeks after treatment utilizing a titanium 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 to the implant platform.

Microbiologic collection and analysis

Plaque samples will be taken from the deepest probing site of each implant before and 12 weeks after PDT. If more than one sites presented similar probing values, the most anterior site would be chosen for ease of obtaining an appropriate sample. 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 titanium implant scalers (Hu-Friedy). 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.

PISF collection and analysis

PISF will be collected from the sulcus around the target dental implant using paper strips (PerioPaper, Oraflow). In patients with multiple implants, preference will be given to the implant included in the study. 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 dental implant 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 implant 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.

c. Final Study Visit

The twelve weeks follow up will be the subjects last visit for this clinical trial. The patient will have clinical measurements taken as previously mentioned as well as possible radiographic measurements. If the patient has been diagnosed with peri-implant mucositis, recommendations will be made for continued maintenance of the diseased implant(s) and follow every 3 months. If the patient has been diagnosed with peri-implantitis, recommendations will be made for the possible need for surgical treatment.

d. Post Study Follow up and Procedures

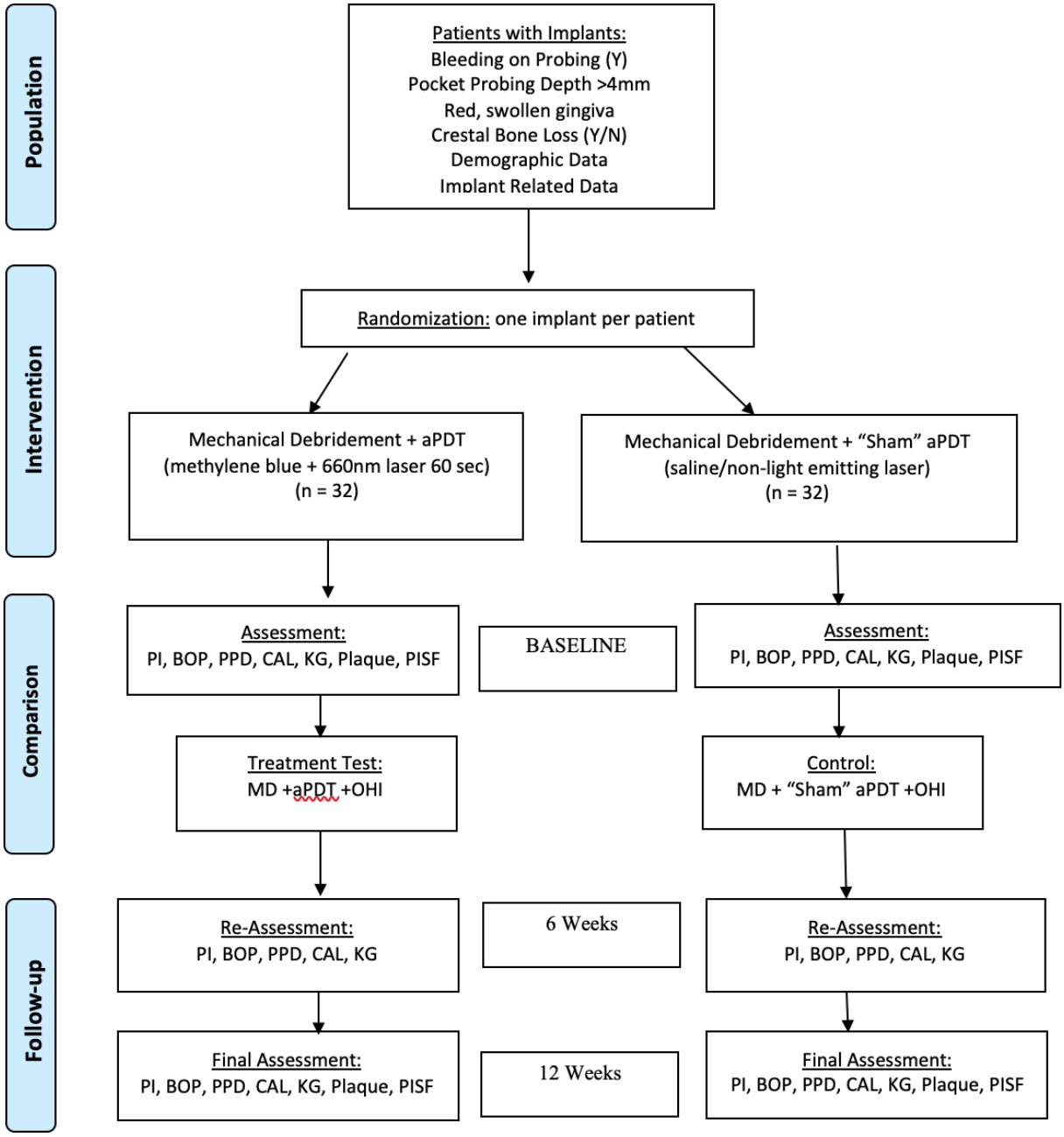
The subjects will continue having routine maintenance appointments and/or treatments to follow up the outcomes related to the surgery and periodontal health in the periodontics clinic of UTSD. If the patients have symptoms or complications, the necessary treatments, will be performed.

e. 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 implant 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.



7. TRIAL MATERIALS

7.1 Trial Products

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 implant 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. Saffarpur et al. (2018) through an in vitro studied evaluated implants treated with aPDT and found no change in the implant surface. Neither the dye nor the low-level energy laser changed the implant surface characteristics. aPDT applied to rough titanium or zirconia surfaces have maintained the surface integrity of the treated implants. (Azizi et al. 2018, Giannelli et al. 2017). The photosensitizer, such as methylene blue, undergoes a strong cationic charge, 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).

8. TREATMENT

8.1 Rationale for selection of treatment

Managing peri-implant disease with adjuncts like antiseptics and systemic antibiotics has a few disadvantages. Systemic antibiotic usage can produce antimicrobial resistance. Conventional mechanical debridement of the implant surface is limited by the contaminated microstructure of the implant surface. Bacteria might be protected by hiding in micro-irregularities of the titanium implant 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 micro-irregularities of the implant surface. Multiple in vitro studies have shown the antimicrobial properties of aPDT on various titanium surfaced seeded with periodontopathic microbes (Haas, Azizi, Huang, Marotti).

8.2 Specific Restrictions/ Requirements

No post operative restrictions or requirements are required after treatment. Oral hygiene instructions will be reviewed with patients after treatment including instruction on brushing and flossing.

8.3 Blinding

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 examiners will be blinded. The patients will be informed of benefits of all the procedures and realize that a minimum they are receiving the gold standard of peri-implant treatment, mechanical debridement.

9. SAFETY MEASUREMENTS

9.1 Definitions

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.

9.2 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.

9.3 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.

10. DATA ANALYSIS

10.1 Data Quality 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.

11. SAMPLE SIZE AND STATISTICAL METHODS

11.1 Determination of Sample Size

We will recruit 74 patients in total, and with a dropout rate of 15%, we will have a sample size of 64. With 64 patients, we can detect a large effect size with 1 degree of freedom at an alpha equal to 0.05 with 80% power between the two treatments.

11.2 Statistical and Analytical Plans

a. General Consideration

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

b. Safety Analyses

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.

c. Statistical Analysis Plan

We will analyze these data sets using generalized linear mixed effects models with the appropriate error structure (e.g., binomial, poisson) and specifying treatment (control mechanical debridement vs mechanical debridement + aPDT) as a fixed factor and repeated measures of individuals over 0, 6, and 12 weeks as a random factor. Demographic data collected from patients will be included in analysis and will be adjusted for any confounders. All quantitative analyses will performed in R Statistical Software (R Core Team 2017).

For microbiologic analysis, microbial 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.

For immunologic analysis, the PerioPaper 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.

12. Ethical Consideration

12.1 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 trial. 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

12.2 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.

12.3 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.

13. PUBLICATIONS

Following completion of the study, the investigator is expected to publish the results of this research in a scientific journal. The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov (De Angelis et al. 2004), which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies. For grants and cooperative agreements, it is the institution's responsibility to register the trial in an acceptable registry. The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase I trials), would be exempt from registering trials in a public registry such as ClinicalTrials.gov

14. RETENTION OF TRIAL DOCUMENTS

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.

Appendix 1 **Data collection form**

Appendix 2 **Informed Consent**

References

Elani, Starr, Da Silva, Gallucci. Trends in dental implant use in the US 1999-2016, and projections to 2026. 2018. Journal of dental research. 97(13) 1424-1430.

Jung, Pjetursson, Glauser, Zembic, Zwahlen, Lang. A systematic review of the 5 year survival and complication rates of implant-supported single crowns. 2008. Clinical Oral Implants Research. 19. 119-130.

Pjetursson, Asgeirsson, Zwahlen, Sailer. Improvements in implant dentistry over the last decade: comparison of survival and complication rates in older and new publications. 2014. The International Journal of Oral & Maxillofacial implants. 29. 308-324

Heitz-Mayfield and Salvi. Peri-implant mucositis. Journal of periodontology. 2018;89. S257-S266.

Schwarz, Derks, Monje, Wang. Peri-implantitis. Journal of periodontology. 2018;89. S267-S290

Renvert, Persson, Pirih, Camargo. Peri-implant health, peri-implant mucositis, and peri-implantitis: Case definitions and diagnosistic considerations. Journal of periodontology. 2018;89. S304-S312.

Salvia, Aglietta, Eick, Sculean, Lang, Ramseier. Reversibility of experimental peri-implant mucositis compared with experimental gingivitis in humans. Clinical Oral Implants Research. 2011. 23, 182-190.

Figuero, Graziani, Sanz, Herrera, Sanz. Management of peri-implant mucositis and peri-implantitis. Periodontology 2000. 2014;66, 255-273

Morrison E C, Ramfjord S P, Hill R W. Short-term effects of initial non-surgical periodontal treatment (hygiene phase). J Clin Periodontol 1980; 7:199-211.

Schwarz, Derks, Monje, Wang. Peri-implantitis. Journal of Periodontology. 2017.

Mizutani, Aoki, Coluzzi, Yukna, Wang, Pavlic, Izumi. Lasers in minimally invasive periodontal and peri-implant therapy. Periodontology 2000. 2016;71,185-212.

Bassetti, Schar, Wicki, Eick, Ramseier, Arweiler, Sculean, Salvi. Anti-infective therapy of peri-implantitis with adjunctive local drug delivery or photodynamic therapy: 12 month outcomes of a randomized controlled clinical trial. Clinical Oral Implants Research. 2014. 25, 279-287

Karimin, Hasani, Khosroshahian. Efficacy of antimicrobial photodynamic therapy as an adjunctive to mechanical debridement in the treatment of peri-implant diseases: A randomized controlled clinical trial. Journal of Lasers in Medical Sciences. 2016. 7(3): 139-145

Lin, Lopez del Amo, Wang. Laser therapy for treatment of peri-implant mucositis and peri-impantitis: An American academy of periodontology best evidence review. Journal of periodontology.2017

Takasaki, Aoki, Mizutani, Schwarz, Sculean, Wang, Koshy, Romanos, Ishikawa, Izumi. Application of antimicrobial photodynamic therapy in periodontal and peri-implant diseases. Periodontology 2000. 2009;51 109-140.

Scaffarpur, Nozari, Fekrazad, Saffarpour, Heibati, Iranparvar. Microstructural evaluation of continated implant surface treated by laser, photodynamic therapy, chlorhexidine 2%. The international Journal of Oral and Maxillofacial Implants. 2018;33:1019-1026

Haas, Mensdorff-Pouilly, Maitlath. Elminiation of bacteria on different implant surface through photosensitization and soft laser. Clinical Oral Implant Research. 1997. 8: 249-254.

Azizi, Budimir, Mehmeti, Jakovljevic, Bago, Gjorgievska, Gabric. Antimicrobial efficacy of photodynamic therapy and light activated disinfection against bacterial species on titanium dental implants.

Huang, Chen, Ding, Chen. Antimicrobial efficacy of methylene blue-mediated photodynamic therapy on titanium alloy surfaces in vitro. Photodiagnosis and Photodynamic Therapy. 2019. 25: 7-16.

Marotti, Tortamano, Cai, Ribeiro, Franco, Campos. Decontamination of dental implant surfaces by means of photodynamic therapy. Lasers Med Sci. 2013. 28:303-309

Recker, Erica N, Gustavo Avila-Ortiz, Carol L. Fischer, Keyla Pagan-Rivera, Kim A. Brogden, Deborah V. Dawson, and Satheesh Elangovan. A cross-sectional assessment of biomarker levels around implants versus natural teeth in periodontal maintenance patients. Journal of periodontology. 2015. 86 (2): 264-272.