Induced Gingivitis & Intra-oral Imaging
Protocol Number CRO-2018-06-EXG-JG

ClinicalTrials.gov ID: NCT03750955

Protocol Date: July 18, 2018

Sponsored by:

Colgate-Palmolive Technology Center
909 River Road
Piscataway, NJ 08855

Protocol Approval

Signature Page

Dr. Maria Ryan
Vice President and Chief Dental Officer

Date

Dr. LaTonya Kilpatrick-Liverman
Word Wide Director – Oral Care Clinical Research

Date

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PRINCIPAL INVESTIGATOR APPROVAL AND AGREEMENT

I, the undersigned, have reviewed this protocol and I agree to conduct this protocol in accordance with the Guidelines for Good Clinical Practice, the ethical principles set forth in the Declaration of Helsinki, and with the U.S. Code of Federal Regulations governing the protection of human subjects (21 CFR 50) and the institutional review boards (21 CFR 56).

_____ _____

Richard Darveau, PhD
Principal Investigator

Date

STUDY AIM:

To evaluate the ability of an intra-oral imaging device to assess the level of gingival inflammation present and the process of resolution using a localized experimentally induced gingivitis model.

BACKGROUND AND SIGNIFICANCE:

This project is significant for public health because it aims to validate a non-invasive imagining examination for determining gingival (gum) inflammation. Given that the prevalence of gingivitis in the US population is greater than 50%, and that gingivitis can progress to periodontitis and tooth loss if left untreated, the proposed non-invasive imaging method can be an invaluable asset in clinicians' quivers for the early prevention of this prevalent disease.

STUDY DESIGN

This study is a modification of classical experimental gingivitis model. The study will prospectively enroll subjects and a maxillary sextant to an intervention, while the other sextant will serve as control in a split-mouth design of localized experimental gingivitis, utilizing localized stent-induced biofilm overgrowth model (SIBO). The intervention will consist of cessation of oral hygiene versus an active control of oral hygiene with a fluoridated toothpaste that will be provided by the investigators. The intervention, i.e. oral hygiene cessation will manipulate the participants' oral environment by leading to reversible inflammation of the gingival tissues. This is a well established protocol that was first introduced in 1965 (Loe's experimental gingivitis) and is safe, reliable and widely utilized in periodontal clinical trials. The purpose of this study is to evaluate the effects of oral hygiene cessation in the gingival tissues by an innovative non-invasive microimaging technique, i.e. OCT.

The study will include the following phases – 1) Hygiene phase of 2 weeks; 2) Gingivitis induction phase with stent lasting up to 2 weeks; and 3) Resolution phase of 2 weeks. This pilot study will enroll up to 15 generally healthy adults aged 18-35 years that are self-referred or referred to the UW School of Dentistry. Recruitment will be completed in 3 waves. The study will include an oral health history review, probing depths, plaque & gingival evaluations, biospecimens collection and imaging of the buccal gingiva of teeth #5 - 12.

RECRUITMENT

All study participants will be patients of record at the UW School of Dentistry, or referred. All study participants will undergo informed consent process, prior to enrollment in the study. Categorization of participants and sites will be based on the clinical assessments. Other than the study-related oral health history recorded, there is no intention to obtain information from the participants' other dental or medical records. The first participant will be assigned a number, and all the following subjects will be assigned consecutive numbers by accession.

Inclusion criteria:

- Aged 18-35 years
- In good general health, ASA I
- No clinical signs of gingival inflammation at >90% sites observed
- Probing Depth(PD) $\leq$ 3.0 mm
- Attachment Loss (AL) = 0 mm
- Gingival health at baseline visit (Day 0): Gingival Index (GI) $\leq$ 0.5, Bleeding on probing (BOP)(-)
- Non-smokers
- Fluent in English

Exclusion criteria:

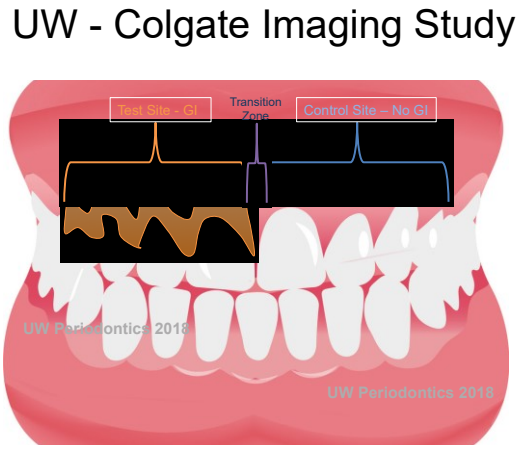
- Medical condition which requires premedication prior to dental treatments/visits
- Subjects unable or unwilling to undergo informed consent process.
- Subjects currently using anti-gingivitis oral rinses (washout period of 1 week)
- History of periodontal disease
- History of systemic inflammatory or immune conditions
- Use of antibiotic or anti-inflammatory drugs within 30 days of enrollment
- Pregnant or breastfeeding at time of screening
- Concurrent orthodontic treatment
- Untreated carious lesions and/or inadequate restorations, implants, crowns on maxillary non-molar teeth
- Participation in any other clinical study or test panel within 1 week prior to enrollment into this study
- Use of tobacco products
- Subjects who must receive dental treatment during the study dates
- Orthodontic bands, appliances, or crowns and bridges, or removable partial dentures affecting the non-molar maxillary teeth
- Immune compromised individuals (HIV, AIDS, immuno suppressive drug therapy)

METHODS

Induced Gingivitis Model

In each participant, one maxillary quadrant will be assigned as test quadrant (experimental gingivitis) and contralateral quadrant as control quadrant. Assignment of quadrants will be random using a computer program for randomization.

At the initial visit all participants will undergo the informed consent process, with thorough discussion of study participation details. Full clinical assessments, biospecimen collections and imaging will be performed and maxillary impression will be taken for acrylic stent. Maxillary acrylic stents will be fabricated to cover teeth on test quadrant extending at least 4-5 mm apical to the marginal gingiva both buccal and palatal and will be relieved around interproximal areas to prevent plaque disturbance. An oral prophylaxis will be administered and thorough oral hygiene instructions will be given. Participants will return 14 days after this initial visit and will have baseline measurements and biospecimen collections taken. These measurements include an abbreviated health assessment with a periodontal probe to measure pocket depths, gingival and plaque index and Imaging of the buccal gingival on eight maxillary teeth (#'s 5-12), and collection of crevicular fluid and plaque samples[37].



At the baseline visit the acrylic stent will be given to participant with detailed instructions for use. Participants will be given instructions not to brush teeth on test quadrant (under the provided stent) and not to use any other measure of oral hygiene (flossing, interdental aids, xylitol gum, and mouth rinses). Participants will be provided with Colgate Cavity Protection Toothpaste and a soft bristle toothbrush to use during their entire participation in this study. In order to prevent accidental brushing of the experimental sites, subjects will be asked to wear the maxillary stent during regular brushing. Participants will be

rechecked at Day 2, 4, 7 & 14 and a full assessment of their plaque and gingival status will be carried out, specimens collected, and imaging performed each time using the same criteria as at the baseline examination. The course of the "no-brushing" part of the experiment will last up to 14 days to allow all the participants to develop gingivitis. Following clinical assessment and biospecimen collections at day 14, a thorough prophylaxis will be administered.

Participants will be given detailed instructions in oral hygiene methods using a manual toothbrush and Colgate Cavity protection toothpaste. Subjects will also usedental floss beginning the same day and continuing once in the morning and once at night for the duration of the study. Assessment of plaque and gingival condition along with imaging will continue during the entire reversal phase, Day 21 and Day 28. At a point where the Gingival Index and Plaque Index scores approach zero (approximately day 28), all measurements and biospecimen samples will be taken, along with imaging and that visit will be recorded as end-of-study.

Medical imaging device

The medical imaging device is a home-built multi-functional OCT system (MI-OCT), which is also illustrated in Figure1. The functionality of the system includes:

- Anatomical structure imaging (OCT)
- Functional angiography (OCTA)
- Molecular imaging: Red and green autofluorescence imaging (MI)

The components of the system include:

- A laser light source (NIR, 1300nm peak, 100nm bandwidth, peak power 3.5mW)
- Optical components that transmit OCT light beam into biological tissue, including: optical fiber and optics table, galvanometer mirror scanners, and several other optics components (lenses, dichroic mirrors, prism, etc.)
- CMOS camera for molecular imaging (MI)
- An excitation light source in violet-near UVA wavelength (405±5 nm)
- A computer and monitor for real-time imaging display, data processing, and data analysis.

The home-built MI-OCT device include OCT module, which is similar to commercially FDA approved OCT devices, and was also approved by the IRB as ‘Non-Significant’ risk for skin imaging (IRB No. 41841) and for periodontal imaging (IRB No. 2827). The difference between MI-OCT and the aforementioned units are:

- Violet-near UVA excitation light source (LED ring, 405±5 nm wavelength). CMOS 3-channel red-green-blue camera with added optics (optical filter, lenses, etc.)
- Wiring and cable harness, providing power to the LED ring and CMOS camera

Although the wavelength is within the visible spectrum and the LED ring is operated at lower power level (25mW, radiating over a surface of 10×10cm2, which yields 0.25 mW/cm2), participants will be given UVA-protective goggles to reduce the risk of exposure to possible residual UVA emissions from the LED. The length of exposure will also be limited to maximum 15 minutes in the MI mode (2 minutes for each scanning sites, total 6 scan sites)

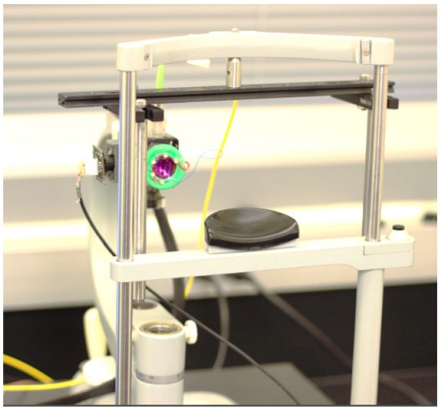


Fig 1a.

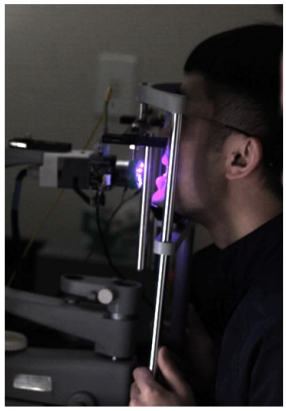


Fig. 1b

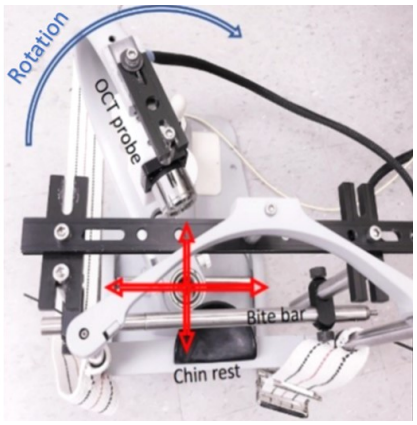


Fig. 1c

Figure 1 a) MI-OCT probe which is attached to a stable frame, b) MI-OCT in operation, showing visible violet excitation light when operated in autofluorescence mode, c) components of the stable frame

Imaging procedure

Participants will be instructed to wear a dental retractor to expose the maxillary incisors, canines, and 1st bicuspids prior to the scan. The scanning protocols for each scan-site (6 in total) are as followed:

- Positioning: participants will be asked to rest their chins on the chin-rest of the stable frame. They will also be asked to bite on the bite bar (Figure 1c) during the whole scanning procedure. Operator will re-position the scanning probe for each scan-site. This step takes approximately 1 to 3 minutes for each scan-site.
- Real-time autofluorescence imaging and registration: participants will be exposed to 405nm excitation light source for approximately 2 minutes; autofluorescence images are captured and addition registration (re-positioning of the probe) is performed when needed. This step takes approximately 2-3 minutes
- OCT scan: participants will be asked to stay particularly still in this step. The scan will be performed within a window of less than 1 minute.

OUTCOMES

In the present study, we will be examining the sensitivity, specificity, and practicality of a new microimaging technique for assessing experimental gingivitis, as compared with standard health-related biomedical outcomes (i.e. gingival index and gingival crevicular fluid volume).

Abbreviated periodontal health assessment

Clinical data will be documented based on probing depth (PD), visible plaque index (VPI), gingival index (GI) and bleeding on probing (BOP) [38]. All clinical measurement will be conducted using a manual periodontal probe. BOP will be recorded within 20 seconds of probing. The following four maxillary teeth will be used in each test and control quadrant: first premolar – central incisor. For each tooth, all measurements and biospecimen collection will be taken on mesiobuccal and mesiopalatal surfaces. Staggering of sites (mesiobuccal & mesiodistal) will be performed for plaque collection.

Visible Plaque Index

The surfaces of the study teeth will be scored and given either a “0” no plaque or “1” plaque assessment:

0 = No plaque

1 = Visible Plaque

Loe and Silness Gingival Index (GI)
The surfaces of the study teeth will be evaluated using light air and a periodontal probe. A score is given based on the following scale:
0 = Normal gingiva
1 = Mild inflammation, slight change in color, slight edema, no bleeding on probing
2 = Moderate inflammation, moderate glazing, redness, bleeding on probing
3 = Severe inflammation, marked redness and hypertrophy, ulceration, tendency to spontaneous bleeding

Gingival Crevicular Fluid (GCF) collection

Prior to GCF collection the plaque index will be recorded. The sites to be sampled will be isolated with cotton rolls and gently air-dried. GCF samples will be collected with sterile paper strips (Periopaper strips, Oraflow Inc.) that will be inserted into the gingival crevice until mild resistance is felt and left in place for 30 seconds.

Amount of GCF samples collected will be quantified with a Periotron 6000 (Oraflow, Inc.) and will be expressed in microliter.[39] GCF will be collected from the mesiobuccal and mesiopalatal surfaces of teeth #'s 5- 12. Paper strips visibly contaminated with saliva and blood will be excluded from the study. All samples will be collected in micro centrifuge tubes. They will be transported on ice and stored within 24 hours at -80oC until processing.

Plaque collection

Sub-gingival plaque will be collected using sterile paper points inserted in the gingival sulcus for 30s from the mesiobuccal and mesiodistalsurfaces of teeth #'s5- 12. Plaque samples will be placed in micro centrifuge tubes then transported on ice to -80oC freezer in lab.

Analysis of GCF chemokines

GCF samples will undergo assay of 3 angiogenesis chemokines using commercially available kits. The following list of chemokine will be analyzed: angiopoietin-1, vascular endothelial growth factor, and hypoxia-inducible factor 1 alpha. The expression of these chemokines have all been shown to contribute to angiogenesis and possibly homeostasis in gingival tissue. Commercially available ELISA kits will determine the concentration of Myeloperoxidase (MPO).

Analysis of microbial composition

Plaque samples will be stored in the Periodontics Repository for future studies. The samples will not be analyzed as part of this study.

Imaging

1) Autofluorescence imaging

Autofluorescence images are captured and analyzed for two purposes:

a. Plaque (biofilm) detection (red autofluorescence)
b. Enamel absorption and anomalies (green autofluorescence)

The images are registered in each subsequent visit. This registration step allows observation on the progression of a) plaque and b) tissues response over the whole study period.

2) OCT and OCTA

Data is captured for each visit and is analyzed off-line. The following features are extracted and monitored:

a. The rete-ridges structure (OCT) in free gingiva. During inflammation, the rete-ridges layer becomes thinner and is replaced by connective tissues.
b. The depth of the free gingiva (OCT+OCTA), which is defined by a line joining: i) the

surface of the gingiva, ii) projected perpendicular to the gingival sulcus line, and iii) passing through the point where connective blood vessels in free/attached gingiva disappear

c. The vasculature (OCTA) in the free gingiva.
d. Possible changes in other morphology (reserved for exploratory parameters).

Recovery and Termination from the Study

All subjects will be evaluated weekly during induction phase. In the case that participants develop GI score 3 in 25% or more of their experimental teeth, they will be immediately moved to the resolution phase. Otherwise the participants will finish the full induction phase and then be moved into resolution phase. All subjects will be followed until gingivitis condition is completely resolved, beyond the two weeks period if necessary. If any participants have persistent gingivitis that does not resolve, they will be referred to the Graduate Periodontics Clinic at the University of Washington School of Dentistry.

Tooth Stent Construction

An alginate impression will be taken of one maxillary quadrant of each subject for the construction of an individual toothshield stent for use during the trial period. The quadrant will be randomly selected. A working model cast will be prepared immediately from the impression using dental stone and labeled with the subject's ID. After curing for an hour, the cast will be removed from the impression mold, trimmed to the desired shape and size, then dried at room temperature overnight.

The toothshield stents will be constructed from 3-mm-thick plastic mouthguard material to include only the teeth and gingival margin and to eliminate contact with the cervical margin of each tooth, thereby reducing the risk of plaque being disturbed during insertion or removal of the shield. Elimination of cervical contact is accomplished by blocking out around the gingival margin and proximal surfaces using a spacer made from 1-mm-thick mouthguard material. Dipping the cast in water, allowing it to drain, and then adapting the 1-mm-thick material by using a vacuum former. After cooling, the material is scribed with a scalpel to a height one-third of the clinical crowns, following the contour of the gingivae; removed from the cast; and trimmed with scissors to ~2 mm above the gingival margin. The 1-mm-thick spacer is placed on the model; coated with silicone spray; then the 3-mm-thick material is vacuum-adapted over the model. When cooled, the mouthguard material is removed from the model and the spacer peeled out. The 3-mm material is trimmed vertically on the buccal side to a length just short of the vestibule and frenum attachments, and on the lingual side to 2 mm above the gingival margin. It is trimmed mesially to the midline, and distally behind the first premolar.

A label with the subject's ID and the date of preparation is affixed to the buccal surface, and then 1-mm-thick mouthguard material is vacuum-adapted over the toothshield and trimmed to the original contours. The shields are prepared prior to the prophylaxis, chemically disinfected, and packed in individual plastic bags with each subject's name.

Statistical Analysis Plan

Multiple sites within each subject will be evaluated using a Gingival Bleeding Index at baseline and subsequent timepoints during clinical exams. The gingivitis bleeding scores will be averaged to provide a mean score per subject for each exam period. Product means and standard deviations will be calculated for each time period. Analysis of Variance techniques (possibly including Analysis of Covariance using the baseline scores as the covariant) will be used to compare the difference between the two test treatments using the 95% confidence level ($p \leq 0.05$).

Based upon our initial study the selection of 15 participants is expected to identify statistically significant differences in clinical parameters, GCF chemokines level, MPO expression and microbial composition over time among the subjects of the study. Participants will be enrolled in cohorts of 5 and we have planned for 3 waves to reach the final sample size N=15.

REPORT OF ADVERSE REACTIONS

Subjects will be told of any possible adverse reactions or side effects from lack of oral hygiene and stent use, i.e. oral irritation. At the all clinic visits/examinations each participant will be asked if they have experienced any discomfort or oral irritation. In addition, all subjects will be examined for signs of epithelial desquamation (sloughing) and discoloration of the teeth and/or tongue. The investigators will record any and all adverse reactions. If the evidence indicates that the adverse reaction may be due to lack of oral hygiene, the subject will be instructed to resume oral hygiene and appropriate treatment will be provided. If an adverse event occurs it is expected to be mild and abate (stop) when oral hygiene is resumed. In the event of an adverse experience, emergency or other problems or questions regarding participation in this study subjects will be advised to contact the following investigator(s):

Richard Darveau, MS, PhD
Office: 206-543-5043

In the event of a medical emergency, subjects will be instructed to contact their physician.

Definitions

Adverse Events (AEs) and Serious Adverse Events (SAEs) are defined by the ICH Guideline for Good Clinical Practice (ICH GCP) as follows:

Adverse Event: Any untoward medical occurrence in a patient or clinical investigations subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

AEs include any clinically significant deterioration of a subject's medical status, after enrolled and signing an Informed Consent Form. The AE may involve any organs or systems and can be represented by the new onset or the deterioration of a disease, a syndrome, a symptom, a physical sign, as well as by findings and results of instrumental examinations and laboratory tests. Any medically relevant and untoward change from baseline, including frequency or pattern changes for a fluctuating condition (e.g., migraine), occurring after the first administration of study medication is an adverse event. All such occurrences must be recorded and reported accordingly, whether they appear causally related to the study medication, or not.

Serious Adverse Event: Any adverse event occurring at any dose that results in any of the following outcomes:

- Death
- Life threatening adverse event
- Inpatient hospitalization, or prolongation of existing hospitalization
- Persistent or significant disability/incapacity
- Congenital anomaly/birth defect

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Examples of such important medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

IMPORTANT NOTES: The concepts of Adverse Event / Experience[1] (AE) and Serious Adverse Event / Experience (SAE) represent **regulatory** instruments used to evaluate and monitor the safety of clinical trial subjects. Therefore, these terms only apply in light of their regulatory definition. The term "serious", in a regulatory sense, does not necessarily mean "severe". All adverse events (serious and non-serious) reported during a study will be taken into account when analyzing the study data and establishing the safety profile of the investigation, and will be included in the final study report. The SAE concept is primarily used to identify, during the conduct of the trial, those adverse events that may require an expedited reporting procedure to regulatory authorities.

Death: The outcome of death requires that the AE that resulted in death be reported as an SAE. Death, in and of itself, is not an AE; it is only an outcome. The cause of death is the AE; therefore, the investigator should make every effort to obtain and document the cause of death for all subjects who die during the study. If, despite all efforts, the cause of death remains unknown, the AE it should be documented as "unspecified fatal event".

Life-threatening Adverse Event: Any adverse event that places the patient or subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred (*i.e.,* it does not include a reaction that had it occurred in a more severe form, might have caused death).

Hospitalization: It should be noted that hospitalization, in and of itself, does not represent an SAE. It is the adverse event leading to the subject's hospitalization that becomes "serious" when it requires inpatient care. Consequently, SAE should not be reported in case of pre-planned hospitalizations for pre-existing conditions that did not worsen during the study.

Disability: A substantial disruption of a person's ability to conduct normal life functions.

Documenting and Reporting Adverse Events

General Procedures for All Adverse Events

All clinical complaints, symptoms, or signs that meet the adverse event definition will be recorded on the Adverse Reaction Form using a recognized medical term or diagnosis that accurately reflects the event. Source documentation should be maintained that allows for clear identification of each adverse event and the following parameters required for the form:

- AE description
- Date of onset
- Date of resolution
- Outcome
- Severity
- Seriousness
- Relationship to study drug (causality)
- Actions taken

Adverse events will be assessed by the investigator or designee for severity, relationship to the study intervention, possible etiologies, and whether the event meets the criteria as a serious adverse event and therefore requires immediate notification of the sponsor.

For data collection purposes, the outcome of all adverse events recorded on the Adverse Reaction Form will be designated as of the completion of the final evaluation or examination. However, the investigator is responsible for following all adverse events until resolution or until no longer of clinical concern, and providing these data to the sponsor.

Reporting Procedures for Serious Adverse Events

Any adverse event that is serious or potentially serious requires additional detailed reports and follow-up. A serious adverse event must be reported via telephone to the sponsor's representative immediately (within 24 hours) so as to facilitate discussion and implementation of necessary follow-up measures, and to enable the sponsor to submit necessary reports to regulatory authorities and other investigators. Following the initial telephone notification, the investigator must complete and submit a Serious Adverse Event Report Form to the sponsor within five calendar days. Serious Adverse Event Report Forms will be provided to the investigator upon initiation of the study. Once the sponsor reviews the Serious Adverse Event Report Form, additional information may be requested from the investigator to allow appropriate medical evaluation and determine the regulatory reporting requirements.
The investigator is responsible for following all adverse events, especially those deemed "serious", until resolution or until the event is no longer of clinical concern, and for providing these data to the sponsor in an agreed-upon format. The investigator is also responsible for reporting all serious adverse events to the Institutional Review Board (IRB) overseeing the conduct of the study at the respective study center, according to the rules and procedures established by the IRB.

COMPENSATION

Participants in this study will receive up to $500 in gift cards for their participation. A $50 gift card will be given at the end of each visit, and subjects who attended all study visits will receive an additional $100 gift card at the final visit (28-day visit). Payment will be prorated for visits attended only. No therapeutic or other benefit will be received by participating in the study.

QUALITY ASSURANCE/CONDUCT OF THE STUDY

This clinical research study will be conducted in compliance with this protocol and U.S. Federal Regulations governing informed consent (21 CFR 50), Institutional Review Board (21 CFR 56), applicable regulations governing Investigator conduct (21 CFR 312) and/or any local regulatory agency (where applicable).

It is the responsibility of the Investigator to ensure that all subject data are collected and reported according to the study protocol. Subject records will indicate subject and examination information such as visit dates, examiners, etc. that is unique to this study and the subjects. Proper documentation of all adverse events and final resolutions will be maintained. Case report forms will be used for recording all clinical data. All CRFs used in this study will be provided by the study sponsor. The Investigator will be responsible for maintaining original consent forms, case report forms (CRFs), and other source documentation.

Data Management Responsibilities

Data collection is the responsibility of the clinical research site staff under the direction of the Principal Investigator. The Investigator will keep a copy of every document (clinical and laboratory) related to the research study.

Data generated at the clinical site will be transferred to the sponsor by the Co-Investigator via mail or electronic email for statistical analyzes. The principal Investigator and study manager are responsible for the review and interpretation of the analyzed data.

Subject Termination/Procedures

A genuine effort will be made to determine the reason(s) why a subject fails to return for the necessary visit(s) or is dropped from the study. Subjects could be dropped from the study if any of the following occur:

1. Subject fails to substantially comply with the protocol requirements.

2. Subject fails to report for a scheduled examination.
3. Subject is treated with medications during the course of the study, which may interfere with the parameters of the study.
4. Subject develops an adverse reaction. The study Investigator will immediately notify the study monitor and information will be recorded on Adverse Reaction Form.
5. Subject receives emergency dental or medical treatment, which may interfere with the parameters of the study.
6. Sponsor elects to terminate the study.
7. Subject elects to terminate participation in the study.

Removal of Subjects From Study

Subjects will be dropped from the study if they receive emergency dental treatment, which in the opinion of the monitor could influence the parameters of the study. Any subject treated with an antibiotic during the study will be dropped from the study. Either the investigator or the sponsor may terminate the study at any time for well documented reasons, provided written notice is submitted at a reasonable time in advance of the intended termination.

Pregnancy

No pregnant women will intentionally be enrolled in this study. All female subjects being considered for this study will be asked about their pregnancy status. All enrolled female subjects will be required to report to the clinical/research investigator if they become pregnant during the course of the 6 week study. In the event a women enrolled in this clinical research study becomes pregnant at any time during this study, participation in this study will be terminated upon the clinical staff's notification of the event. The subject's medical records used in this study will be updated to reflect the pregnancy and there will be follow- up contact until the end of the pregnancy to record the outcome in the clinical file.

ETHICAL & REGULATORY REQUIREMENTS/HUMAN SUBJECT PROTECTION

Protocol Approval, Study Monitoring and Compliance

Prior to initiation of the study, the Investigator will obtain approval from Product Safety and Regulatory for the study protocol, the informed consent document, study instructions, and any forms of advertising in compliance with regulations. The reviewers will also review any change(s) in the protocol before the change is initiated.

Adherence to Protocol/Amendment(s)

The Investigator will be required to adhere to the final protocol. Any changes to the protocol, except those necessary to eliminate apparent hazards, will require prior approval by the local reviewers through the submission of a protocol amendment. These changes to the protocol must be implemented only through formal written protocol amendments and only upon joint approval by the sponsor and investigator. If a protocol amendment requires changes to the informed consent form, the revised consent form must also be approved by any local board. Departures from eligibility requirements may be allowed on a case-by-case basis by the medical monitor or other authorized sponsor representative. Such departures must be medically and scientifically justified, must be pre-authorized, and must be documented in the case report form and tracked as official eligibility waivers.

Institutional Review Board

Prior to initiation of the study, the Investigator will obtain Institutional Review Board approval for the study protocol, the informed consent document, study instructions, and any forms of advertising in compliance with regulations. The IRB will review the investigation at least once a year and will review any significant change in the protocol before the change is initiated. The Investigator will maintain all

original correspondence. IRB approval for this study will be obtained from:

Human Subjects Division
University of Washington Box 359470
Seattle, Washington 98195
Telephone: (206) 543-0098

Advertising

No newspaper, radio, or television advertising will be used for recruitment purposes. A flyer will be created to recruit participants. The flyer will be approved by an Institutional Review Board prior to posting or dissemination.

Informed Consent Process

Written informed consent will be obtained from all subjects prior to their enrollment into the study. The purpose and description of the study in lay language, possible adverse reactions, risks and benefits of participation and the subject's right to withdraw without prejudice at any time must be explained to each subject in the presence of a witness. The subject must read, understand and sign the informed consent form provided. The informed consent form and any other written information for subjects should meet local requirements of language and interpretation (i.e., non-English speaking subjects must be presented with informed consent forms in a language that they can understand). The consent form will comply with all applicable regulations governing protection of the participating subjects in the study, and include basic elements specified in the U. S. Code of Federal Regulations, 21 CFR 50.25(a) and 50.27 and ICH-GCPs, Chapter 4, subpart 4.8. The Informed Consent form will be reviewed and approved by the clinical site's IRB.

Each subject will be given unlimited time to read the consent form and ask questions. Subjects who agree to participate will be asked to sign and date an IRB-approved informed consent form. A copy of the signed and dated consent form will be given to each subject prior to their participation in the study. The original signed and dated informed consent document will be retained by the Investigator. All informed consent forms will be documented in a log by date and subject ID; the log will be kept as source documentation. All study procedures must be explained in non-technical terms. Study personnel will assure that participants are clearly informed regarding their roles and obligations to protect vulnerable subjects and ensure they are not under coercion or undue influence. Subjects have the right to withdraw consent at any time.

Confidentiality

All records of subject participation in this study are confidential and these records are available only to the Investigator, supervising dentist, sponsoring company and possibly the Food and Drug Administration of the United States. The results of this study may be published in a scientific journal or a government public clinical database. If any publication occurs, subjects will not be identified.

New Findings

Subjects will be informed of any significant new findings related to study products or procedures when they become known during the course of this clinical research study. Such information may affect the subject's decision to continue participation in the study.

Publication

All manuscripts or presentations based upon this study, including press statements and internal public notices and memoranda must be submitted to the sponsor for review and approval prior to release for publication or presentation. This review period will be up to 60 days in duration. Review of an abstract may be expedited in some circumstances.

ADMINISTRATIVE ASPECTS

Curriculum Vitae

The investigator will complete the FDA form 1572 and provide the sponsor with copies of his/her curriculum vitae and those of all sub-investigators listed on the form, at sponsor's request.

Data collection in the case report form

All study data will be recorded in the case report form supplied by the sponsor. All entries will be written clearly in black ink. Only the principal investigator, sub-investigators, or study coordinators may make entries in the case report forms. If erroneous data are entered on the case report forms, corrections to the data must be made by crossing out the incorrect entry with a single line (such that the initial entry remains legible) and entering the correction. All corrections on a case report form will be initialed and dated by the investigator, sub-investigator, or study coordinator making the correction.

Documentation of Consent and storage of study documents

All informed consent forms will be documented in a log by date and subject ID; the log will be kept as source documentation. Informed consent forms will be stored in a secure locked room designated for research charts storage.

Study Management

Under the direct supervision of the Principal Investigator, certain duties may be delegated during the course of the study. These responsibilities will be documented on the Transfer of Responsibilities form maintained in the Investigator's clinical file for the study.

Study monitoring

Appropriate phone calls or visits will be made by the monitor to the investigative site in order to review the protocol and to ascertain that the facility is adequate for satisfactory conduct of the study, as well as to discuss the obligations of both the sponsor and the investigator.

The investigator will permit a representative of the sponsor or the sponsor's designate and the FDA, if requested, to inspect all case report forms and corresponding portions of the study subjects' original office, hospital and/or clinic medical records at regular intervals throughout the study. These inspections are for the purpose of assessing the progress of the study, verifying adherence to the protocol, determining the completeness and exactness of the data being entered on the case report forms. During on-site visits, case report forms will be examined by the study monitor(s) and the data verified by comparison with corresponding source data (such as clinic, hospital and/or office records). Due to the geographic distance of the clinical site from the sponsor, phone conferences or teleconferences may be used to complete the monitoring requirement.

Final Report

Following the completion of the study, the Investigator shall prepare a final study report. The final report will include a general description of the conduct of the study including protocol deviations, subject withdrawals, discussion of any adverse events, safety and efficacy data, laboratory data, and statistical analysis of the data if available. This report will be approved and signed by the Principal Investigator.

Record Retention and Access to Source Data/Documents

Source documents must be kept for at least six (6) years after terminating the study. The Investigator will maintain all study documentation for all subjects entered into the study in a secure area ensuring the confidentiality of the information collected. Securing records includes placing written forms in locked file cabinets and/or sealed and labeled storage boxes in a locked room

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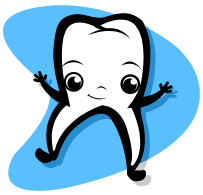
Appendix A: draft flyer



How do **unhealthy** gums become **healthy** again?

Researchers at the University of Washington want to learn more about the mechanisms in gingivitis.

Sign Up and Help Us Learn!

You may participate in this study if you:

- Are between the ages of 18 and 35
- Have healthy gums and overall are generally healthy
- Are a non-smoker

What happens in the research?

- You will have a dentist examine your gums
- If qualified, you will be asked to attend several dental visits
- Participants can receive up to $**500** in Gift Cards
- Contact us to learn more details!

APPENDIX B

Protocol Title	Induced Gingivitis and Intra-oral Imaging
Study Duration	Approximately 42 days total 14 day "no brushing" period

Pre-experimental (Hygiene) phase (Day -14 to 0) Visit at Day -14	At Day -14 visit:
	• Screening (may be done at separate visit)
	• Informed Consent
	• The following measurements will be taken on six surfaces of all teeth
	  ○ Plaque Evaluation
	  ○ Gingival Index Evaluation
	  ○ Probe pocket depths
	  ○ Attachment Level
	  ○ Bleeding on Probing
	• The following Biospecimen will be taken from mesiobuccal and mesiopalatal surfaces of teeth #'s 5- 12,
	  ○ Gingival Crevicular Fluid
	  ○ Plaque sample
	• Prophylaxis (scaling and polishing)
	• Oral hygiene instructions
	• Impression for maxillary stent

Baseline Visit at Day 0	• The following measurements will be taken from mesiobuccal and mesiopalatal surfaces of teeth #'s5- 12
	  ○ Plaque Evaluation
	  ○ Gingival Index Evaluation
	  ○ Probe pocket depths
	  ○ Attachment Level
	  ○ Bleeding on Probing
	  ○ Gingival Crevicular Fluid
	  ○ Plaque sample
	• Stent will be delivered
	• Imaging buccal gingival teeth #'s 5 - 12
	• Begin oral hygiene abstinence

Gingivitis induction phase (14 Days)
Visit at Day 2
Visit at Day 4
Visit at Day 7
Visit at Day 14
	Assessments over 14 days (4 visits)
	• The following measurements will be taken from mesiobuccal and mesiopalatal surfaces of teeth #'s5- 12
	  ○ Plaque Evaluation
	  ○ Gingival Index Evaluation
	  ○ Probe pocket depths
	  ○ Attachment Level
	  ○ Bleeding on Probing
	  ○ Gingival Crevicular Fluid
	  ○ Plaque sample
	• Imaging buccal gingival teeth #'s 5 - 12
	• At Day 14 after data collection:
	  ○ Prophylaxis (scaling and polishing)
	  ○ Reinstitution of regular oral hygiene

Resolution Phase (14 Days)
Visit at Day 21
Final Visit at Day 28
	• The following measurements will be taken from mesiobuccal and mesiopalatal surfaces of teeth #'s5- 12
	  ○ Plaque Evaluation
	  ○ Gingival Index Evaluation
	  ○ Probe pocket depths
	  ○ Attachment Level
	  ○ Bleeding on Probing
	  ○ Gingival Crevicular Fluid
	  ○ Plaque sample
	• Imaging buccal gingival teeth #'s 5 - 12