Efficacy of Er:YAG Laser in Decontamination of Dental Implants: An In-Vitro Study

---

Principal Investigator:
Robert Gyurko, D.M.D., Ph.D.

MS Student Co-Investigator:
Hani AlMoharib, B.D.S.

Co-Investigators:
Driss Zoukhri, Ph.D.
Bjorn Steffensen, D.D.S., M.S., Ph.D.
Matthew Finkelman, Ph.D.

Study Location:
Tufts University School of Dental Medicine
One Kneeland Street
Boston, MA 02111

NCT number: NCT03100435

Protocol Version Date:
Aug 10, 2017

I. Introduction

Key Question

Is Er:YAG laser a more effective method in decontamination of titanium surfaces compared to carbon fiber curettes?

Hypothesis

Er:YAG removes biofilm more effectively from titanium surfaces than carbon fiber curettes.

Aim

The aim of this study is to compare the amount of residual biofilm on titanium discs after decontamination with Er:YAG laser and carbon fiber curette.

Background and Rationale

Dental Implants

The American Association of Oral and Maxillofacial Surgeons estimated that 69% of adults ages 35 to 44 have lost at least one permanent tooth. The advent of oral implants has revolutionized dental practice and industry as a whole.[1] Implants provide a solution for tooth loss that is predictable with long-term success.[1] A success rate of 90% has been reported for both fully or partially edentulous restorations.[2-5] Different implant systems have been developed with various sizes, shapes, surfaces, and prosthetic components.[6] The success of dental implants depends on osseointegration, a concept defined as a direct structural connection at light microscopic level between the bone and surface of the implant.[7] The dental implant and prosthetic market in the U.S is projected to reach $6.4 billion by 2018.

Peri-Implantitis

As well as teeth, dental implants are not immune to inflammatory lesions. The first European Workshop on Periodontology[8] identified two disease entities surrounding implants. They have been recognized as peri-implant mucositis and peri-implantitis.[8] Peri-implant mucositis is defined as a reversible inflammation in the soft tissues surrounding the implant, while peri-implantitis is designated as inflammation associated with loss of supporting bone around an implant.[8] Heitz-Mayfield[9] identified several diagnostic criteria for peri-implant diseases such as probing depth, attachment level changes, bleeding on probing, suppuration, mobility, and radiographic bone changes. The article[9] also presented oral hygiene, history of periodontitis, diabetes, smoking, and alcohol consumption as risk factors in developing peri-implantitis. In their recent meta-analysis, Derks et al.[10] estimated the prevalence of peri-implant mucositis and peri-implantitis to be 43% and 22% respectively.[10] These high prevalence rates led to development of different methods for implant surface decontamination.[11]

Treatment of Peri-Implant Diseases

Bacterial colonization on the implant surface is the main causative factor in development of peri-implant diseases.[12-16] Formation of adherent bacterial biofilm on

titanium surface is critical for disease development and altering the biocompatibility of the surface.[17] The literature provides evidence of peri-implantitis' microbial etiology that is very similar to advanced periodontitis, with high levels of spirochetes and non-motile anaerobic Gram-negative bacterium (*Aggregatibacter Actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, Treponema denticola*).[18] Furthermore, the presence of multiple components in a single dental implant, i.e. fixture, abutment, and crown, accumulate more plaque than natural dentition.[19, 20] A study by Pontoriero et al.[21] demonstrated a cause and effect relationship between plaque accumulation and peri-implant mucositis. Surface debridement constitutes the basis of treatment of peri-implant disease.[17] However, complete debridement is not a simple task.

Mechanical hand instrumentation using curettes to remove biofim and calculus is still the basis for any periodontal therapy. Based on the extensive evidence supporting hand instrumentation in treatment of periodontitis, hand instruments were proposed for the treatment of peri-implant diseases. Scalers of different materials were made specifically for implant surfaces. These scalers were made with similar or lower external hardness, in order not to produce scratches on the implant surface and not to make the implant surface more plaque retentive. Furthermore, a damaged surface impairs adhesion of fibroblasts and lowers biocompatibility of the implant.[22, 23] Fox et al.[23] studied the effect of stainless steel, titanium, and plastic curettes on implant surface roughness by scanning electron microscopy (SEM). Surprisingly, the roughest surface was produced by the titanium curettes followed by stainless steel and plastic. The plastic surface alteration was determined to be insignificant. Homiak et al.[24] in their SEM study on implant abutments confirmed that stainless steel instrument produced a rougher surface. Whereas, plastic scalers rounded off sharp edges and smoothened the surface but changes were not extremely dramatic. Carbon fiber curettes are another alternative to plastic curettes, which do not significantly damage the implant surface.[25] Strooker et al.[26] reported decrease in Gingival Index and number of bacterial colonies in patients treated by mechanical instrumentation using carbon fiber curettes.

Karring et al. showed that mechanical non-surgical therapy alone is not sufficient to treat peri-implantitis.[27] Persson et al.[28] compared using curettes with ultrasonic devices, and reported failure of both measures to resolve the disease. Renvert et al. observed mechanical non-surgical therapy could only be effective in peri-implant mucositis cases only, and results could be enhanced by the adjunctive use of antimicrobial rinses.[17] No treatment protocols have received full consensus as a standard of care, as most treatments were unable to achieve complete inflammation resolution or surface decontamination.[17, 29, 30] Therefore, different modalities of treatment have been developed to overcome inadequacies such as sustained antibiotics release, antiseptics, air abrasion, and laser therapy.[17]

<u>Er:YAG Laser</u>

Laser therapy has been advocated for implant surface decontamination.[31, 32] Several types of lasers have been evaluated for this purpose such as erbium-doped yttrium–aluminum–garnet (Er: YAG), carbon dioxide (CO_2), gallium–aluminum– arsenide

(GaAlAs) diode, neodymium-doped yttrium–aluminum–garnet (Nd:YAG) and erbium- and chromium-doped yttrium–scandium–gallium–garnet.[31, 32] CO_2, diode, and Er:YAG lasers have been implicated for irradiation of implant surfaces because they are poorly absorbed by the implant and do not raise surface temperature.[33] The Er:YAG laser appears optimal for implant decontamination as the Er:YAG laser energy is primarily absorbed by water, resulting in vaporization of bacteria and minimal surface alterations on the implant surface.[34, 35]

Several investigations have been carried out on the effectiveness of Er: YAG laser in surface decontamination. Kreisler et al.[36] incubated *Streptococcus sanguinis* over titanium discs and irradiate them with power 60 and 120 mJ/pulse at 10 Hz. Their results reported reduction ranges from 98.3% to 99.94%. Matsuyama et al.[37] evaluated Er: YAG in six contaminated healing abutments. They showed that Er: YAG at 30 mJ/pulse and 30 Hz with water spray was capable of effectively removing plaque and calculus on the implant abutments without injuring their surfaces. Quaranta et al.[38] explored different types of implant surfaces contaminated with *Porphyromonas gingivalis*. They observed values of 76.2% for machine tested implants, 90.9% for titanium plasma spray implants, and 98.3% for sandblasted and etched implants. A minimal residual bacterial presence was observed in all groups. Sennhenn-Kirchner et al.[39] evaluated efficacy against *Candida albicans* incubation. They found Er:YAG laser damaged the fungal cells to a great extent wherever it was applied on direct contact. Decontamination efficacy was dose-dependent with values ranging from 59% following irradiation with 80 mJ/pulse at 5 Hz[40] to 99.94% following irradiation with 120 mJ/pulse at 10 Hz.[36] Schwarz et al.[41] in a canine study showed Er:YAG to be more suitable to promote re-osseointegration than ultrasonics or plastic curettes.

Studies evaluated efficiency of decontamination using scanning light microscopy, scanning electron microscopy (SEM), counting the colony forming units (CFU), bacterial smears, and photometric XTT–formazan evaluations.[31] Molecular Probes' LIVE/DEAD® *Bac*Light™ Bacterial Viability Kits provide a novel two-color fluorescence assay of bacterial viability that has proven useful for a diverse array of bacterial genera. LIVE/DEAD *Bac*Light allow researchers to easily, reliably and quantitatively distinguish live and dead bacteria in minutes, even in a mixed population containing a range of bacterial types.

Since no consensus treatment for peri-implantitis has yet emerged, there is an urgent need for quantitative evaluation of the available treatment options. This study is designed to compare the biofim removal efficiency of the Er:YAG laser with mechanical biofilm removal tools. Our goal is to establish whether Er:YAG laser significantly improves titanium surface decontamination. Our findings may contribute to the development of novel and efficient treatment of peri-implantitis.

Standard of Care in the TUSDM Periodontal Clinic

For patients who are experiencing a failing dental implant, the treatment starts with improving overall oral hygiene and controlling any etiological factors that prevent restoration of optimum oral health. The second step usually involves a surgical

intervention method such as open flap debridement, followed by implant decontamination using Er:YAG, titanium brushes, or plastic curettes. If the bony defect developed by the disease process is contained, bone regenerative therapy may be used.

I) Research Plan

A) Experimental Design

This study is designed to investigate the efficiency of biofilm decontamination of Er:YAG laser compared to carbon fiber curette. The first phase is clinical wherein experimental subjects will be recruited. Full periodontal examination will be performed and alginate impressions will be taken. A custom mouth guard that holds multiple titanium discs will be fabricated. Experimental subjects will be instructed to wear this mouth guard for 72 hours, during which time a natural bacterial biofim will form on the disc surfaces. The second phase of the study will be performed after collecting the discs from the subjects and exposing them *ex vivo* to one of four treatment groups 1) Er:YAG laser, 2) carbon fiber curette, 3) titanium brush, and 4) no treatment (control). The biofilm will be stained with LIVE/DEAD *Bac*Light dye and the residual biofilm will be visualized under fluorescence microscopy. Quantification of residual biofilm will be performed using image analysis software (Image J, NIH). Statistical methods will be used to determine the significance of each treatment modality. The primary outcome of the study is the percent area of the titanium disc covered by biofilm.

B) Power Calculation

A power calculation was performed via the statistical software nQuery Advisor (Version 7.0). Using the results of Schwarz et al.[42] for anticipated means and standard deviations, and assuming that the mean for the combination group is equal to the mean of the Er:YAG laser group, a sample of 8 subjects, allowing for ~20% dropout with a final projected sample size of 6 (8 discs per subject, 64 total discs) is sufficient for a Type I error rate of 5% and a power over 99%. Discs retrieved from each mouth guard will be randomized over the 4 treatment groups using the "sample" function of the statistical software package R (Version 3.1.2), so that each subject will contribute two discs to each treatment group.

C) Statistical analysis

Descriptive statistics (means, standard deviations, medians, and inter-quartile ranges) will be computed by group. Statistical significance will be assessed via repeated-measures ANOVA if the data are normally distributed, and Friedman's test if the data are not normally distributed. $P<0.05$ will be considered statistically significant. The analysis will be conducted using SPSS Version 22.

D) Products

Titanium discs will be supplied by Straumann USA (Andover, MA).

E) Subject Characteristics

Inclusion criteria:

- Adults (18 years or older).
- Presence of enough teeth to support the mouth guard.
- English proficiency.
- Subjects diagnosed with clinical health, gingivitis, or slight chronic periodontitis defined as periodontal inflammation with slight (1-2mm) attachment loss. Subjects will not be stratified based on oral health.

Exclusion criteria:

- Subjects with insufficient dental support for the mouth guard
- Subjects diagnosed with moderate or severe chronic periodontitis.
- Subjects with known allergy to acrylic or titanium.
- Subjects who smoke cigarettes, cigars, snuff tobacco, or any other form of smoking.
- Subjects with a history of antibiotic treatment within the last six months.
- Subjects with contraindications to wearing a mouth guard, such as chronic obstructive pulmonary disease or severe sleep apnea.
- Subjects with uncontrolled or debilitating medical conditions, including but not limited to subjects with uncontrolled diabetes, hematologic disorders, cancers, immunosuppression, severe cardiovascular disease, or uncontrolled thyroid disease
- Subjects that are currently pregnant according to self-report as hormonal changes may affect the outcomes of the study.
- Subjects who are unable or unwilling to sign the informed consent form (ICF).
- Subjects may not participate in this study if they concurrently participate in another research study.

Subject withdrawal and termination criteria:

- Subject may withdraw from the study at any time. Subjects may remain patients of TUSDM if they decide to withdraw from the study.
- Investigators may terminate subjects if they no longer fulfill inclusion criteria, if an exclusion criterion is met, or if they do not show up for scheduled study visits. Subjects may remain patients of TUSDM if they are withdrawn from the study. If a subject withdraws from the study early and returns the mouth guard, the mouth guard and the titanium discs will not be analyzed and will be disposed of. Subjects withdrawing from the study early will not be eligible for the $25 gift card.
- Unanticipated Adverse Device Event

The Principal Investigator will determine whether subjects (either withdrawn subjects or subjects completing the study) are in need of additional treatment and/or follow-up observation. Potential unforeseen adverse events may include allergic reaction to acrylic or titanium, or irritation of the gum by the mouth guard.

Subjects and/or their insurance will be responsible for the cost of any standard of care follow-up visits or additional treatment that is not part of this study.

F) Assessment

1) Risk

Risks associated with standard of care periodontal examination for periodontal patients include sensitivity, discomfort, pain and bleeding during examination. Swelling is not expected.

Risks associated with the mouth guard include speech alteration and esthetic concerns. Abstaining from the use of toothpaste and mouthwash for 3 days poses minimal health risk as the principal factor in oral hygiene is the mechanical plaque removal by the toothbrush bristles. The risk associated with brushing without toothpaste or not using mouthwash for 3 days is halithosis (bad breath). Also, disc de-attachment presents another risk of swallowing or aspiring. That risk will be minimized by close adaptation of the mouth guard to the disc and the use of adhesive (PeriAcryl).

Potential unforeseen adverse events may include allergic reaction to acrylic or titanium, or irritation of the gum by the occlusal mouth guard.

Loss of confidentiality is also a risk of participation. This risk will be minimized by following the procedures outlined in the confidentiality section.

2) Benefits

There are no specific benefits to study participants in this study. Potential benefits to society might include improved treatment protocols for peri-implantitis. Potential benefits to science might include increased understanding of methods used to decontaminate implant surfaces.

3) Alternatives

The alternative is to not participate in the study and receive standard of care periodontal treatment at the TUSDM clinic at standard clinic costs.

G) Study Procedures

<u>Visit 1: Screening and consent (Approximately 90 minutes)</u>

Subjects will be asked to read the informed consent form (ICF). Subjects will be given ample time to have any questions answered. If a subject decides to participate, he or she will be asked to sign the ICF. A copy of the ICF will be given to the subject.

Medical history and demographic information will be collected. To determine eligibility periodontal examination will be performed according to standard of care, including

measuring pocket depth, recession, attachment level, and bleeding on probing. If full periodontal examination was performed at TUSDM no more than 6 months ago, then existing records may be used to determine eligibility. As periodontal measurements are not a primary outcome, existing periodontal measurements collected by other TUSDM dentists may be used.

Inclusion and exclusion criteria will be considered and eligibility for the study will be determined.

Alginate impression for the maxillary teeth will be taken using stock plastic trays according to subject's arch size in conformance with standard of care practice.

<u>After Visit 1 (In the Laboratory): Impression pouring, cast, and mouth guard fabrication</u>

After the visit, alginate impressions will be disinfected and poured in the dental laboratory using dental stone. Subjects are not required to stay for the length of time of laboratory procedures. Dental stone will be allowed to set for 45 minutes before cast retrieval. After retrieval, casts will be trimmed according to laboratory standards. Titanium discs (5mm diameter, 1 mm thick, 4 discs each on the left and right premolar/molar areas, a total of 8 discs per subject, supplied by Straumann USA) will be fixed on the buccal surfaces of posterior teeth of the casts. A computerized positive pressure thermal-molding machine (Biostar, Great Lakes Orthodontics, Tonawanda, NY) will be used to fabricate a 1mm thick mouth guard on top of the cast, covering the attached titanium discs. The mouth guard will be removed from cast and trimmed to proper size. New titanium discs of the same size and count will be secured in mouth guard compartments with surgical adhesive (PeriAcryl, Salvin Dental Specialties, Charlotte, NC, Figure 1). The final shape of the appliance will resemble an occlusal mouth guard. The mouth guard will be disinfected in 0.12% chlorhexidine.

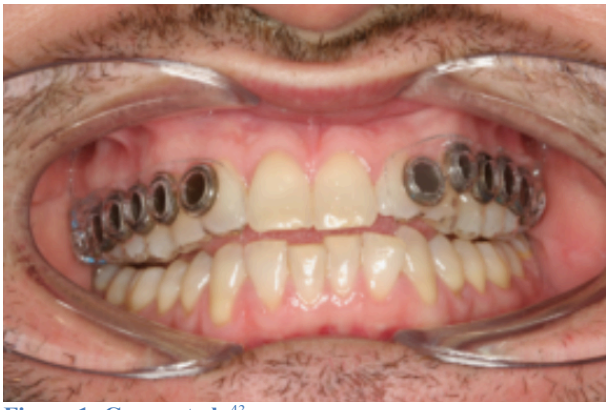

Figure 1: Gosau et al. [43]

<u>Visit 2: Within 2 months of Visit 1- Mouth guard delivery (approximately 60 minutes)</u>

Medical history will be reviewed. Eligibility criteria will be re-evaluated. The mouth guard will be inserted into subject's mouth and adjusted for complete fitting and comfort. Intra-oral photograph will be taken in frontal view of the subject's teeth and gums while wearing the mouth guard.

Subject will be asked to use the mouth guard for 72 hours continuously except during eating. Patient will be instructed to brush their teeth twice daily without the use of toothpaste and to floss once daily. Mouth rinses will be prohibited. These instructions will be given verbally with demonstration facing a mirror. The same instructions will be provided to study subjects in writing as well.

Visit 3: 3 days after Visit 2- Mouth guard retrieval (approximately 30 minutes)

Medical history will be reviewed. Eligibility criteria will be evaluated. Mouth guard will be retrieved from patient and stored until analysis.

After Visit 3(In the laboratory): Group Assigning, Experimental Treatments, and Measurements

After the visit, titanium discs will be separated from the mouth guard. Subjects are not required to stay for the length of time of laboratory procedures. Care will be taken not to disturb the surface biofilm. The back side of each disc will be numbered 1-64 and the subject and intraoral location will be recorded. Discs retrieved from each occlusal mouth guard will be randomized over the 4 treatment groups using the "sample" function of the statistical software package R (Version 3.1.2), so that each subject will contribute two discs to each treatment group.

After treatment, discs will be placed individually in a 24-well tissue culture plate, rinsed with saline and stained using the LIVE/DEAD *Bac*Light dye Kit (Thermo Fisher Scientific, Waltham, MA). Equal volumes of Component A and Component B will be combined in a microfuge tube. The dye mixture will be added to the discs and incubated at room temperature in the dark for 15 minutes. The discs will be observed in a fluorescence microscope equipped with FITC/TRITC filter sets (Zeiss Axiovert 200, Carl Zeiss AG, Oberkochen, Germany). Percent biofilm covered area will be calculated with digital image analysis software (ImageJ, NIH).

Appointment / Procedures	Visit 1 Screening/ Baseline	Visit 2 Baseline (Treatment Day 0)	Visit 3 Retrieval (Treatment Day 3)
Consent Form	X		
Demographics	X		
Medical/Dental History	X	X	X
Inclusion/Exclusion Criteria	X	X	X
Oral Examination	X		
Digital Photography		X	
Alginate Impressions	X		
Compensation			X

Mouth guard Delivery		X	
Mouth guard Instructions		X	
Mouth guard Retrieval			X

H) Subject Safety

1) Adverse Event Reporting

Adverse Events

An adverse event is any untoward or unfavorable medical occurrence in a human subject, including any abnormal physical exam or laboratory finding, symptom, or disease, temporally associated with a subject's participation in the research. An adverse event in this study may be the loss of the mouth guard or the loss of a titanium disc.

Adverse events will be recorded in source documents and on case report forms. All adverse events and non-serious situations will be recorded, monitored, and reported to the IRB at time of continuing review or at the study's termination if this occurs before the study's next continuing review.

Adverse events will be recorded in source documents and on case report forms. All adverse events and non-serious situations will be recorded, monitored, and reported to the IRB at time of continuing review.

Serious Adverse Events

A serious adverse event is one that results in death, or is life-threatening, or results in hospitalization or prolongation of existing hospitalization, or results in a persistent or significant disability/incapacitation, or results in a congenital anomaly/birth defect, or may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed above. Based on clinical experience with mouth guards it is unlikely that study subjects will experience any serious adverse event. Should a serious adverse events occur, will be recorded in source documents and on case report forms. Serious Adverse Events that meet the criteria of an unanticipated problem will be reported to the IRB within 5 business days following the Reportable New Information Policy. Serious adverse events that do not meet the criteria for an unanticipated problem will be recorded in source documents and on case report forms, and included in the summary reporting form at the continuing review or at the study's termination if this occurs before the study's next continuing review.

Unanticipated Problems

An unanticipated problem is an incident, experience, or outcome that meets all of the following criteria: 1) The nature, severity, or frequency is unexpected for the subject population or research activities as described in the current IRB approved protocol, supporting documents, and the ICF(s); 2) it is related or possibly related to

participation in the research; 3) it suggests the research may place the subject or others at a greater risk of harm then was previously recognized.

Unanticipated problems will be recorded in source documents and on case report forms. Unanticipated problems will be reported to the IRB within 5 business days after the PI/study team becomes aware of the problem. A Reportable New Information Form will be submitted to the IRB no later than 5 business days after the PI/study team becomes aware of the problem.

Subject Participation

2) Screening

   Dr. AlMoharib will conduct screening as outlined in Visit 1.

3) Informed Consent

   Dr. AlMoharib will introduce the study.

   Written consent will be obtained following "SOP: Written Documentation of Consent (HRP-091)." Consenting will take place in a private clinic bay area and the patient will be given as much time as he/she needs to consider participation. The participant will be invited to include or exclude any associates (e.g., loved ones) in the consent process.

   Patients will be asked to read the consent form and given ample opportunity to have their questions answered. To avoid coercion, the consenting investigator will read through the copy of the consent form with the participant section by section, making sure the participant understands each section and has an opportunity to ask questions. If at any time the participant indicates s/he is not interested in participation, the meeting will end.

   If after going through the consent form, the participant indicates s/he would like to discuss the study with associates or think about participating, then the meeting will be ended and the participant will be asked to contact the study when s/he makes her decision. If the participant contacts the study in the future for participation, s/he will be invited back to the clinic, and if informed consent is given at that time, study activities will begin then.

   If the participant indicates s/he may be interested in participating after going through the consent form with the investigator, and the investigator determines the participant has the capacity to provide informed consent, the participant will be asked to provide informed consent at that time. Patients will certify their willingness to participate in the study by signing and dating the IRB approved informed consent document. The subject will be given a copy of the consent form.

If any new finding requires any change to the informed consent form, the subject will be re-consented.

Non-English speaking subjects will not be enrolled in the study because study staff at this time are not certified, prepared, or trained to translate or communicate in any language other than English. The study budget does not allow for the payment of translation services at this time. There are no benefits to the subject by participating in this study.

4) Study Location

Tufts University School of Dental Medicine

5) Personnel
   - Robert Gyurko, PI: supervision of the study, contact with IRB, data analysis
   - Hani AlMoharib, Co-investigator, Resident: obtaining informed consent, performing study visits, recording data
   - Bjorn Steffensen, Co-Investigator, advisor
   - Driss Zoukhri, Co-Investigator, advisor
   - Matthew Finkelman, statistician, data analysis

6) Payment for Participation
   (a) Compensation
   - Amazon gift card ($25, given at the completion of Visit 3)
   - Subjects will not be able to keep the mouth guard fabricated for the study. If they wish to have a mouth guard, they will be referred to TUSDM for the fabrication of a new mouth guard. Subjects will be responsible for payment of a new mouth guard.

   (b) Transportation
   - No reimbursement for travel.

   (c) Payment and Insurance
   - Subjects and their insurance will not be billed for any study procedures.

7) Study Results

If interested, study results will be presented to a subject upon their request, either in person or via mail according to their preference, upon completion of the study. An internal log will be kept of the participants who have requested to receive study results.

8) Confidentiality

To ensure confidentiality of subject information, each subject enrolled in the study will be assigned a unique alphanumeric code. Subjects' files will be kept in a secure, locked cabinet in a secure room (PI's office) when the files are not being reviewed. The information will only be shared between the researchers. All HIPAA requirements

will be followed. All information collected in this study will be stored in a secure location TUSDM and kept strictly confidential except as may be required by law. All electronic files will be kept on a password protected computer in a secure, locked office. If any publication results from this study, subjects will not be identified by name without prior consent.

(a) Coding

Once eligibility has been determined by the inclusion and exclusion criteria, subject identification number and treatment group will be assigned. Identification numbers will be assigned sequentially. The full subject identification number will consist of the three letters from the subject's initials and their enrollment number.

(b) Access

Only study personnel will have access to data. Investigators will permit monitoring, audits, and regulatory inspections and will provide direct access to study related documentation.

9) New Findings

The subject will be informed of any significant new findings discovered during the course of this study that might influence the subject's continuation and participation in the study. Subjects will be told at a study appointment or via telephone of new findings during the study. Costs of treatment for any new findings will not be covered by the study. If new findings require revisions to the ICF, the subject will be re-consented.

10) Data Safety Monitoring Plan

Study personnel will monitor this trial for all safety related issues to determine whether an unreasonable risk to subjects develops. Quality control measures include routine inspection of case report forms, source documents, data tabulations, and tracking of adverse events.

I) Record Retention
1) Study Records

The Principal Investigator will maintain all study records and documents during the study period. All paper files and documents will be kept in a locked file cabinet, within a locked room. Electronic records will be kept on a password protected computer and only be accessible to study personnel.

2) Long Term Retention

The investigator will maintain all study records following completion or termination of this study in accordance to state law and institutional policy (a minimum of 7 years).

J) Reporting

Unanticipated problems and adverse events will be reported per the Tufts MC/TUHS IRB Reportable New Information Policy.

The IRB will be notified of any deviations from the protocol in cases of medical emergencies when the change is necessary to eliminate an apparent immediate hazard to the subject

Progress reports on the investigation shall be submitted to the IRB at regular intervals, but in no event less often than yearly, e.g., at continuing review.

K) Protocol Deviations

No protocol changes or deviations will be made without prior agreement by the IRB unless implemented to protect the health, safety or welfare of subjects. Protocol changes or deviations that affect the scientific soundness of the study or the rights, safety or welfare of human subjects will be made by a formal amendment subject to IRB approval. All such changes or deviations will be included in the final study report.

L) Study Termination

This study may be terminated for the following reasons:

Discovery of unforeseen risk that could jeopardize the dental/physical well-being of subjects.

Enrollment or recall rates that are not likely to produce sufficient data for evaluation of safety and efficacy

Non-compliance with the clinical investigational plan, the Investigator

Agreement, applicable FDA regulations or conditions of approval imposed by the reviewing IRB

Withdrawal of IRB approval

In the event of study termination, the Principal Investigator will determine whether subjects are in need of additional treatment and/or follow-up observation as a result of participation in this trial.

M) Subject Recruitment/Advertising

Flyers will be posted in TUSDM by the elevators, in the Periodontology clinic waiting room and on the pre-doctoral clinic floors next to the huddle boards. Permission is not required for these posting locations. Flyers will remain posted until enrollment goals are met. The same flyer will be emailed to TUSDM pre-doctoral and postdoctoral students and dental school faculty (see attached flyer). Subjects will be recruited through responding to posted study advertisements. These posted advertisements will be visible to faculty, staff, students, and patients.

Investigators may also inform clinic patients about the study. Investigators may send messages to colleagues via axiUm or inform colleagues verbally, asking for their help in recruiting eligible subjects. In addition subjects will be recruited from TUSDM pre-doctoral clinics through verbal requests to Practice Coordinators. All of the forms of recruitment will be submitted for IRB approval prior to use.

A screening interview/questionnaire or screening script will be used for recruitment in response to potential subjects who answer advertisements. Subject name, contact information and screening criteria (specified in phone script) will be collected and recorded during the calls so that the study team can follow-up with the interested people for an in-person screening.

Screen failure data will be retained by PI. Confidentiality of subjects that screen fail will be maintained following procedures outlined in the confidentiality section. Screening ID number and demographic information will be recorded. Identifiable information will not be recorded in the screening log.

N) References

1. Iacono VJ. Dental implants in periodontal therapy. *J Periodontol* 2000;71:1934-1942.
2. Albrektsson T, Dahl E, Enbom L, et al. Osseointegrated oral implants. A Swedish multicenter study of 8139 consecutively inserted Nobelpharma implants. *J Periodontol* 1988;59:287-296.
3. Albrektsson T, Lekholm U. Osseointegration: current state of the art. *Dent Clin North Am* 1989;33:537-554.
4. Roos J, Sennerby L, Lekholm U, Jemt T, Grondahl K, Albrektsson T. A qualitative and quantitative method for evaluating implant success: a 5-year retrospective analysis of the Branemark implant. *Int J Oral Maxillofac Implants* 1997;12:504-514.
5. Sbordone L, Barone A, Ciaglia RN, Ramaglia L, Iacono VJ. Longitudinal study of dental implants in a periodontally compromised population. *J Periodontol* 1999;70:1322-1329.
6. Binon PP. Implants and components: entering the new millennium. *Int J Oral Maxillofac Implants* 2000;15:76-94.
7. Albrektsson TO, Johansson CB, Sennerby L. Biological aspects of implant dentistry: osseointegration. *Periodontol 2000* 1994;4:58-73.
8. Zitzmann NU, Berglundh T. Definition and prevalence of peri-implant diseases. *J Clin Periodontol* 2008;35:286-291.
9. Heitz-Mayfield LJ. Peri-implant diseases: diagnosis and risk indicators. *J Clin Periodontol* 2008;35:292-304.
10. Derks J, Tomasi C. Peri-implant health and disease. A systematic review of current epidemiology. *J Clin Periodontol* 2015;42 Suppl 16:S158-171.
11. Koldsland OC, Scheie AA, Aass AM. Prevalence of peri-implantitis related to severity of the disease with different degrees of bone loss. *J Periodontol* 2010;81:231-238.
12. Augthun M, Conrads G. Microbial findings of deep peri-implant bone defects. *Int J Oral Maxillofac Implants* 1997;12:106-112.
13. Furst MM, Salvi GE, Lang NP, Persson GR. Bacterial colonization immediately after installation on oral titanium implants. *Clinical oral implants research* 2007;18:501-508.
14. Leonhardt A, Renvert S, Dahlen G. Microbial findings at failing implants. *Clinical oral implants research* 1999;10:339-345.
15. van Winkelhoff AJ, Goene RJ, Benschop C, Folmer T. Early colonization of dental implants by putative periodontal pathogens in partially edentulous patients. *Clinical oral implants research* 2000;11:511-520.
16. Canullo L, Penarrocha-Oltra D, Covani U, Rossetti PH. Microbiologic and Clinical Findings of Implants in Healthy Condition and with Peri-Implantitis. *Int J Oral Maxillofac Implants* 2015;30:834-842.
17. Renvert S, Roos-Jansaker AM, Claffey N. Non-surgical treatment of peri-implant mucositis and peri-implantitis: a literature review. *J Clin Periodontol* 2008;35:305-315.
18. Hultin M, Gustafsson A, Hallstrom H, Johansson LA, Ekfeldt A, Klinge B. Microbiological findings and host response in patients with peri-implantitis. *Clinical oral implants research* 2002;13:349-358.

19. Quirynen M, Bollen CM. The influence of surface roughness and surface-free energy on supra- and subgingival plaque formation in man. A review of the literature. *J Clin Periodontol* 1995;22:1-14.
20. Quirynen M, van der Mei HC, Bollen CM, et al. An in vivo study of the influence of the surface roughness of implants on the microbiology of supra- and subgingival plaque. *Journal of dental research* 1993;72:1304-1309.
21. Pontoriero R, Tonelli MP, Carnevale G, Mombelli A, Nyman SR, Lang NP. Experimentally induced peri-implant mucositis. A clinical study in humans. *Clinical oral implants research* 1994;5:254-259.
22. Dmytryk JJ, Fox SC, Moriarty JD. The effects of scaling titanium implant surfaces with metal and plastic instruments on cell attachment. *J Periodontol* 1990;61:491-496.
23. Fox SC, Moriarty JD, Kusy RP. The effects of scaling a titanium implant surface with metal and plastic instruments: an in vitro study. *J Periodontol* 1990;61:485-490.
24. Homiak AW, Cook PA, DeBoer J. Effect of hygiene instrumentation on titanium abutments: a scanning electron microscopy study. *The Journal of prosthetic dentistry* 1992;67:364-369.
25. al FdACe. instrumentation of dental implants: a literature review. *RSBO 2013 Jan-Mar;10(1):82-8* 2012.
26. Strooker H, Rohn S, Van Winkelhoff AJ. Clinical and microbiologic effects of chemical versus mechanical cleansing in professional supportive implant therapy. *Int J Oral Maxillofac Implants* 1998;13:845-850.
27. Karring ES, Stavropoulos A, Ellegaard B, Karring T. Treatment of peri-implantitis by the Vector system. *Clinical oral implants research* 2005;16:288-293.
28. Persson GR, Samuelsson E, Lindahl C, Renvert S. Mechanical non-surgical treatment of peri-implantitis: a single-blinded randomized longitudinal clinical study. II. Microbiological results. *J Clin Periodontol* 2010;37:563-573.
29. Schwarz F, John G, Mainusch S, Sahm N, Becker J. Combined surgical therapy of peri-implantitis evaluating two methods of surface debridement and decontamination. A two-year clinical follow up report. *J Clin Periodontol* 2012;39:789-797.
30. Claffey N, Clarke E, Polyzois I, Renvert S. Surgical treatment of peri-implantitis. *J Clin Periodontol* 2008;35:316-332.
31. Kamel MS, Khosa A, Tawse-Smith A, Leichter J. The use of laser therapy for dental implant surface decontamination: a narrative review of in vitro studies. *Lasers in medical science* 2014;29:1977-1985.
32. Goncalves F, Zanetti AL, Zanetti RV, et al. Effectiveness of 980-mm diode and 1064-nm extra-long-pulse neodymium-doped yttrium aluminum garnet lasers in implant disinfection. *Photomedicine and laser surgery* 2010;28:273-280.
33. Kreisler M, Al Haj H, Gotz H, Duschner H, d'Hoedt B. Effect of simulated CO2 and GaAlAs laser surface decontamination on temperature changes in Ti-plasma sprayed dental implants. *Lasers in surgery and medicine* 2002;30:233-239.
34. Ferreira CF, Babu J, Migliorati EK, Stein S, Garcia-Godoy F. Assessment of the Effect of CO2 Laser Irradiation on the Reduction of Bacteria Seeded on Commercially Available Sandblasted Acid-Etched Titanium Dental Implants: An In Vitro Study. *Int J Oral Maxillofac Implants* 2015;30:588-595.
35. Kreisler M, Gotz H, Duschner H. Effect of Nd:YAG, Ho:YAG, Er:YAG, CO2, and GaAIAs laser irradiation on surface properties of endosseous dental implants. *Int J Oral Maxillofac Implants* 2002;17:202-211.
36. Kreisler M, Kohnen W, Marinello C, et al. Bactericidal effect of the Er:YAG laser on dental implant surfaces: an in vitro study. *J Periodontol* 2002;73:1292-1298.
37. Matsuyama T, Aoki A, Oda S, Yoneyama T, Ishikawa I. Effects of the Er:YAG laser irradiation on titanium implant materials and contaminated implant abutment surfaces. *Journal of clinical laser medicine & surgery* 2003;21:7-17.
38. Quaranta A, Maida C, Scrascia A, Campus G, Quaranta M. Er:Yag Laser application on titanium implant surfaces contaminated by Porphyromonas gingivalis: an histomorphometric evaluation. *Minerva stomatologica* 2009;58:317-330.
39. Sennhenn-Kirchner S, Schwarz P, Schliephake H, Konietschke F, Brunner E, Borg-von Zepelin M. Decontamination efficacy of erbium:yttrium-aluminium-garnet and diode laser light on oral Candida albicans isolates of a 5-day in vitro biofilm model. *Lasers in medical science* 2009;24:313-320.
40. Tosun E, Tasar F, Strauss R, Kivanc DG, Ungor C. Comparative evaluation of antimicrobial effects of Er:YAG, diode, and CO(2) lasers on titanium discs: an experimental study. *Journal of oral and maxillofacial surgery : official journal of the American Association of Oral and Maxillofacial Surgeons* 2012;70:1064-1069.

41. Schwarz F, Jepsen S, Herten M, Sager M, Rothamel D, Becker J. Influence of different treatment approaches on non-submerged and submerged healing of ligature induced peri-implantitis lesions: an experimental study in dogs. *J Clin Periodontol* 2006;33:584-595.
42. Schwarz F, Sculean A, Romanos G, et al. Influence of different treatment approaches on the removal of early plaque biofilms and the viability of SAOS2 osteoblasts grown on titanium implants. *Clinical oral investigations* 2005;9:111-117.
43. Gosau M, Hahnel S, Schwarz F, Gerlach T, Reichert TE, Burgers R. Effect of six different peri-implantitis disinfection methods on in vivo human oral biofilm. *Clinical oral implants research* 2010;21:866-872.

O)