IFI16 is a Periodontitis Modulating Protein

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STATEMENT OF COMPLIANCE

The study will be conducted in accordance with the International Conference on Harmonisation guidelines for Good Clinical Practice (ICH E6), the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46), and the NIDCR Clinical Terms of Award. All personnel involved in the conduct of this study have completed human subject's protection training.

The investigator will ensure protection of subject personal data and will not include subject names on any forms, reports, publications or in any other disclosures. The Investigator will ensure that each study subject, or his/her legally acceptable representative, is fully informed about the nature and objectives of the study and possible risks associated with participation. The Investigator, or a person designated by the Investigator, will obtain written informed consent from each subject, or the subject's legally acceptable representative, before any study-specific activity is performed. The informed consent form used in this study, and any changes made during the course of the study, must be prospectively approved by local Institutional Review Board (IRB) before use. The Investigator will retain the original of each subject's signed consent form.

Individuals will be given an Informed Consent document to read and sign. Before consent is obtained, the Investigator, or a person designated by the Investigator, will provide the subject with ample time and opportunity to inquire about the details of the research study, and to decide whether or not to participate. All questions about the study should be answered to the satisfaction of the subject. The subject will have all of their study related questions answered by the Investigator or designated staff, and if they agree, they will sign the Informed Consent document.

Details on the administrative structure of this study (e.g., principal Investigator, sub-Investigator, steering committee, administration, monitoring and evaluation committees, institutions, statistician, central laboratory facilities, external service provider (ESP or CRO), or clinical study supply management) will be maintained in the Investigator's Master File throughout the study for inclusion in the clinical study report.

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this study will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Principal Investigator or Clinical Site Investigator:

Signed: _____ Date: _____

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
BOP	Bleeding on Probing
CAL	Clinical Attachment Level
CFR	Code of Federal Regulations
CRF	Case Report Form
FF	Federal Financial Report
FWA FFR	Federalwide Assurance Federal Financial Report
FWA	Federalwide Assurance
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
ICH	International Conference on Harmonisation
IRB	Institutional Review Board
N	Number (typically refers to participants)
NIDCR	National Institute of Dental and Craniofacial Research
NIH	National Institutes of Health
OHSP	Office for Human Research Protections
PPC	Periodontal Profile Classes
PI	Plaque Index
PD	Probing Depth
SAE	Serious Adverse Event/Serious Adverse Experience
SIBO	Stent-Induced Biofilm Overgrowth
SOP	Standard Operating Procedure
SRP	Scaling and Root Planing
US	United States

PROTOCOL SUMMARY

Title: **IFI16 is a periodontitis modulating protein**

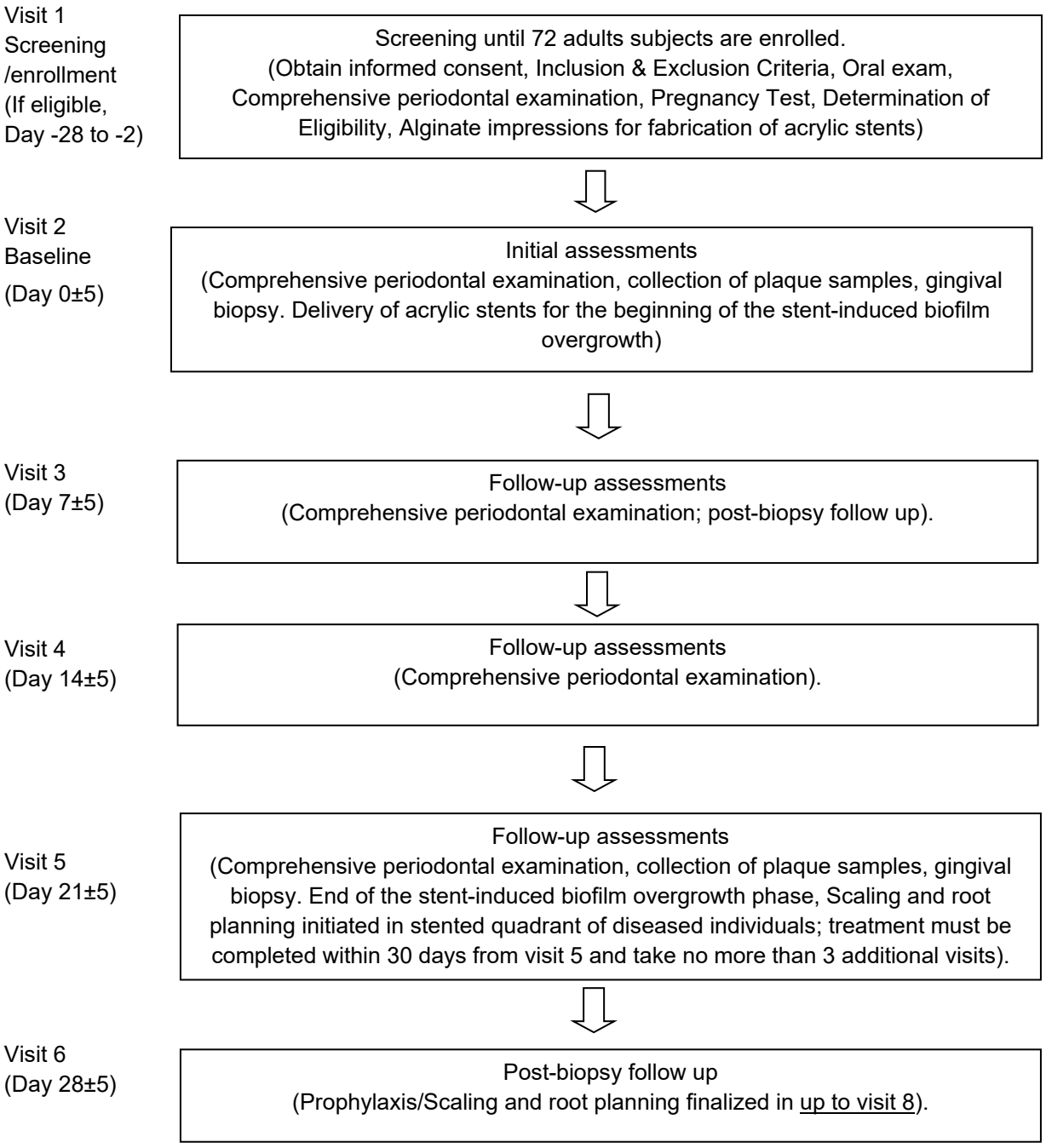
Précis: A total of 72 subjects 18 years or older will be recruited and examined for this 1-8 visit, up to 56 days research study. Assuming a 10% dropout in both groups, healthy individuals (n=36) and individuals with severe periodontal disease (n=36) will be evaluated at 2 main timepoints: baseline and 21-days of biofilm overgrowth (visit 5). Visit 1 includes enrollment and alginate impressions for a stent fabrication; visit 2 includes plaque collection, gingival biopsy and delivery of the stent; visits 3 and 4 are designed for monitoring disease; visit 5 includes collection of gingival biopsies and dental plaque and discontinuation of the stent usage. Analysis will include characterization of the expression of IFI16 and AIM2 (mRNA and protein) in gingival tissues in chronic and acute inflammation, and further characterization of the main cells expressing these proteins. Clinical outcomes will include measurements of periodontal disease, including as CAL, PD, BOP, GI, and PI. Plaque samples will be evaluated for the presence of periodontal pathogens. Visits 6-8 will be completed in 30 days from visit 5 and are for prophylaxis and SRP based on individual need.

Objectives: Primary: To characterize the expression of IFI16 and AIM2 in gingival tissues in an inflammatory response using the stent-induced biofilm overgrowth model.

Population: Adult subjects will be recruited from the patients, students and staff at the University of North Carolina, as well as the general population in or near Chapel Hill NC. Inclusion criteria for subjects includes a diagnosis of a healthy and severe periodontal disease according to periodontal and tooth profile classification system (1). Individuals will be classified based on an algorithm that relies on the periodontal clinical parameters, presence of teeth and crowns. Subject exclusion criteria include a history of autoimmune diseases, diabetes, immunocompromised subjects, neurologic or psychiatric disorders, systemic infections, use of chronic medications

	known to affect the periodontium, smokers, and untreated dental conditions.
Number of Sites:	General and Oral Health Center, UNC School of Dentistry
Study Duration:	60 Months
Subject Participation Duration:	Up to 56 Days
Estimated Time to Complete Enrollment:	36 Months

Figure 1: Schematic of Study Design:



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2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

The host response is recognized as the major contributor to periodontal tissue damage by supporting a non-resolving inflammation and sustaining dysbiosis (2). Our group has recently shown that interferon, gamma-inducible protein 16 (IFI16) and absent in melanoma (AIM2) are potentially involved in the pathogenesis of periodontal disease (3, 4). IFI16 and AIM2 are intracellular recognition sensors that modulate inflammatory responses against several microorganisms. Our data shows that IFI16 and AIM2 are expressed in multiple cells of human gingival tissues (4). In murine experimental periodontitis, these proteins significantly increase in comparison to mice with healthy gingival tissues. This suggests that pathways of IFI16/AIM2 could be interesting for a future therapeutic development to treat periodontal diseases. However, the expression of IFI16 and AIM2 has not been fully characterized in human gingival tissues. Thus, we propose to characterize the gingival tissue expression of IFI16/AIM2 in distinct periodontal conditions represented in healthy and periodontal diseased subjects to elicit chronic and acute responses using the stent-induced biofilm overgrowth model. This master protocol document describes the procedures for such an experiment that represents the clinical portion and the first aim of a larger three aim study of IFI16.

2.2 Rationale

Periodontal disease is a biofilm-induced inflammatory disease that affects the integrity of the tissues that surround and support the teeth (5). Severe periodontitis is the 6th most prevalent disease worldwide, with an overall prevalence of 11.2% and 734 million people affected. In the U.S. Periodontal disease affects 47% of the adult population (6, 7). Periodontitis is a major cause of tooth loss in adults, having subsequent impact on the person's masticatory dysfunction, quality of life and self-esteem (8). The global cost of lost productivity from severe periodontitis alone has been estimated to be 54 billion USD/year. Such burden of periodontitis will continue to increase with the growing ageing population with increased tooth retention globally (8-10). While treatment of periodontal disease is successful in the majority of cases, up to 30% of patients with moderate chronic periodontitis respond poorly to treatment (i.e. refractory periodontitis) (11, 12).
Periodontal disease is a multifactorial disease that has microorganisms and a susceptible host as key players. The tooth-associated biofilm is required but not sufficient to induce periodontitis because it is the host inflammatory response to this microbial challenge that ultimately can cause destruction of the periodontium (13). The host response is now recognized as the major contributor to periodontal tissue damage by supporting a non-resolving inflammation and sustaining dysbiosis (2). Both innate and acquired immune responses are shown to be important for disease initiation and progression of periodontal disease (14). A recent consensus by the European Workshop on Periodontology recognizes that all current biological approaches to improve periodontal treatment have been focusing on direct microbial managements (antibiotic treatments) instead of approaches that will assist host response modulation (15). Concerns about the ever-increasing development of antimicrobial resistance

additionally supports methods for modulating the host response instead of drugs targeting a broad spectrum of microorganisms.

The innate immune function depends on recognition of pathogen-associated molecular patterns (PAMPS) derived from invading pathogens by germline-encoded pattern-recognition receptors (PRRs). Activation of PPRs triggers downstream, signaling cascades and leads to production of pro-inflammatory cytokines and chemokines (16). The balance between pro- and anti-inflammatory mediators determines the severity and extent of tissue destruction in the periodontium (17, 18). In order to re-establish the balance between mediators, anti-inflammatory pharmacologic agents [non-steroidal anti-inflammatory drugs (NSAIDs)] have been used to prevent and slow the progression of experimental and human periodontitis (15, 18, 19). While this host-modulation approach has shown to be successful, the side effects of NSAIDs have made the usage impossible for the routine treatment of periodontitis. Therefore, further understanding of the host response is a mean for future therapeutic development.

Inflammasomes are multimeric protein complexes that assemble in the cytosol after sensing PAMPs and danger-associated molecular patterns (DAMPs). Several inflammasome sensor molecules can trigger the formation of inflammasomes, including the nucleotide-binding domain, leucine-rich repeat containing proteins (NLRs, the largest family of inflammasomes), absent in melanoma 2 (AIM2)-like receptors and interferon gamma-inducible protein 16 (IFI16) (20). Inflammasome names denote from the protein forming the scaffold. While there are differences between inflammasomes dependent upon stimuli, classic inflammasomes serve as a scaffold to recruit inactive caspase-1. Oligomerization of pro-caspase-1 proteins induces their auto-cleavage into active caspase-1, which cleaves precursor cytokines into active IL-1β and IL-18 (21). Inflammasomes are shown to play either causative or contributing roles in the initiation of a variety of auto-inflammatory and autoimmune diseases, including neurodegenerative diseases (multiple sclerosis, Alzheimer's disease and Parkinson's disease), and metabolic disorders (atherosclerosis, type 2 diabetes and obesity). Expression of several inflammasome components are altered in cells and tissues from diseased individuals (22). Recent in vitro and in vivo studies have pointed towards promising therapeutics that target inflammasome activity in inflammatory diseases, including inhibition of the NLRP3-inflammasome for the reduction of atherosclerosis lesions (22, 23).

AIM2 is the first member of the PYHIN protein family to be linked to the inflammasome (24). AIM2 is a cytosolic protein that is a well-characterized sensor of viruses and bacteria (25-27). Expression of AIM2 is increased in inflammatory conditions, including psoriasis, inflammatory bowel disease, atopic dermatitis and periodontitis, supporting a role of this protein in inflammatory diseases (4, 28-32). In vitro, Porphyromonas gingivalis (Pg) pro-inflammatory IL-1β response is supported by AIM2-inflammasome activation (33). Therefore, AIM2-inflammasome may be important in driving the periodontal host response. The role of AIM2-inflammasome in the host response to other important periodontal pathogens and in experimental periodontitis has never been explored. While AIM2 is shown to be increased in chronic periodontitis (30, 31), the effect of this protein in inflammatory bone loss is unknown.

Recent studies from our group identified IFI16 as a potential protein important for periodontal disease pathogenesis (3) and recently published manuscript by the

applicant. Our study is the first to demonstrate expression of IFI16 in periodontal tissues **(4)**. Expression of IFI16 is observed in multiple cells of the periodontal tissues, including epithelial cells, endothelial cells, fibroblasts and leukocytes.
IFI16 (mouse ortholog ifi204) is a multifunctional protein that has been well characterized as a pathogen sensor that leads to inflammasome activation and production of type I interferon and a pro-inflammatory response (25, 34, 35). IFI16 is also reported to be increased in inflammatory conditions, including psoriatic epidermis, inflammatory bowel disease (32, 36). Like other inflammasome proteins, the majority of studies support IFI16 as a pro-inflammatory molecule. However, IFI16 has also demonstrated an anti-inflammatory action *in vitro* by directly binding to AIM2 and suppressing activation of caspase-1 and further host response (37, 38) (Figure 2). **This suggests that, dependent on the type of stimuli and disease, IFI16 can have an anti-inflammatory function by dampening the AIM2-mediated inflammatory response.** However, these *in vitro* studies did not evaluate IFI16 as a modulator of inflammation upon bacterial infection. In addition, currently no *in vivo* study has evaluated IFI16 as a modulator of inflammation via AIM2 inflammasome. We continued our studies by inducing experimental periodontitis in *Ifi204*$^{-/-}$ animals. Analysis of periodontal tissues shows that ***Ifi204***$^{-/-}$ **mice have a significant increase in the extent of alveolar bone loss** in experimental periodontitis ($p \leq 0.05$). Mean alveolar bone loss is 28.8% for WT and 47.74% for Ifi204ko mice in a 10-day period of ligature, representing **1.6-fold increase in the amount of bone loss.** Together, the data suggests that IFI16 acts as an anti-inflammatory protein by altering the expression of cytokine/chemokine responses. Better characterization of the cellular and molecular biology of inflammasomes will help identify important potential therapeutic targets for the treatment and prevention of periodontal diseases (39). The importance of IL-1β and IL-18, in periodontal disease pathogenesis is shown in several clinical studies (40-45). Currently, therapies targeting IFI16 and AIM2 have not been developed. Studying these proteins individually and collectively, along with upstream and downstream events, will yield to new approaches to potentially treat periodontal disease severity and progression. This project will form the basis of a future project involving inflammasomes and alternative therapeutic approaches.

2.3 Potential Risks and Benefits

All research involves risks. This may include physical, psychological, social, legal and economic risks. There may be uncommon or previously unknown risks that might occur. Risks for this study are similar to those associated with other related dental procedures. Every participant will be encouraged to report any problems and/or health changes while enrolled in this research study. Participants will be given any new information gained during the course of the study that might affect their willingness to continue participation.

In spite of safety measures, a participant might develop a reaction or injury from being in this study. If such problems occur, the researchers will help get medical care for the

participant, but any costs for the medical care will be billed to the participant. The principal investigator or The University of North Carolina at Chapel Hill has not set aside funds to pay for any such reactions or injuries, or for the related medical care.

2.3.1 Potential Risks

Subjects may experience short-term discomfort and bleeding of the gums from periodontal probing as a result of the periodontal clinical examinations. This exam is not different from what would normally be performed in a dental or periodontal office to evaluate the condition of the periodontal tissues and extent of disease. No dental radiographs will be taken.

Subjects may experience short-term gingival inflammation, halitosis or bad mouth taste, dental plaque accumulation, and bleeding of the gums from refraining from all oral hygiene procedures (i.e., tooth brushing, flossing or use of interdental aids and/or mouthwashes) in the two selected sextants (up to 6 teeth in each arch) for 21 days. Subjects may continue plaque control procedures for the remaining four sextants using fluoride dentifrice and toothbrush. At the completion of the study, adult prophylaxis (for orally healthy individuals) or Scaling and Root Planing (SRP) (for individuals with periodontal disease) will be completed to restore gingival health eliminating gingival bleeding, dental plaque accumulation, and halitosis. Subjects will be monitored for safety every week and after the induction of experimental biofilm overgrowth through 21 days. Any site undergoing clinical attachment loss of >2mm from the baseline measurement will be deemed as “progressing” and participant will exited from the study and given scaling and root planing treatment as a rescue therapy at the same day of the visit.

Subjects may experience mild to moderate discomfort, and bleeding during the scaling and planing procedure or adult prophylaxis. Transient bacteremia can occur after dental prophylaxis and SRP. Standard of care practices will be utilized for all scaling and root planing, adult prophylaxis and dental exams performed. In order to minimize discomfort, subjects will receive local anesthesia prior to scaling and root planing procedures. Although not required, subjects may receive topical anesthesia prior to and during the adult prophylaxis.

Subjects may experience moderate discomfort following treatment procedures, which will be performed under local anesthesia. Topical anesthesia is used initially to minimize the discomfort associated with local anesthesia injections. Local anesthesia may cause swelling, drooling, dizziness, tachycardia or muscle pain that should be temporary. There may be post-operative tenderness, sensitivity to hot or cold, slight bleeding or oozing, or development of a transient or permanent temporomandibular joint disorder. Some gingival recession or loss of papillae height may occur. Subjects receiving scaling and root planing procedures will be encouraged to use over the counter analgesics, according to the subject’s medication tolerance, to control any post-operative pain.

Subjects may experience moderate discomfort following treatment or biopsy procedures, which will be performed under local anesthesia. Topical anesthesia is used initially to minimize the discomfort associated with local anesthesia injections. Local

anesthesia may cause swelling, drooling or muscle pain that should be temporary There may be post-operative tenderness, sensitivity to hot or cold, slight bleeding or oozing, or development of a transient or permanent temporomandibular joint (TMJ) disorder. Some gingival recession or loss of papillae height may occur. Subjects receiving biopsy and/or scaling and root planing procedures will be encouraged to use over the counter analgesics, according to the subject's medication tolerance, to control any post-operative pain.

In this study, the following events will not be considered reportable AEs:

- Short-term discomfort, bleeding or oozing of the gums, tooth sensitivity, cheek biting, gingival soreness,mild gingival inflammation, mild gingival bleeding, mild tooth mobility, mild gingival abrasion or irritation,as a result of the periodontal assessments, local anesthesia or periodontal treatment.
- Gingival recession or loss of gingival papillae
- Short term jaw soreness resulting from periodontal assessments or periodontal treatment

2.3.2 Potential Benefits

This study will provide mechanistic insight into the underlying causes and molecular level pathogenesis of periodontal diseases. We will identify key mechanisms that potentially confer risk and protection. Ultimately this may lead to new and improved diagnostics and therapeutics. Since IFI16 and AIM2 are reported to be involved in other diseases, clarifying the role of these proteins in periodontal disease will also provide insights into the role of these proteins in other inflammatory conditions.

Orally healthy individuals will receive adult prophylaxis that is recommended for healthy individuals every 6 months. Subjects with periodontal conditions will have therapeutic benefit from the treatment. Diseased subjects will be offered referral to the UNC School of Dentistry graduate clinics for additional care at reduced fees, based upon the UNC School of Dentistry graduate student fee schedule, to treat their periodontal condition beyond initial periodontal therapy.

3 OBJECTIVES

3.1 Study Objectives

We will establish 2 new cohorts of individuals: healthy with acute inflammation (SIBO) and chronic periodontitis with acute inflammation (SIBO) to study the role of IFI16 and AIM2 in the pathogenesis of periodontitis. Studying these proteins individually and collectively, along with upstream and downstream events, will yield to new approaches to potentially treat periodontal disease severity and progression. This project will form the basis of a future project involving inflammasomes and alternative therapeutic approaches. The study objectives are to:

1) (Primary) compare the expression of AIM2 and IFI16 between healthy (PPC-A, PPC-B) and severe periodontitis (PPC-E, PPC-G) subjects during an acute inflammatory response using the stent-induced biofilm overgrowth model (SIBO).

2) (Exploratory) correlate the expression of these proteins with periodontal pathogens

3.2 Study Outcome Measures

Primary study outcomes measured for individuals in each group (healthy and severe periodontitis):

- Expression of AIM2 and IFI16 levels will be quantified by IHC and qRT-PCR
- Periodontal clinical measurements
- Composition of oral microbiota by 16S rDNA (Illumina)

4 STUDY DESIGN

A total of 72 subjects will be enrolled. Specifically, a sufficient number of adults 18 years and older will be screened until 36 healthy (PPC-A, PPC-B) subjects and 36 subjects with severe periodontal disease (PPC-E, PPC-G) will be enrolled based on the algorithm previously defined on Morelli et al. 2017. Eligibility for study participation will be determined during the screening session. Barring dropout, subject participation will include 1 to 8 visits lasting over a maximum period of 56 days. The last 3 visits (visit 5-8) will depend on the individual need of the subject for providing SRP. Clinical data and medical history data will be collected at the screening visit to ascertain eligibility. All subjects will have dental plaque and a gingival biopsy collected at baseline. Enrolled subjects will be included in an experimental gingivitis model (SIBO) for 21 days. Individuals will return for safety checks every week during the 21-day period. At 21-days, plaque samples and a gingival biopsy will be collected. For individuals with severe periodontal disease, SRP will initiate during the 21-day visit at the SIBO quadrant. Visit 6 (28 days) will include the post-biopsy follow up, prophylaxis (for healthy individuals) and SRP (diseased individuals). Subjects receiving SRP may have 2 additional visits (visit 7 at day 35 and visit 8 at day 42) for completing the SRP of all quadrants and will be dependent on individual need. Medical histories, demographics, height and weight, clinical and biological data described above will be recorded and stored on a secure server located at the University of North Carolina. Each participant enrolled into the study will have a unique identification number that has been stripped of any information that could be used by non-study members to identify the subject.

5 STUDY ENROLLMENT AND WITHDRAWAL

All subjects will be seen in the General and Oral Health Center, which is a dental research clinic at the University of North Carolina at Chapel Hill School of Dentistry. The General and Oral Health Center is affiliated with the UNC CTSA. There are no collaborating sites where human subject's research will be performed.

5.1 Subject Inclusion Criteria

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

- Subjects must have read, understood and signed an informed consent form.
- Subjects must be able and willing to follow study procedures and instructions.
- Subjects must be adult males or females with a minimum of 18 years (inclusive).
- Subjects must present with at least 20 teeth in the functional dentition, excluding third molars.
- Subjects must have at least 2 natural adjacent teeth in two posterior sextants that will be selected for baseline gingival biopsy and SIBO gingival biopsy.
- Subjects must be in good general health, as evidenced by medical hx (exclusion conditions defined under subject exclusion criteria below).
- Females of child-bearing capacity must be willing to have pregnancy test to confirm they are not pregnant.
- Subjects must be in the healthy (PPC-A, PPC-B) or severe periodontitis (PPC-E, PPC-G) categories according to the PPC (1).

5.2 Subject Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

- If the sextants identified for the analysis has implants
- All individuals who meet criteria for anti-infective prophylaxis prior to dental procedures should be excluded from this study.
- Chronic disease with oral manifestations including diabetes mellitus.
- Current smoker or one that has stopped smoking less than 2 years prior to enrollment (self-report).
- Gross oral pathology other than the periodontal disease.

- Treatment with antibiotics for any medical or dental condition within 1 month prior to the screening examination.
- Chronic treatment (i.e., two weeks or more) with any medication known to affect periodontal status (e.g., phenytoin, calcium antagonists, cyclosporin, coumadin, non-steroidal anti-inflammatory drugs, aspirin) within one month of the screening examination.
- Ongoing medications initiated less than three months prior to enrollment (i.e., medications for chronic medical conditions must be initiated at least three months prior to enrollment).
- Significant organ disease including impaired renal function, heart murmur, history of rheumatic fever or valvular disease, or any bleeding disorder.
- Individuals with prosthetic material used for intra-cardiac repair (e.g. for congenital heart disease), or intra-cardiac devices, cardiac transplant, infective endocarditis and individuals who have had previous infectious complications of prosthetic joint infections
- Infectious diseases such as hepatitis, HIV or tuberculosis.
- Anemia or other blood dyscrasias.
- Anticoagulant therapy or drugs, such as heparin or warfarin.
- Severe unrestored caries, or any condition that is likely to require antibiotic treatment over the trial.
- Pregnant, or expect to become pregnant within the next several months.
- Females of child-bearing capacity not using any form of contraceptive methods
- Anything that would place the individual at increased risk or preclude the individual's full compliance with or completion of the study.

5.3 Strategies for Recruitment and Retention

Before initiation of the study, the investigator will obtain written approval of the research protocol and informed consent form from the UNC IRB complying with the provisions specified in 21 CFR Part 56 and applicable pertinent governmental regulations.

All recruitment plans including advertising, email postings, and flyers will be submitted to the IRB and must be approved before study initiation. Our plan is to use campus-wide list server postings, website postings, our IRB approved patient registry, newspaper ads and postings within UNC including the dental school and the local community. Our dental school has a lottery-based patient acceptance program and many individuals are on waiting lists to become patients within the dental school clinics. While waiting to be accepted at the dental school, subjects become patients of the General and Oral Health

Center and are able to receive dental care per study protocol as well as referrals to other dental clinics within the school as appropriate.

Monetary compensation of $200 after completion of 2 biopsy procedures will be provided at the end of visit 5. Free parking vouchers will be given to each subject for every completed visit. Periodontal treatment that includes scaling and root planning may be offered to each study participant according to his/her level of need. Study participants will be called by phone and/or emailed, depending upon their indicated preferred contact method, to be reminded of planned study visits or to reschedule missed study visits.

5.4 Subject Withdrawal

Subjects may withdraw from the study at any time at their own request, or they may be withdrawn at any time at the discretion of the Investigator for safety, behavioral or administrative reasons (i.e. protocol compliance). If a participant withdraws consent, no further evaluations will be performed on that participant and no attempts will be made to collect additional data regarding that participant. If a participant fails to present for a scheduled study visit, study staff will attempt three times to reschedule the participant utilizing the means of contact provided by the participant.

5.4.1 Reasons for Withdrawal

The Investigator may discontinue a subject if, in the opinion of the Investigator, the subject is no longer a suitable candidate for the study.

Possible reasons for the discontinuation of a subject are including but not limited to:

- ✓ Adverse events (i.e. delayed healing)
- ✓ Protocol deviations (i.e. subject compliance with protocol)
- ✓ Failure to meet or maintain eligibility

5.4.2 Handling of Subject Withdrawals

All participants that prematurely discontinue from the study, regardless of cause will be given the opportunity to be seen for a final evaluation and complete a dental prophylaxis. Withdrawn subjects with unresolved oral disease will be offered referral for dental care within the UNC School of Dentistry. Subjects will only be compensated after two biopsy procedures are completed.

Any participant with an AE that is ongoing at the time of discontinuation will be followed until the event returns to baseline, resolves, or stabilizes. If the AE does not meet these outcomes within 30 days after discontinuation, the participant will be offered a referral to an appropriate practitioner for continued care.

5.5 Premature Termination or Suspension of Study

This study may be suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party. If the study is prematurely terminated or suspended, the principal investigator will promptly inform the IRB and will provide the reason(s) for suspension or termination. No interim analyses will be done to determine futility. It is expected that valuable results will be obtained from the 200 participants in the five-week SIBO experiment even if there is an unanticipated finding that no biomarkers vary across the four groups under strong error rate control. Therefore, futility analyses for potential early stopping of the trial are not applicable.

Circumstances that may warrant termination include, but are not limited to:

- ✓ Determination of unexpected, significant, or unacceptable risk to subjects.
- ✓ Insufficient adherence to protocol requirements.

6 STUDY SCHEDULE

6.1 Visit 1: Screening/enrollment

(Day -28 +/- 2 days)

- Informed Consent: Before recruitment and enrollment, each prospective candidate will be given a full explanation of the study, and allowed to read the informed consent form that has been approved by the Institutional Review Board. Qualified study personnel will directly ask the subject if he/she understands the implications of participating in the study. Subject will have the opportunity to ask questions and have answers related to the study participation. No protocol procedures will be performed prior to confirmation that the subject understands the procedures and risks. Once the subject confirms this understanding verbally, the subject will be asked to give consent to participate in the study by signing the informed consent form. The study personnel will provide copies of the signed informed consent forms to the subject.
- Study personnel will obtain medical history and demographics related to the participant, height and weight
- Study personnel will record the subject's vital signs (blood pressure and pulse).
- Female participants capable of child bearing will be given a urine-based pregnancy test.
- A dental examiner (calibrated dentist or dental hygienist) will perform an intraoral examination to verify no presence of oral pathology.
- Dental examiner will perform a comprehensive periodontal screening examination that includes Plaque Index (PI), Gingival Index (GI), Pocket Depth (PD), Bleeding on Probing (BOP) and Clinical Attachment Levels (CAL).
- Subjects in the categories of healthy (PPC-A, PPC-B) or severe disease (PPC-E, PPC-G) will be considered eligible for the study.
- If the participant meets eligibility criteria during the clinic visit, the participant will be invited to participate in the research study at that time. If determined non-eligible, the individual will be informed at that visit, or by telephone, to avoid an unnecessary visit. All subjects that complete visit 1 will receive a free parking voucher.
- If eligible, an alginate impression will be taken for stent fabrication

- The interproximal region of a tooth will be selected by a dental examiner for the first gingival biopsy (occurs at visit 2).
- The subject will receive a parking voucher for the Dogwood deck and be scheduled for visit 2

6.2 Visit 2: Baseline

(Day 0 +/- 5 days)

- Study personnel will update medical history, including concomitant medication, enquire about potential adverse events related to the dental exam performed during screening and ask continuance criteria questions.
- Study personnel will record the subject's vital signs (blood pressure and pulse).
- A dental examiner will verify the previously performed intraoral exam and assess for potential adverse events.
- Dental examiner will perform a comprehensive periodontal screening examination that includes Plaque Index (PI), Gingival Index (GI), Pocket Depth (PD), Bleeding on Probing (BOP) and Clinical Attachment Levels (CAL).
- The dental examiner will collect two separate subgingival plaque samples from the interdental sulcus of the teeth that are selected for a gingival biopsy (see appendix II). Dental implant sites will not be considered for subgingival plaque samples.
- A small gingival tissue biopsy sample will be removed from underneath the papillae, buccally and lingually, to include the col area of depth of the osseous crest. Biopsies will be collected by a study dentist and performed under local anesthesia. Absorbable sutures will be used to close the interproximal papillae.
- The dental examiner will deliver 1 customized acrylic stent that will be used in a different sextant from the one that the biopsy was performed. The sextant selected will be the right posterior sextant (maxillary or mandibular), except when fewer than two teeth are present and adjacent without significant diastema.
- The participant will be instructed to defer oral hygiene measures in the experimental sextant, to wear the customized stents during brushing and to discontinue flossing throughout his/her mouth. The participant will continue to use his/her standard toothpaste and toothbrush. Participants will be informed to avoid crunchy or sticky food (e.g. apples and carrots), and use of mouthwashes during baseline through visit 5 (Day 21).
- Subjects will be monitored for safety every week during the induction of experimental biofilm overgrowth through 21 days. Any site undergoing CAL

increase of >2 mm from the baseline measurement will be deemed as "progressing." That site will not be included in data analysis, and will receive scaling and root planing treatment as a rescue therapy.

- Subjects will be given parking vouchers for the UNC Dogwood parking deck and scheduled to return for visit 3.

6.3 Visit 3

(Day 7 +/- 5 days)

Visit 3 may occur within 7 days of visit 2 (+/-5 days). The following procedures will be performed:

- Study personnel will update medical history, including concomitant medication, enquire about potential adverse events related to the dental procedures and ask continuance criteria questions.
- Study personnel will record the subject's vital signs (blood pressure and pulse).
- A dental examiner will verify the previously performed intraoral exam and assess for potential adverse events.
- Dental examiner will perform a comprehensive periodontal screening examination that includes Plaque Index (PI), Gingival Index (GI), Pocket Depth (PD), Bleeding on Probing (BOP) and Clinical Attachment Levels (CAL).
- A follow-up for verifying the healing of the gingival biopsy will be completed.
- The participant will be re-instructed to defer oral hygiene measures in the selected sextant and to continue wearing the customized stents during brushing of the non-selected sextants.
- Adverse events will be monitored and recorded as necessary.
- The subject will be given a parking voucher for the Dogwood deck, and Visit 4 will be scheduled.

6.4 Visit 4

(Day 14 +/- 5 days)

Visit 4 may occur within 7 days +/-5 days of day 7. The following procedures will be performed:

- Study personnel will update medical history, including concomitant medication, enquire about potential adverse events related to the dental procedures and ask continuance criteria questions.
- Study personnel will record the subject's vital signs (blood pressure and pulse).

- A dental examiner will verify the previously performed intraoral exam and assess for potential adverse events.
- Dental examiner will perform a comprehensive periodontal screening examination that includes Plaque Index (PI), Gingival Index (GI), Pocket Depth (PD), Bleeding on Probing (BOP) and Clinical Attachment Levels (CAL).
- The participant will be re-instructed to defer oral hygiene measures in the selected sextant and to continue wearing the customized stents during brushing of the non-selected sextants.
- Adverse events will be monitored and recorded as necessary.
- The subject will receive a parking voucher to the Dogwood parking deck, and Visit 5 will be scheduled.

6.5 Visit 5

(Day 21 +/- 5 days)

Visit 5 may occur within 7 days +/-5 days of day 14. The following procedures will be performed:

- Study personnel will update medical history, including concomitant medication, enquire about potential adverse events related to the dental procedures and ask continuance criteria questions.
- Study personnel will record the subject's vital signs (blood pressure and pulse).
- A dental examiner will verify the previously performed intraoral exam and assess for potential adverse events.
- Dental examiner will perform a comprehensive periodontal screening examination that includes Plaque Index (PI), Gingival Index (GI), Pocket Depth (PD), Bleeding on Probing (BOP) and Clinical Attachment Levels (CAL).
- The dental examiner will collect two separate subgingival plaque samples from the interdental sulcus of the teeth that are selected for a gingival biopsy (see appendix II). Dental implant sites will not be considered for subgingival plaque samples.
- A small tissue biopsy sample will be removed from underneath the papillae, buccally and lingually, to include the col area of depth of the osseous crest of a tooth included in the SIBO model. The site for biopsy will be selected based on the tooth (included in the SIBO model) with the highest GI score. The biopsy will be collected by a study dentist and performed under local anesthesia. Absorbable sutures will be used to close the interproximal papillae.

- SRP may be initiated in individuals with severe periodontitis and will include the quadrant that received the gingival biopsy to allow optimal healing
- Adverse events will be monitored and recorded as necessary
- The subject will receive compensation for study participation, a parking voucher to the Dogwood deck. and visit 6 will be scheduled.

6.6 Visit 6: Final Visit (Post-biopsy follow-up; Prophylaxis; SRP may be completed up to visit 8 based on individual needs)

(Day 28 +/- 5 days)

Participants will be seen for a final visit at day 28 (+/-5 days). The following procedures will be performed at this visit:

- Study personnel will update medical history, including concomitant medication, enquire about potential adverse events related to the dental procedures and ask continuance criteria questions.
- Study personnel will record the subject's vital signs (blood pressure and pulse).
- A dental examiner will verify the previously performed intraoral exam and assess for potential adverse events.
- Dental examiner will perform a comprehensive periodontal screening examination that includes Plaque Index (PI), Gingival Index (GI), Pocket Depth (PD), Bleeding on Probing (BOP) and Clinical Attachment Levels (CAL).
- A follow-up for verifying the healing of the gingival biopsy will be done
- Prophylaxis will be performed on healthy individuals and prophylaxis/SRP will be performed on individuals with severe periodontal disease where the need is clinically indicated. Participants will be compensated for their participation in the study.
- Adverse events will be monitored and recorded as necessary.

Scaling and root planing may involve up to 2 additional visits (visit 7 and 8) that must be completed within 4 weeks of visit 6 (+/-5 days)

6.7 Withdrawal Visit

Subjects will be exited from the experimental component of the research protocol following the first visit or following visit 6. All subjects will be exited from the study and assessed by the General and Oral Health Center dental personnel for additional periodontal care within the General and Oral Health Center or for referral to another dental clinic as appropriate.

6.8 Unscheduled Visit

Unscheduled study visits will be documented in the electronic data capturing tool (CDART 2.0 which is the clinical research data management system designed and developed by UNC's Collaborative Studies Coordinating Center (CSCC) and NC TraCS), utilized for this research study. This documentation will be recorded in the clinical notes section of the participant and will include date of visit, reason for visit, procedures performed during the visit, if a follow up visit will be required and the initials of the study staff recording the clinical note.

7 STUDY PROCEDURES/EVALUATIONS

7.1 Study Procedures/Evaluations

Prior to the first subject study visit, study examiner(s) will be trained and calibrated for accuracy and repeatability in using the UNC Modified Gingival Index (GI); Loe and Sillness, UNC Modified Plaque Index (Green & Vermillion), periodontal pocket depths, and clinical attachment levels using a periodontal probe. All examiners have a kappa > 0.90 for all calibration sessions.

Calibration training will be conducted on an annual basis according to the guidelines set forth in this document including: Periodontal determination of plaque (PI), gingival inflammation (GI), gingival bleeding (BOP), pocket probing depths (PD) and clinical attachment levels (CAL). These parameters are customarily used in clinical dental studies to measure the disease status of gingivitis and periodontitis. Probe penetration and depth may vary with the degree of inflammation, probing force, angulation, position and instrument tip diameter.

Other confounding factors include patient discomfort, accuracy of probe markings, anatomical differences in tooth crown and roots, and technique variability within and between examiners. Thus, all of these measurements are important to consider in periodontal clinical studies and this training session is designed to minimize the inter-examiner variance.

Stent-Induced Biofilm Overgrowth (SIBO) Model

A customized stent will be fabricated for each subject. The stent will be fabricated to resemble an acrylic mouth-guard but extended to cover approximately 2mm over gingival margins. The stent will form a seal and rest on the gingiva, but will be relieved on the tooth and tissue side except for the occlusal surfaces to avoid disturbing plaque or gingival tissues. The acrylic stent will cover the area in one sextant where no brushing and flossing teeth is to occur during 21 days. The acrylic stent will be worn only during tooth brushing. Subjects will be monitored for safety every week and after the induction of experimental biofilm overgrowth through 21 days.

The SIBO model only induces a transient gingival inflammation without any addition of further periodontal attachment loss. The model was previously used and results support the safety of the model with no further loss of periodontal attachment (47-49). If test sites demonstrate a clinical attachment loss of more than 2 mm during the study, subjects will be removed from the study and receive rescue therapy (Scaling and Root Planing) at the same day of the visit. Clinical attachment loss will be automatically calculated by the entry software used in the study (Dental ToolKit) and compared longitudinally to the baseline clinical attachment level measurement. Previous publications from our group

demonstrated that the SIBO model is safe by having no subjects required to withdrawn previous SIBO studies due to clinical attachment loss (47-49).

Clinical Assessments and Data Collection

UNC Modified Gingval Index (GI), Loe and Silness

Full mouth gingival scores shall be visually assessed by segmenting marginal and papillary units, 6 sites per tooth: distobuccal, buccal, mesiobuccal and distolingual, lingual, mesiolingual surfaces.

0 = Normal gingiva
1 = Mild inflammation; slight change in color, slight edema. No bleeding on probing.
2 = Moderate inflammation:redness, edema, and glazing. Bleeding on probing
3= Severe inflammation; marked redness and edema. Ulceration. Tendency for spontaneous bleeding.

UNC Modified Plaque Index (Green & Vermillion)

Full mouth plaque assessment shall be assessed using the UNC Modified Plaque Index (Greene and Vermillion). Plaque scores shall be visually assessed at **6 sites per tooth** (distobuccal, buccal, mesiobuccal and distolingual, lingual, mesiolingual surfaces) on a scale of 0-3.

0 =No debris or stain present on the clinical crown.
1 =Soft debris covering not more than 1/3 of the clinical crown (cervical 3rd), or presence of extrinsic stains without other debris regardless of surface area covered.
2 =Soft debris covering more than 1/3, but not more than 2/3 (middle 3rd) of the clinical crown.
3 =Soft debris covering more than 2/3 of the clinical crown

Pocket depth (PD): Linear distance from the gingival margin (GM) to base of the pocket. If a PD reading falls between two millimeter readings, the rule shall be to round down and the lower of the two readings will be recorded.

Bleeding on Probing (BOP): Presence or absence of bleeding to manual probing recorded as a dichotomous variable.

0- No bleeding within 10 seconds after probing.
1- Bleeding within 10 seconds after probing.

Clinical Attachment Level (CAL): Linear distance from the cemento-enamel junction (CEJ) to base of the pocket. If a CAL reading falls between two millimeter readings, the rule shall be to round down and the lower of the two readings will be recorded.

7.2 Laboratory Procedures/Evaluations

Dental plaque samples and gingival tissue samples will be collected at the dental research clinic of the UNC School of Dentistry (General and Oral Health Center). All bar coded de-identified biological samples will be transported to the laboratory of the principal investigator for processing with a chain of custody recording for each sample. All information collected for this study will only be used for the study, and not as part of another study. Additional grant request may be submitted in the future to complete additional analyses on stored biological specimens.

All measurements will be conducted using standard techniques that will be carried out in the investigator's laboratory and in collaborations previously established by the investigator (Histology Research Core Facility, Microbiome Core Facility at UNC).

7.3 Study Specific Biospecimens

7.3.1 Specimen Collection Procedures

Specimen Collection Procedures

Plaque: The dental examiner will collect two separate, facial, sublingual plaque samples, from the interdental sulculs of the teeth that are selected for a gingival biopsy .Dental implants will not be considered for plaque samples. Subgingival plaque samples will be obtained with a sterile Gracey dental curette. The curette tip shall be inserted into to the depth of the sulcus and plaque shall be collected in a single coronal stroke movement. After plaque collection, the curette is then placed in an Eppendorf tube containing 500 ul of TE buffer (10mM Tris-HCl, 0.1 mM EDTA, pH 7.6) and shaken for a few seconds to release the bacterial cells from the curette into the solution. The plaque sample can then remain at the clinic until the end of the day, when all samples are taken to the laboratory, where they are stored at -80 ˚C for later bacterial microbiological analysis. All samples will be taken to the UNC Cytokine Analysis Lab for validation of collection and storage.

We will evaluate the microbial content determined by sequencing the V3-V4 variable region of 16S rRNA gene using next generation sequencing (50). The bacterial diversity and relative abundance (% of total reads) of each genus and taxon will be evaluated using the QIIME pipeline analysis on the 16S rRNA Illumina sequencing with references databases (SILVA, Greengenes and the Human Oral Microbiome Database). This is a high-throughput state-of-the art technology (50, 51).

Gingival Tissue/Biopsy: Biopsy samples of gingival tissues will be collected from all individuals at baseline and 21-days of biofilm-overgrowth. A trained dentist will use a scalpel blade to collect 2 biopsy samples per subject for a total of 128 samples. Samples will be obtained using topical and local anesthesia. Each biopsy shall be ≤0.5 cm thick. Biopsies will only be collected from the papillae of posterior teeth. The scalpel blade shall be oriented in an inverse bevel direction to capture the base of the periodontal pocket or gingival sulcus. Each biopsy will contain oral epithelium, gingival connective tissues and loosely adherent cells lining the periodontal pocket. Papillae height will be retained by using vertical mattress suture technique, minimizing facial reduction, and coronal reposition of the flap. The biopsy wound will be undermined with minimal flap reflection and closed with a suture. Post-operative instructions, including placing ice over the surgical area and avoiding hot liquids for the remainder of the day, will be given to the subject.

Gingival biopsy samples will be bi-sectioned for immunohistochemistry/immunofluorescence imaging and mRNA expression.

1. For **RNA** extraction, samples will be immediately placed in RNAlater® (Ambion Inc.) which is an aqueous, non-toxic tissue storage reagent that rapidly permeates tissue to stabilize and protect cellular RNA *in situ* in unfrozen specimens. Samples will be placed in a microfuge tube containing ~1ml of RNAlater and kept at 4°C overnight, then RNA later is removed. Samples will stored at -80º C until used for analysis.

2. For **Immunohistochemistry/Immunofluorescence**, samples will be fixed in 4% paraformaldehyde and stored at -20°C for 24 hours and then placed in 70% ethanol for further analysis.

7.3.2 Specimen Preparation, Handling, and Storage

All biological samples are collected into pre-labeled barcoded vials for laboratory processing. Clinical data are collected and stripped of all subject identifiers and provided a bar-coded ID that link the subject's clinical data to the study visit, examiner, date and dataset. Biological samples are analyzed by the laboratory in a blinded manner and at the data closeout the ID links are established to connect the clinical data to the laboratory data. None of that data contains any patient identifiers. Identification design uses a barcode that includes the study ID, subject ID, visit ID and biological specimen ID.

Example barcode:

- "ITS" is the acronym that identifies the specific IRB approved research study.
- "1001" is the subject identifier
- "03" is the visit ID (visit 3)

- “904” is the specific biological sample ID

7.3.3 Specimen Shipment

Biological specimens will not be shipped to an outside location for analysis, except for plaque samples. Specimens will be taken, by GO Health staff, to the principal investigator’s laboratory, 3412 Koury Oral Health Sciences Building and processed or stored for future analysis. Plaque samples will be stored at -80˚C for later bacterial microbiological analysis at the Microbiome core facility. Tissue samples will be stored at -80º C until use for RNA extraction and at 4˚C for histological processing.

7.4 Questionnaire Administration

There are no questionnaires in place for this research study.

8 ASSESSMENT OF SAFETY

The principal investigator is responsible for all aspects of the conduct of the study. There will be a primary study coordinator assigned to track patients, IRB compliance, adverse events and protocol adherence and study deviations. The principal investigator will meet weekly with the study coordinator to track progress, enrollment and study adherence.

8.1 Specification of Safety Parameters

Safety monitoring reporting for this study will focus on unanticipated problems involving risks to participants, including unanticipated problems that meet the definition of a serious adverse event. Any adverse experience that is determined to be reportable to the Regulatory Authorities will be promptly reported to the Institutional Review Board (IRB).

8.1.1 Unanticipated Problems

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to subjects or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- 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
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.1.2 Serious Adverse Events

A serious adverse event (SAE) is one that meets one or more of the following criteria:

- Results in death
- Is life-threatening (places the subject at immediate risk of death from the event as it occurred)
- Results in inpatient hospitalization or prolongation of existing hospitalization
- Results in a persistent or significant disability or incapacity
- Results in a congenital anomaly or birth defect

An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.2 Reporting Procedures

The principal investigator will be responsible to monitor the overall study that includes the research data as well as the clinical procedures. All adverse events will be reported by the PI to the IRBs and appropriate agencies.

The Principal Investigator and study coordinator will review adverse events weekly and report them to the Institutional Review Board within 30 days. All “unanticipated” adverse events will be reported to the IRBs within 2 week of the investigator becoming aware of the event. Any related and unexpected serious adverse events (SAE) will be reported to the IRB within 24 hours of the principal investigator becoming aware of the event. Unanticipated patient deaths will be immediately reported to the IRB’s and NIH. A summarized copy of all adverse events will be submitted to the IRB’s annually or as requested.

Incidents or events that meet the Office of Human Research Protections (OHRP) criteria for **unanticipated problems** require the creation and completion of an unanticipated problem report form. OHRP recommends that investigators include the following information when reporting an adverse event, or any other incident, experience, or outcome as an unanticipated problem to the IRB:

- appropriate identifying information for the research protocol, such as the title, investigator’s name, and the IRB project number;
- a detailed description of the adverse event, incident, experience, or outcome;
- an explanation of the basis for determining that the adverse event, incident, experience, or outcome represents an unanticipated problem;
- a description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the unanticipated problem.

Adverse Event (AE)

An adverse event is any unfavorable and unintended diagnosis, sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the study intervention, whether or not related to the intervention. Adverse events include new events not present during the pre-intervention period and pre-existing conditions that have increased in severity.

Expected clinical/dental Adverse Events (AE's)

- Apthous and canker lesions
- Tooth sensitivity
- Cheek bite
- Linealba
- Gingival soreness
- Mild gingival inflammation
- Mild gingival bleeding
- Mild tooth mobility
- Mild gingival abrasion or irritation
- TMJ pain and soreness

Unexpected Adverse Event

An unexpected adverse event is an adverse reaction, the nature or severity of which is not consistent with the clinical course of a subject receiving the study procedures. Expected events are untoward clinical occurrences that are perceived by the investigator to occur with reasonable frequency in the aforementioned study procedures. Examples of adverse events that are expected include apthous and canker lesions,tooth sensitivity, cheek bite, linealba, gingival soreness, mild gingival inflammation, mild gingival bleeding, mild tooth mobility, mild gingival abrasion or irritation, and TMJ pain and soreness. Such events, will not be considered reportable unless the event is considered by the investigator to be unexpectedly severe or frequent for an individual patient .If expectedness of an event is unclear, the study's Principal Investigator will be consulted to make this determination.

Serious Adverse Event (SAE)

A serious adverse event is any untoward medical occurrence that meets the definition of adverse event and results in death, is life-threatening, requires or prolongs hospitalization, causes persistent or significant disability/incapacity, results in congenital anomalies/birth defects or, in the opinion of the investigators, represents other significant hazards or potentially serious harm to research participants or others.

Grading of Adverse Events

The following scale will be used to grade the severity of adverse events noted during the study:

Severity Criteria:

1 = Mild adverse event – Did not interfere with normal activity

2 = Moderate adverse event – Interfered with normal activity to some extent

3 = Severe adverse event – incapacitating with inability to work or do usual activity

Serious Criteria:
1 = Caused or prolonged hospitalization
2 = Resulted in persistent or significant disability/incapacity
3 = Congenital anomaly/birth defect
4 = Life threatening
5 = Fatal
6 = Other event considered serious by investigator. Site investigators may use their discretion in classifying events as serious if they represent a significant hazard or potentially serious harm to research participants or others.

Attribution of Adverse Events
Site investigators will also evaluate the association of each AE with the periodontal intervention according to the following categories:
a. Unrelated: Adverse event(s) clearly not be related to investigational procedure(s)/agent(s) or other intervention.
b. Unlikely: Adverse event(s) doubtfully related to investigational procedure(s)/agent(s) or other intervention.
c. Possibly: Adverse events(s) may be related to investigational procedure(s)/agent(s) or other intervention; known to occur but the temporal relationship is unclear; other causes are possible or biologically very plausible.
d. Probably: Adverse event(s) likely associated with investigational procedure(s)/agent(s) or other intervention; known to occur and temporal relationship is appropriate or improvement is seen upon withdrawal of study drug; other causes are unlikely.
e. Definitely: Adverse event(s) clearly associated with the investigational procedure(s)/agent(s) or other intervention; known to occur and clear temporal relationship is noted or improvement is seen upon withdrawal of study drug; other causes are very unlikely. If re-challenge is attempted, the adverse event must return.

*An adverse event will be considered associated with the study procedures if in the estimation of the local investigator the event is at least possibly related to the study procedures procedures.

Reporting Adverse Events

All AEs and SAEs will be recorded on the electronic AE Eorm incorporated in the CDART “Dental Toolkit” Module. Adverse events that are (1) unexpected, (2) related or possibly related to participation in the research, and (3) serious or suggest that there are new or increased risk(s) to subjects or others is promptly reportable to UNC Office

of Human Research Ethics (OHRE) within 7 calendar days of the investigator becoming aware of the information. The Principal Investigator will assure that documentation of each event is adequate to permit accurate inferences regarding causation (e.g., temporal associations, onset, course, response to patient or physician intervention, alternative etiologies) and severity.

All unanticipated problems will be reported to **NIDCR's centralized reporting system** via Rho Product Safety:

- Product Safety Fax Line (US): 1-888-746-3293
- Product Safety Fax Line (International): 919-287-3998
- Product Safety Email: rho_productsafety@rhoworld.com

General questions about SAE reporting can be directed to the Rho Product Safety Help Line (available 8:00AM – 5:00PM Eastern Time):

- US: 1-888-746-7231

  International: 919-595-6486

9 STUDY OVERSIGHT

The investigator will be responsible for study oversight, including monitoring safety and ensuring that the study is conducted according to the protocol and ensuring data integrity. The principal investigator will review the data for safety concerns and data trends at regular intervals, and will promptly report to the IRB and NIDCR any Unanticipated Problem (UP), protocol deviation, or any other significant event that arises during the conduct of the study.

10 CLINICAL SITE MONITORING

Clinical site monitoring will not be done for this study; however, the NIDCR reserves the right to conduct independent audits or clinical monitoring as necessary.

11 STATISTICAL CONSIDERATIONS

11.1 Study Hypotheses

The central hypothesis of this proposal is that IFI16 functions to attenuate the host response that drives periodontitis. We propose to investigate whether macrophage-derived IFI16 expression modulates tissue inflammation via AIM2. Human tissues will be used for confirming results generated by the principal investigator from in vitro and in vivo models that show high expression of AIM2 and IFI16 under inflammatory conditions (4). Better characterization of the cellular and molecular biology of inflammasomes will help identify important potential therapeutic targets for the treatment and prevention of periodontal diseases. The following “hypothesis” corresponding to Study Objective 1 (section 3.1) is:

Primary hypotheses corresponding to the second study objective ***will be tested***:

1a. There will be a temporal change in expression of AIM2 (mRNA fold change and percentage of positive cells for histological analysis) induced by SIBO in healthy and severe periodontitis subjects

1b. There will be a temporal change in expression of IFI16 (mRNA fold change and percentage of cells for histological analysis) induced by SIBO in healthy and severe periodontitis subjects

1c. There will be differences in temporal changes in expression of AIM2 (mRNA fold change and percentage of cells for histological analysis) induced by SIBO between healthy and severe periodontitis subjects

1d. There will be differences in temporal changes in expression of IFI16 (mRNA fold change and percentage of cells for histological analysis) induced by SIBO between healthy and severe periodontitis subjects

Exploratory hypotheses corresponding to the second study objective will be assessed:

2a. The stent-induced change in expression of protein AIM2 will correlate with differentially expressed (between healthy and severe periodontitis subjects) periodontal pathogens identified by 16S rDNA (% of total reads) at baseline (visit 2)

2b. The stent-induced change in expression of protein IFI16 will correlate with differentially expressed (between healthy and severe periodontitis subjects) periodontal pathogens identified by 16S rDNA (% of total reads) at baseline (visit 2)

2c. The stent-induced change in expression of protein AIM2 will correlate with the temporal change in periodontal pathogens identified by 16S rDNA (% of total reads)

2d. The stent-induced change in expression of protein IFI16 will correlate with the temporal change in periodontal pathogens identified by 16S rDNA (% of total reads)

11.2 Sample Size Considerations

In order to evaluate the expression of IFI16 and AIM2 in subjects, 72 (36 subjects/group) will be recruited from the patients, students, staff and general population at the University of North Carolina-School of Dentistry. We will enroll 72 subjects to accommodate a 10% drop-out rate. Individuals with healthy tissues and severe periodontitis will be enrolled based on Morelli et al 2017 (1). Sample size calculations are based, in part, on preliminary cross-sectional (non-SIBO experiment) data on expression levels of Aim2 from immunohistochemistry reported in Xue et al. (2015; Table 3) who reported statistically significant differences in healthy (n=10; mean=0.50; s.d.=0.14) and severe periodontitis (n=10; 0.97; s.d.=0.27) subjects with respect to AIM2 expression from immunohistochemical analyses. For the first study objective (section 3.1), there will be 99% power to detect differences in AIM2 and IFI16 expressions at Visit 2 between healthy and severe periodontitis at these levels using two-sided t-tests at the 0.05 significance level. When comparing change in AIM2 expression levels from visit 2 to visit 5, assumptions of a common standard deviation of 0.25 and an intra-subject correlation of 0.40 across the two time points indicate that power will be at least 80% to determine differences in change between healthy and severe periodontitis subjects that are as small as 0.40; in a worst case (but not realistic) scenario, an intra-subject correlation of 0 would give 80% power to detect differences in temporal change that are as small as 0.50. Xue et al (2015; Figure 1c) found no statistically significant cross-sectional differences between healthy and severe periodontitis subjects with respect to mRNA expression of AIM2; they did not report this data numerically precluding power analysis.

11.3 Final Analysis Plan

1) (Primary) compare the expression (fold-change relative to baseline) of AIM2 and IFI16 between healthy (PPC-A, PPC-B) and severe periodontitis (PPC-E, PPC-G) subjects during an acute inflammatory response using the stent-induced biofilm overgrowth model

3) (Exploratory) correlate the expression of these proteins with periodontal pathogens

For the primary hypotheses involving gingival tissue samples, univariate and multivariate nonparametric testing procedures for SIBO experiments (Preisser, Sen and Offenbacher, 2011) will be utilized. In particular, a multivariate Wilcoxon Signed Rank test will use all available data to jointly test for changes in AIM2 and IFI16 expression levels over time (visit 2 to visit 5). If the overall multivariate test is statistically significant at the 0.05 level, then univariate Wilcoxon Signed Rank tests will be applied to AIM2 and IFI16 proteins individually. Similar procedures will be applied for expression levels from mRNA and from immunohistochemistry. Additionally, multivariate and univariate Wilcoxon Rank Sum Tests will be applied at the 0.05 significance level to compare SIBO-induced temporal change in Aim2 and IFI16 expression levels between healthy and severe periodontitis subjects.

Parametric statistical procedures will be used to summarize and test periodontal clinical measurements related to secondary hypotheses. Means and their 95% confidence intervals will be used to summarize measurements at each time point in healthy and severe periodontitis subjects. As these measurements will be collected at visits 2, 3 4 and 5, linear mixed models will be used to assess change over time within groups and differences in change over time between healthy and severe periodontitis groups.

Using 16s rRNA sequencing, expression counts for a large number (~20,000) of Operational Taxonomic Units (OTUs) with known taxonomy will be obtained from 72 samples at baseline and among them approximately 64 samples at Day 21 of SIBO. A few hundred taxa will be identified by collapsing expression data within genera corresponding to candidate periodontal pathogens. Change in pathogen levels from baseline to Day 21 among severe periodontitis subjects will be assessed with univariate Wilcoxon Signed Rank tests applied to each OTU (hypothesis 2f) or Wilcoxon Rank Sum Tests for comparison of Day 21 minus baseline differences in expression counts between healthy and severe periodontitis groups (hypothesis 2h). False discovery rate (FDR; Benjamini and Hochberg, 1995) and family-wise error rate (FWER; Hochberg, 1988) methods, e.g., as described in Preisser et al. (2011), will be applied to resulting *p*-values for exploratory and confirmatory testing, respectively, to identify statistically significant taxa.

Temporal changes in periodontal pathogen levels that are statistically significant controlling for multiple testing as described above will be correlated with stent-induced changes in AIM2 and IFI16 proteins using Spearman rank correlation coefficients and corresponding 95% confidence intervals.

Statistical computation for analysis of the data will be done my Mr. Moss in consultation with Dr. Marchesan and Dr. Preisser.

All hypothesis tests that are observed to be NOT statistically significant will be reported as being inconclusive.

12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

As part of the responsibilities assumed by initiating and conducting the study, the principal investigator will maintain adequate case histories for the subjects recruited under this research protocol. These case histories include source documentation, hard copy case report forms and electronic files.

To ensure compliance with the Health Insurance Portability and Accountability Act (HIPAA) guidelines, all research data entered into the database will be de-identified. Accordingly, there will be no identification of subjects by name, geographic residence (smaller than a state), date, telephone or fax number, electronic mail address, social security number, medical record number, health plan beneficiary number, account number, certificate or license number, vehicle identifiers and serial numbers (including license plate number), device identifiers and serial numbers, web universal resource locators (URLs), internet protocol (IP) address numbers, biometric identifiers (including finger and voice prints) full face photographic images or any comparable images, or any other number, characteristic or code derived from actual identifiers.

Data will be collected on desktop and/or laptop computers using the web based data entry program module, Dental Toolkit. This system resides in a secure server environment within a hardened data center on the UNC campus and is governed by standard UNC and UNC Schools of Medicine and Dentistry information security guidelines.

Source documentation for this study will consist of a combination of the dental eCRF (electronic case report form) containing medical histories, demography, vital signs, height and weight, body mass index, oral assessments and indices, sample tracking, AE's, protocol violations, subject disposition and paper based CRF's including IRB approved consent forms. All final data recorded in the dental eCRF will be copied onto CDs and kept with paper CRF's at the clinical site.

Computers and hard copies of the data sheets are kept in a locked office. Research samples will be identified only by unique numbers assigned to the participants. Biological specimens will also be coded. Linkage to these codes will be retained by the study coordinator and kept in locked workrooms accessible only by study personnel. Data will not be shared outside the immediate research team.

Study staff will maintain appropriate medical and research records for this study, in compliance with ICH E6, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects. Study staff will permit authorized representatives of NIDCR and regulatory agencies to examine (and when required by applicable law, to copy) research records for the purposes of quality assurance reviews, audits, and evaluation of the study safety, progress and data validity.

13 QUALITY CONTROL AND QUALITY ASSURANCE

Clinical examiners will be calibrated prior to commencement of the study for training of study procedures and for documentation of acceptable intra- and inter-examiner measurement reliability. All study examiners will have participated in an examiner calibration session conducted at the University of North Carolina, School of Dentistry in the GO Health Center prior to becoming a certified study examiner. Examiner calibration is done annually at the General and Oral Health Center as a standard protocol.

Trained quality control personnel will ensure that the research study is conducted, recorded and reported in accordance with the protocol, Standard Operating Procedures (SOPs), Good Clinical Practice (GCP) and the applicable regulatory requirements.

A data quality assurance program will be established that will consist of 1) real-time detection and correction of errors within the Dental Toolkit, and 2) periodic data review by study personnel. At the time of data entry, the Dental Toolkit alerts the user to missing, out-of-range, and inconsistent values and provides the user the opportunity to correct errors in real time. In accordance with federal regulations, the system records all elements of data entry (i.e., time, date, verbatim text, and the name of the person performing the data entry) in an electronic audit trail. For data not directly entered into the Dental Toolkit, study personnel will verify key outcome measurements by comparing data in the system with source documentation. Other data will be entered directly into the Dental Toolkit without source documentation. The frequency and the number of records reviewed are described in the Clinical Quality Management Plan. Missing values will be documented.

This study coordinator will monitor source documentation daily to ensure that data fields are complete and to verify that any transcribed data are accurately reflected on the study electronic case report forms. All study staff will receive training on the electronic data capturing tool, Dental Toolkit, prior to first subject being enrolled.

14 ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1 Ethical Standard

The investigator will ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, as drafted by the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and/or the ICH E6.

14.2 Institutional Review Board

The protocol, informed consent form(s), recruitment materials and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented in the study.

UNC IRB and Office of Research Ethics
Medical School Bldg. 52
105 Mason Farm Road
Chapel Hill, NC 27599-7097
(919) 966-3113
(919) 966-7879 (f)
FWA#4801

14.3 Informed Consent Process

Informed consent is a process that is initiated prior to the individual agreeing to participate in the study and continues throughout study participation. Extensive discussion of risks and possible benefits of study participation will occur with each potential participant.

Before any study procedures or assessments are performed, an IRB-approved consent form describing in detail the study procedures and risks of the study, will be given to each subject to read. Once trained study staff is assured that an individual understands the implications of participating in the study, the subject will be asked to give consent to participate in the study by signing the informed consent form. Consent forms will be IRB-approved, and the participant is required to read and review the document or have the document read to him or her. The investigator or designee will explain the research study to the participant and answer any questions that may arise. The participant will sign the informed consent document.

Participants 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 study. A copy of the signed informed consent document will be offered to each participants for their records. The rights and welfare of the participants

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.

14.4 Exclusion of Women, Minorities, and Children (Special Populations)

There is no planned involvement of special classes of subjects, such as fetuses, neonates, women, children, prisoners, institutionalized individuals. Periodontal disease is a chronic condition that affects individuals over 30. Children will be excluded from this study, because we are seeking to understand the expression of IFI16 and AIM2 in severe periodontitis.

14.5 Participant Confidentiality

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.

Authorized representatives may inspect all study documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) for the study participants. The clinical study site will permit access to such records. Participant confidentiality is strictly held in trust by the investigators, study staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to any study information relating to participants.

14.6 Future Use of Stored Specimens and Other Identifiable Data

Plaque samples and gingival biopsies that are not utilized for the current proposal will be stored for later analysis. Subjects will be consented using a separate biorepository consent form approved by UNC IRB (#15-1814) for stored specimens.

15 DATA HANDLING AND RECORD KEEPING

15.1 Data Management Responsibilities

The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. Data collection and accurate documentation are the responsibility of the study staff under the supervision of the investigator. The investigators will maintain adequate case histories of study participants, including accurate case report forms (CRFs), and source documentation. All source documents and laboratory reports must be reviewed by the study team and data entry staff, who will ensure that they are accurate and complete. Unanticipated problems must be reviewed by the investigator or designee.

15.2 Data Capture Methods

Data will be collected on desktop and/or laptop computers within the UNC School of Dentistry, GO Health Center. Research staff that have specifically been trained on the software data collection tools will be performing data entry. Other bar-coded data will be actually scanned into the software via desktop scanners. These computers are stored in locked offices, and are password protected. Data backup will be performed on password protected network.

15.3 Types of Data

Data captured will include subject's demographics, medical and dental histories, vital signs, body mass index, clinical oral exams and indices, adverse events, protocol deviations, clinical and laboratory notes and tracking of biological specimens. Reports may include any captured data retained on this module.

15.4 Schedule and Content of Reports

Customized reports will be generated from the data capturing module. Reports may include any captured data retained on this module. Data will not be shared with anyone outside those listed as IRB approved personal or investigators. Biannual progress reports will be provided to the NIDCR and/or the medical monitor. Report may include data regarding enrollment and retention, unanticipated problems and protocol deviations, disposition of biological specimens, outcome measures, quality management findings and other relevant parameters.

15.5 Study Records Retention

Study records will be maintained for at least three years from the date that the grant federal financial report (FFR) is submitted to the NIH.

15.6 Protocol Deviations

A protocol deviation is any noncompliance with the clinical study protocol, Good Clinical Practice, or Manual of Procedures requirements. The noncompliance may be on the part

of the subject, the investigator, or study staff. As a result of deviations, corrective actions are to be developed by the study staff and implemented promptly.

These practices are consistent with investigator and sponsor obligations in ICH E6:

- Compliance with Protocol, Sections 4.5.1, 4.5.2, 4.5.3, and 4.5.4.
- Quality Assurance and Quality Control, Section 5.1.1
- Noncompliance, Sections 5.20.1 and 5.20.2.

All deviations from the protocol must be addressed in study subject source documents and promptly reported to NIDCR and the local IRB, according to their requirements.

16 PUBLICATION/DATA SHARING POLICY

This study will comply with the NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

17 REFERENCES

1. Morelli T, Moss KL, Beck J, Preisser JS, Wu D, Divaris K, Offenbacher S. Derivation and Validation of the Periodontal and Tooth Profile Classification System for Patient Stratification. Journal of periodontology. 2017;88(2):153-65. doi: 10.1902/jop.2016.160379. PubMed PMID: 27620653; PMCID: PMC5288277.
2. Meyle J, Chapple I. Molecular aspects of the pathogenesis of periodontitis. Periodontology 2000. 2015;69(1):7-17. doi: 10.1111/prd.12104. PubMed PMID: 26252398.
3. Offenbacher S, Divaris K, Barros SP, Moss KL, Marchesan JT, Morelli T, Zhang S, Kim S, Sun L, Beck JD, Laudes M, Munz M, Schaefer AS, North KE. Genome-wide association study of biologically-informed periodontal complex traits offers novel insights into the genetic basis of periodontal disease. Human molecular genetics. 2016. doi: 10.1093/hmg/ddw069. PubMed PMID: 26962152.
4. Marchesan JT, Jiao Y, Moss K, Divaris K, Seaman W, Webster-Cyriaque J, Zhang S, Yu N, Song C, Bencharit S, Teles R, Offenbacher S. Common Polymorphisms in IFI16 and AIM2 Genes Are Associated With Periodontal Disease. Journal of periodontology. 2017;88(7):663-72. doi: 10.1902/jop.2017.160553. PubMed PMID: 28387608.
5. Hajishengallis G. Immunomicrobial pathogenesis of periodontitis: keystones, pathobionts, and host response. Trends Immunol. 2014;35(1):3-11. doi: 10.1016/j.it.2013.09.001. PubMed PMID: 24269668; PMCID: PMC3947349.
6. Kassebaum NJ, Bernabe E, Dahiya M, Bhandari B, Murray CJ, Marcenes W. Global burden of severe periodontitis in 1990-2010: a systematic review and meta-regression. Journal of dental research. 2014;93(11):1045-53. doi: 10.1177/0022034514552491. PubMed PMID: 25261053; PMCID: PMC4293771.
7. Eke PI, Dye BA, Wei L, Thornton-Evans GO, Genco RJ. Prevalence of periodontitis in adults in the United States: 2009 and 2010. Journal of dental research. 2012;91(10):914-20. Epub 2012/09/01. doi: 10.1177/0022034512457373. PubMed PMID: 22935673.
8. Tonetti MS, Jepsen S, Jin L, Otomo-Corgel J. Impact of the global burden of periodontal diseases on health, nutrition and wellbeing of mankind: A call for global action. Journal of clinical periodontology. 2017;44(5):456-62. doi: 10.1111/jcpe.12732. PubMed PMID: 28419559.
9. Jepsen S, Blanco J, Buchalla W, Carvalho JC, Dietrich T, Dorfer C, Eaton KA, Figuero E, Frencken JE, Graziani F, Higham SM, Kocher T, Maltz M, Ortiz-Vigon A, Schmoeckel J, Sculean A, Tenuta LM, van der Veen MH, Machiulskiene V. Prevention and control of dental caries and periodontal diseases at individual and population level: consensus report of group 3 of joint EFP/ORCA workshop on the boundaries between caries and periodontal diseases. Journal of clinical periodontology. 2017;44 Suppl 18:S85-S93. doi: 10.1111/jcpe.12687. PubMed PMID: 28266120.
10. Tonetti MS, Bottenberg P, Conrads G, Eickholz P, Heasman P, Huysmans MC, Lopez R, Madianos P, Muller F, Needleman I, Nyvad B, Preshaw PM, Pretty I, Renvert S, Schwendicke F, Trombelli L, van der Putten GJ, Vanobbergen J, West N, Young A, Paris S. Dental caries and periodontal diseases in the ageing population: call to action to protect and enhance oral health and well-being as an essential component of healthy ageing - Consensus report of group 4 of the joint EFP/ORCA workshop on the boundaries between caries and periodontal diseases. Journal of clinical periodontology. 2017;44 Suppl 18:S135-S44. doi: 10.1111/jcpe.12681. PubMed PMID: 28266112.
11. Colombo AP, Haffajee AD, Dewhirst FE, Paster BJ, Smith CM, Cugini MA, Socransky SS. Clinical and microbiological features of refractory periodontitis subjects. Journal of clinical periodontology. 1998;25(2):169-80. PubMed PMID: 9495617.

12. Levine M, Lohinai Z, Teles RP. Low Biofilm Lysine Content in Refractory Chronic Periodontitis. Journal of periodontology. 2017;88(2):181-9. doi: 10.1902/jop.2016.160302. PubMed PMID: 27620654.
13. Darveau RP. Periodontitis: a polymicrobial disruption of host homeostasis. Nat Rev Microbiol. 2010;8(7):481-90. doi: 10.1038/nrmicro2337. PubMed PMID: 20514045.
14. Cekici A, Kantarci A, Hasturk H, Van Dyke TE. Inflammatory and immune pathways in the pathogenesis of periodontal disease. Periodontology 2000. 2014;64(1):57-80. doi: 10.1111/prd.12002. PubMed PMID: 24320956; PMCID: PMC4500791.
15. Tonetti MS, Chapple IL, Working Group 3 of Seventh European Workshop on P. Biological approaches to the development of novel periodontal therapies--consensus of the Seventh European Workshop on Periodontology. Journal of clinical periodontology. 2011;38 Suppl 11:114-8. doi: 10.1111/j.1600-051X.2010.01675.x. PubMed PMID: 21323708.
16. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. Cell. 2010;140(6):805-20. doi: 10.1016/j.cell.2010.01.022. PubMed PMID: 20303872.
17. Kantarci A, Hasturk H, Van Dyke TE. Host-mediated resolution of inflammation in periodontal diseases. Periodontology 2000. 2006;40:144-63. doi: 10.1111/j.1600-0757.2005.00145.x. PubMed PMID: 16398691.
18. Van Dyke TE. Proresolving lipid mediators: potential for prevention and treatment of periodontitis. Journal of clinical periodontology. 2011;38 Suppl 11:119-25. doi: 10.1111/j.1600-051X.2010.01662.x. PubMed PMID: 21323709.
19. Reddy MS, Geurs NC, Gunsolley JC. Periodontal host modulation with antiproteinase, anti-inflammatory, and bone-sparing agents. A systematic review. Annals of periodontology / the American Academy of Periodontology. 2003;8(1):12-37. doi: 10.1902/annals.2003.8.1.12. PubMed PMID: 14971246.
20. Latz E, Xiao TS, Stutz A. Activation and regulation of the inflammasomes. Nat Rev Immunol. 2013;13(6):397-411. doi: 10.1038/nri3452. PubMed PMID: 23702978; PMCID: PMC3807999.
21. Schroder K, Tschopp J. The inflammasomes. Cell. 2010;140(6):821-32. doi: 10.1016/j.cell.2010.01.040. PubMed PMID: 20303873.
22. Guo H, Callaway JB, Ting JP. Inflammasomes: mechanism of action, role in disease, and therapeutics. Nat Med. 2015;21(7):677-87. doi: 10.1038/nm.3893. PubMed PMID: 26121197; PMCID: PMC4519035.
23. van der Heijden T, Kritikou E, Venema W, van Duijn J, van Santbrink PJ, Slutter B, Foks AC, Bot I, Kuiper J. NLRP3 Inflammasome Inhibition by MCC950 Reduces Atherosclerotic Lesion Development in Apolipoprotein E-Deficient Mice. Arterioscler Thromb Vasc Biol. 2017. doi: 10.1161/ATVBAHA.117.309575. PubMed PMID: 28596375.
24. Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. Mol Cell. 2002;10(2):417-26. PubMed PMID: 12191486.
25. Schattgen SA, Fitzgerald KA. The PYHIN protein family as mediators of host defenses. Immunol Rev. 2011;243(1):109-18. doi: 10.1111/j.1600-065X.2011.01053.x. PubMed PMID: 21884171.
26. Rathinam VA, Vanaja SK, Fitzgerald KA. Regulation of inflammasome signaling. Nature immunology. 2012;13(4):333-42. doi: 10.1038/ni.2237. PubMed PMID: 22430786; PMCID: 3523703.
27. Rathinam VA, Fitzgerald KA. Inflammasomes and anti-viral immunity. J Clin Immunol. 2010;30(5):632-7. doi: 10.1007/s10875-010-9431-4. PubMed PMID: 20665097.
28. Dombrowski Y, Peric M, Koglin S, Kammerbauer C, Goss C, Anz D, Simanski M, Glaser R, Harder J, Hornung V, Gallo RL, Ruzicka T, Besch R, Schauber J. Cytosolic DNA triggers inflammasome activation in keratinocytes in psoriatic lesions. Sci Transl Med.

2011;3(82):82ra38. doi: 10.1126/scitranslmed.3002001. PubMed PMID: 21562230; PMCID: PMC3235683.
29. de Koning HD, Bergboer JG, van den Bogaard EH, van Vlijmen-Willems IM, Rodijk-Olthuis D, Simon A, Zeeuwen PL, Schalkwijk J. Strong induction of AIM2 expression in human epidermis in acute and chronic inflammatory skin conditions. Exp Dermatol. 2012;21(12):961-4. doi: 10.1111/exd.12037. PubMed PMID: 23171461.
30. Xue F, Shu R, Xie Y. The expression of NLRP3, NLRP1 and AIM2 in the gingival tissue of periodontitis patients: RT-PCR study and immunohistochemistry. Arch Oral Biol. 2015;60(6):948-58. doi: 10.1016/j.archoralbio.2015.03.005. PubMed PMID: 25841070.
31. Sahingur SE, Xia XJ, Voth SC, Yeudall WA, Gunsolley JC. Increased nucleic Acid receptor expression in chronic periodontitis. Journal of periodontology. 2013;84(10):e48-57. doi: 10.1902/jop.2013.120739. PubMed PMID: 23646855.
32. Vanhove W, Peeters PM, Staelens D, Schraenen A, Van der Goten J, Cleynen I, De Schepper S, Van Lommel L, Reynaert NL, Schuit F, Van Assche G, Ferrante M, De Hertogh G, Wouters EF, Rutgeerts P, Vermeire S, Nys K, Arijs I. Strong Upregulation of AIM2 and IFI16 Inflammasomes in the Mucosa of Patients with Active Inflammatory Bowel Disease. Inflamm Bowel Dis. 2015;21(11):2673-82. doi: 10.1097/MIB.0000000000000535. PubMed PMID: 26313692.
33. Park E, Na HS, Song YR, Shin SY, Kim YM, Chung J. Activation of NLRP3 and AIM2 inflammasomes by Porphyromonas gingivalis infection. Infection and immunity. 2014;82(1):112-23. doi: 10.1128/IAI.00862-13. PubMed PMID: 24126516; PMCID: PMC3911849.
34. Kerur N, Veettil MV, Sharma-Walia N, Bottero V, Sadagopan S, Otageri P, Chandran B. IFI16 acts as a nuclear pathogen sensor to induce the inflammasome in response to Kaposi Sarcoma-associated herpesvirus infection. Cell host & microbe. 2011;9(5):363-75. Epub 2011/05/18. doi: 10.1016/j.chom.2011.04.008. PubMed PMID: 21575908; PMCID: 3113467.
35. Unterholzner L, Keating SE, Baran M, Horan KA, Jensen SB, Sharma S, Sirois CM, Jin T, Latz E, Xiao TS, Fitzgerald KA, Paludan SR, Bowie AG. IFI16 is an innate immune sensor for intracellular DNA. Nature immunology. 2010;11(11):997-1004. Epub 2010/10/05. doi: 10.1038/ni.1932. PubMed PMID: 20890285; PMCID: 3142795.
36. Tervaniemi MH, Katayama S, Skoog T, Siitonen HA, Vuola J, Nuutila K, Sormunen R, Johnsson A, Linnarsson S, Suomela S, Kankuri E, Kere J, Elomaa O. NOD-like receptor signaling and inflammasome-related pathways are highlighted in psoriatic epidermis. Sci Rep. 2016;6:22745. doi: 10.1038/srep22745. PubMed PMID: 26976200; PMCID: PMC4792137.
37. Duan X, Ponomareva L, Veeranki S, Panchanathan R, Dickerson E, Choubey D. Differential roles for the interferon-inducible IFI16 and AIM2 innate immune sensors for cytosolic DNA in cellular senescence of human fibroblasts. Mol Cancer Res. 2011;9(5):589-602. doi: 10.1158/1541-7786.MCR-10-0565. PubMed PMID: 21471287; PMCID: PMC3096691.
38. Veeranki S, Duan X, Panchanathan R, Liu H, Choubey D. IFI16 protein mediates the anti-inflammatory actions of the type-I interferons through suppression of activation of caspase-1 by inflammasomes. PloS one. 2011;6(10):e27040. doi: 10.1371/journal.pone.0027040. PubMed PMID: 22046441; PMCID: PMC3203938.
39. Yilmaz O, Lee KL. The inflammasome and danger molecule signaling: at the crossroads of inflammation and pathogen persistence in the oral cavity. Periodontology 2000. 2015;69(1):83-95. doi: 10.1111/prd.12084. PubMed PMID: 26252403; PMCID: PMC4530468.
40. Kornman KS, Polverini PJ. Clinical application of genetics to guide prevention and treatment of oral diseases. Clin Genet. 2014;86(1):44-9. doi: 10.1111/cge.12396. PubMed PMID: 24702466; PMCID: PMC4233973.
41. Orozco A, Gemmell E, Bickel M, Seymour GJ. Interleukin 18 and periodontal disease. Journal of dental research. 2007;86(7):586-93. PubMed PMID: 17586702.

42. Karimbux NY, Saraiya VM, Elangovan S, Allareddy V, Kinnunen T, Kornman KS, Duff GW. Interleukin-1 gene polymorphisms and chronic periodontitis in adult whites: a systematic review and meta-analysis. Journal of periodontology. 2012;83(11):1407-19. Epub 2012/02/22. doi: 10.1902/jop.2012.110655. PubMed PMID: 22348697.
43. Hart TC, Kornman KS. Genetic factors in the pathogenesis of periodontitis. Periodontology 2000. 1997;14:202-15. PubMed PMID: 9567972.
44. Wu X, Offenbacher S, Lomicronpez NJ, Chen D, Wang HY, Rogus J, Zhou J, Beck J, Jiang S, Bao X, Wilkins L, Doucette-Stamm L, Kornman K. Association of interleukin-1 gene variations with moderate to severe chronic periodontitis in multiple ethnicities. J Periodontal Res. 2015;50(1):52-61. doi: 10.1111/jre.12181. PubMed PMID: 24690098; PMCID: PMC4183738.
45. McDevitt MJ, Wang HY, Knobelman C, Newman MG, di Giovine FS, Timms J, Duff GW, Kornman KS. Interleukin-1 genetic association with periodontitis in clinical practice. Journal of periodontology. 2000;71(2):156-63. doi: 10.1902/jop.2000.71.2.156. PubMed PMID: 10711605.
46. Lobene RR, Weatherford T, Ross NM, Lamm RA, Menaker L. A modified gingival index for use in clinical trials. Clin Prev Dent. 1986;8(1):3-6. PubMed PMID: 3485495.
47. Morelli T, Stella M, Barros SP, Marchesan JT, Moss KL, Kim SJ, Yu N, Aspiras MB, Ward M, Offenbacher S. Salivary biomarkers in a biofilm overgrowth model. Journal of periodontology. 2014;85(12):1770-8. doi: 10.1902/jop.2014.140180. PubMed PMID: 25079398; PMCID: PMC4383599.
48. Paes Batista da Silva A, Barros SP, Moss K, Preisser J, Marchesan JT, Ward M, Offenbacher S. Microbial Profiling in Experimentally Induced Biofilm Overgrowth Among Patients With Various Periodontal States. Journal of periodontology. 2016;87(1):27-35. doi: 10.1902/jop.2015.150328. PubMed PMID: 26430927.
49. Marchesan JT, Morelli T, Moss K, Barros SP, Ward M, Jenkins W, Aspiras MB, Offenbacher S. Association of Synergistetes and Cyclodipeptides with Periodontitis. Journal of dental research. 2015;94(10):1425-31. doi: 10.1177/0022034515594779. PubMed PMID: 26198391.
50. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. QIIME allows analysis of high-throughput community sequencing data. Nature methods. 2010;7(5):335-6. doi: 10.1038/nmeth.f.303. PubMed PMID: 20383131; PMCID: 3156573.
51. Quail MA, Smith M, Coupland P, Otto TD, Harris SR, Connor TR, Bertoni A, Swerdlow HP, Gu Y. A tale of three next generation sequencing platforms: comparison of Ion Torrent, Pacific Biosciences and Illumina MiSeq sequencers. BMC genomics. 2012;13:341. doi: 10.1186/1471-2164-13-341. PubMed PMID: 22827831; PMCID: 3431227.

SUPPLEMENTAL MATERIALS

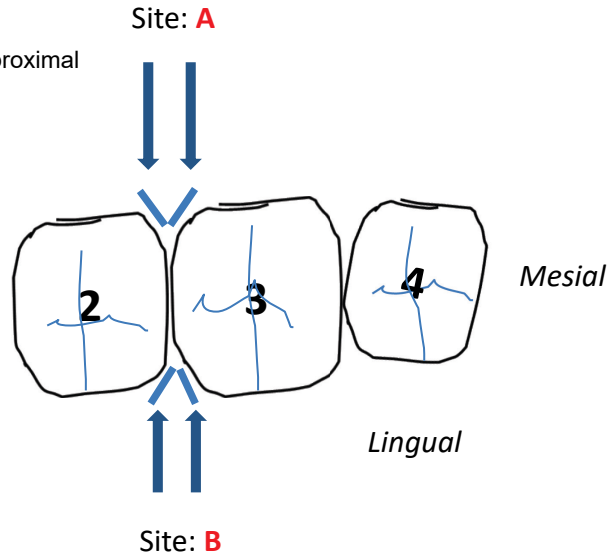
These documents are relevant to the protocol, but they are not considered part of the protocol. They are stored and modified separately. As such, modifications to these documents do not require protocol amendments.

***Appendix I*:** Schedule of Events

Procedures	**Visit 1** Screening/Enrollment *Day -28 to ±2*	**Visit 2** Baseline *Day 0* *±5 days*	**Visit 3** *Day 7* *±5 days*	**Visit 4** Day 14 *±5 days*	**Visit 5** Day 21 *±5 days*	**Visit 6** Day 28 *±5 days*	**Visit 7** Day 35 *±5 days*	**Visit 8** Day 42 *±5 days*
Consenting	X							
Medical Hx/ Update	X	X	X	X	X	X	X	X
Oral Exam	X	X	X	X	X	X	X	X
Pregnancy testing	X							
Stent impression	X							
Deliver stent		X						
Discontinue stent					X			
Adverse Events			X	X	X	X	X	X
Height /Weight	X							
Demographics	X							
Plaque Collection		X			X			
Biopsy Collection		X			X			
PI, GI, PD, CAL, BOP	X	X	X	X	X	X		
Adult Prophylaxis/SRP					X	X	X	X

Appendix II: Gingival biopsy

Example shown for biopsy collection of interproximal biopsy tissue sites between teeth #2 & 3.



Strategy:
During visit (baseline, 0 day), one biopsy sample will be collected from the posterior interproximal unit determined by the dental provider. Sites will not be standardized since dentition will vary from subject to subject.

During visit 5 (day 21 of SIBO), one biopsy sample will be collected from the posterior interproximal unit in SIBO, determined by the dental provider. Sites will not be standardized since dentition will vary from subject to subject.