

Electronic Cover Sheet		
PI: Soh, Dam	Title: Role of microbial pH modulation within biofilms as a determinant of the establishment of the pathogen Porphyromonas gingivalis	
Received: 10/13/2025	Opportunity: PA-24-182	Council: 05/2026
Competition ID: FORMS-I	FOA Title: Mentored Clinical Scientist Research Career Development Award (Parent K08 Independent Clinical Trial Not Allowed)	
1K08DE036315-01	Dual: AI	Accession Number: 5205743
IPF: 5992614	Organization: STATE UNIVERSITY OF NEW YORK AT BUFFALO	
Former Number:	Department: Department of Oral Biology	
IRG/SRG: ZRG1 MSOS-F (22)S	AIDS: N	Expedited: N
<u>Subtotal Direct Costs</u> <u>(excludes consortium F&A)</u> Year 1: 126,430 Year 2: 128,696 Year 3: 131,808 Year 4: 135,014 Year 5: 138,366	Animals: N Humans: Y Clinical Trial: N Current HS Code: 20 HESC: N HFT: N Special Topics: Data Management Sharing	New Investigator: Early Stage Investigator:
<i>Senior/Key Personnel:</i>	<i>Organization:</i>	<i>Role Category:</i>
Dam Soh	State University of New York at Buffalo	PD/PI
Alex Valm Ph.D.	State University of New York at Albany	Other Professional-Co-mentor
Troy Wood Ph.D.	State University of New York at Buffalo	Other Professional-Co-mentor
Hung Ton-That Ph.D.	University of California, Los Angeles	Other Professional-Co-mentor
Patricia Diaz Ph.D.	State University of New York at Buffalo	Other Professional-Mentor

Reference Letters

Chelsie Armbruster	Jacobs School of Medicine and Biomedical Sciences, University of Buffalo	10/13/2025
Mira Edgerton	University at Buffalo	10/13/2025
Keith Kirkwood	University at Buffalo	10/13/2025

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE		State Application Identifier
1. TYPE OF SUBMISSION*		4.a. Federal Identifier
<input type="radio"/> Pre-application <input checked="" type="radio"/> Application <input type="radio"/> Changed/Corrected Application		b. Agency Routing Number
2. DATE SUBMITTED	Application Identifier	c. Previous Grants.gov Tracking Number
5. APPLICANT INFORMATION		UEI*: LMCJKRFW5R81
Legal Name*: The Research Foundation for SUNY on behalf of U. at Buffalo		
Department: Sponsored Projects Services		
Division:		
Street1*: The UB Commons		
Street2: 520 Lee Entrance, Suite 211		
City*: Amherst		
County:		
State*: NY: New York		
Province:		
Country*: USA: UNITED STATES		
ZIP / Postal Code*: 14228-2567		
Person to be contacted on matters involving this application		
Prefix: First Name*: Deirdre Middle Name: Last Name*: O'Rourke Suffix:		
Position/Title: Agreement Administrator		
Street1*: The UB Commons		
Street2: 520 Lee Entrance, Suite 211		
City*: Amherst		
County: Erie		
State*: NY: New York		
Province:		
Country*: USA: UNITED STATES		
ZIP / Postal Code*: 14228-2567		
Phone Number*: 716-645-4421 Fax Number: Email: do8@buffalo.edu		
6. EMPLOYER IDENTIFICATION NUMBER (EIN) or (TIN)*		1-141368361-A1
7. TYPE OF APPLICANT*		X: Other (specify)
Other (Specify): Private, non-profit		
Small Business Organization Type <input type="radio"/> Women Owned <input type="radio"/> Socially and Economically Disadvantaged		
8. TYPE OF APPLICATION*		If Revision, mark appropriate box(es).
<input checked="" type="radio"/> New <input type="radio"/> Resubmission		<input type="radio"/> A. Increase Award <input type="radio"/> B. Decrease Award <input type="radio"/> C. Increase Duration
<input type="radio"/> Renewal <input type="radio"/> Continuation <input type="radio"/> Revision		<input type="radio"/> D. Decrease Duration <input type="radio"/> E. Other (specify):
Is this application being submitted to other agencies?* <input type="radio"/> Yes <input checked="" type="radio"/> No What other Agencies?		
9. NAME OF FEDERAL AGENCY* National Institutes of Health		10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER TITLE:
11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT* Role of microbial pH modulation within biofilms as a determinant of the establishment of the pathogen Porphyromonas gingivalis		
12. PROPOSED PROJECT Start Date* Ending Date* 07/01/2026 06/30/2031		13. CONGRESSIONAL DISTRICTS OF APPLICANT NY-026

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE**Page 2****14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION**

Prefix: Dr. First Name*: Dam Middle Name: Last Name*: Soh Suffix:

Position/Title:

Organization Name*: State University of New York at Buffalo

Department: Department of Oral Biology

Division:

Street1*: 345 Biomedical Research Building

Street2:

City*: Buffalo

County:

State*: NY: New York

Province:

Country*: USA: UNITED STATES

ZIP / Postal Code*: 14214-8024

Phone Number*: 716-829-6284 Fax Number: Email*: damsoh@buffalo.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested* \$713,139.00

b. Total Non-Federal Funds* \$0.00

c. Total Federal & Non-Federal Funds* \$713,139.00

d. Estimated Program Income* \$0.00

16. IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ☐ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ☒ PROGRAM IS NOT COVERED BY E.O. 12372; OR

☐ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

☒ I agree*

* The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION

File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: Deirdre Middle Name: Last Name*: O'Rourke Suffix:

Position/Title*: Agreement Administrator

Organization Name*: The Research Foundation for SUNY on behalf of U. at Buffalo

Department: Sponsored Projects Services

Division:

Street1*: The UB Commons

Street2: 520 Lee Entrance, Suite 211

City*: Amherst

County: Erie

State*: NY: New York

Province:

Country*: USA: UNITED STATES

ZIP / Postal Code*: 14228-2567

Phone Number*: 716-645-4421 Fax Number: Email*: do8@buffalo.edu

Signature of Authorized Representative*

Deirdre ORourke

Date Signed*

10/13/2025

20. PRE-APPLICATION File Name:**21. COVER LETTER ATTACHMENT** File Name:1250-Cover Letter resized.pdf

424 R&R and PHS-398 Specific

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Project/Performance Site Location(s)**Project/Performance Site Primary Location**

☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: State University of New York at Buffalo

UEI: LMCJKRFW5R81

Street1*: 345 BIOMEDICAL RES BLDG

Street2:

City*: Buffalo

County: Erie

State*: NY: New York

Province:

Country*: USA: UNITED STATES

Zip / Postal Code*: 14214-8024

Project/Performance Site Congressional District*: NY-026

Additional Location(s)

File Name:

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* <input checked="" type="radio"/> Yes <input type="radio"/> No	
1.a. If YES to Human Subjects	
Is the Project Exempt from Federal regulations? <input type="radio"/> Yes <input checked="" type="radio"/> No	
If YES, check appropriate exemption number: <input type="text"/> 1 <input type="text"/> 2 <input type="text"/> 3 <input type="text"/> 4 <input type="text"/> 5 <input type="text"/> 6 <input type="text"/> 7 <input type="text"/> 8	
If NO, is the IRB review Pending? <input type="radio"/> Yes <input checked="" type="radio"/> No	
IRB Approval Date: 02-20-2025	
Human Subject Assurance Number 00008824	
2. Are Vertebrate Animals Used?* <input type="radio"/> Yes <input checked="" type="radio"/> No	
2.a. If YES to Vertebrate Animals	
Is the IACUC review Pending? <input type="radio"/> Yes <input type="radio"/> No	
IACUC Approval Date:	
Animal Welfare Assurance Number	
3. Is proprietary/privileged information included in the application?* <input type="radio"/> Yes <input checked="" type="radio"/> No	
4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* <input type="radio"/> Yes <input checked="" type="radio"/> No	
4.b. If yes, please explain:	
4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? <input type="radio"/> Yes <input type="radio"/> No	
4.d. If yes, please explain:	
5. Is the research performance site designated, or eligible to be designated, as a historic place?* <input type="radio"/> Yes <input checked="" type="radio"/> No	
5.a. If yes, please explain:	
6. Does this project involve activities outside the United States or partnership with international collaborators?* <input type="radio"/> Yes <input checked="" type="radio"/> No	
6.a. If yes, identify countries:	
6.b. Optional Explanation:	
7. Project Summary/Abstract*	Filename 1235-Project summary_Soh.pdf
8. Project Narrative*	1236-Project Narrative.pdf
9. Bibliography & References Cited	1237-Bibliography and References cited.pdf
10. Facilities & Other Resources	1238-3. Facilities and Other Resources_Soh.pdf
11. Equipment	1239-3. Equipment Forms_Soh.pdf

Project summary

Periodontitis affects nearly half of U.S. adults and is driven by dysbiotic subgingival microbiomes that trigger host-mediated tissue destruction. *Porphyromonas gingivalis* (*Pg*) is a key pathogen associated with disease progression, yet its colonization is variable, possibly due to ecological factors that influence its establishment. Environmental pH is a critical ecological factor influencing microbial communities, as bacterial metabolic activities may create local pH variations that select for species with specific pH tolerances. While *Pg* requires alkaline conditions (pH 6.7-8.0) for growth and virulence, acid-producing early colonizers like *Actinomyces oris* (*Ao*) may create inhibitory acidic microenvironments. Our preliminary data revealed that *Ao* inhibits *Pg* growth through lactate production which lowers the environmental pH below *Pg*'s tolerance threshold. However, another common subgingival commensal, *Veillonella parvula* (*Vp*), can neutralize the environmental pH by consuming *Ao*-produced organic acids, restoring favorable conditions for *Pg* establishment. In this proposal we will evaluate the relevance of this three species interaction and whether local pH modulation determines the growth and spatial arrangement of *Pg* in model biofilms. In addition, we will examine human subgingival biofilms to determine whether local pH, spatial architecture and the metabolic activities of microbiome species influence *Pg* establishment. Our hypothesis is that the establishment of *Pg* in subgingival biofilms is influenced by the metabolic activities of other microbial species which modify local pH, either selecting out or supporting *Pg*'s growth through the creation of distinct pH-defined microenvironments. Aim 1 will use imaging to map pH gradients within model three-species biofilms and correlate these patterns with *Pg* colonization, including studies with a lactate-deficient *Ao* mutant to directly test the role of acid production as a determinant of biofilm spatial arrangement and *Pg* biomass. Aim 2 will employ a carrier system to develop intact human subgingival plaque in situ, evaluating pH gradients, species spatial arrangements and *Pg* colonization. In addition, the relationship of community-level pH-modulating functions and metabolites with *Pg* colonization will be assessed by metatranscriptomics and LC-MS profiling. Completion of these aims will elucidate how pH gradients and metabolic interactions shape the spatial organization of multispecies biofilms and establishment of *Pg*. The integration of advanced imaging with multi-omics offers unprecedented resolution of structure–function relationships in intact biofilms, providing mechanistic insight into pH-mediated biofilm organization. Under the guidance of an expert mentorship team, Dr. Soh will gain training in clinical research methods, biofilm model development, advanced microscopy, metabolomics, bioinformatics, scientific communication, and leadership skills essential for launching a successful independent career as a clinician–scientist in oral biology and periodontal disease mechanisms.

Project Narrative

The proposed research will employ advanced imaging and multi-omics approaches to visualize pH gradients within subgingival plaque biofilms and determine how interspecies metabolic interactions influence spatial community organization and colonization by the pathogen *Porphyromonas gingivalis*. This study will significantly advance our understanding of how subgingival plaque is formed providing critical insights into how varying microenvironmental conditions either facilitate or inhibit pathobiont colonization and proliferation, with direct implications for understanding periodontal disease progression and developing targeted therapeutic interventions.

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Facilities and Other Resources

Laboratory:

The research described within this application will be carried out in Dr. Patricia Diaz's laboratory. Dr. Diaz's laboratory is located in the Department of Oral Biology at the University at Buffalo (UB), housed within the Biomedical Research Building, adjacent to the UB School of Dental Medicine. The laboratory has spaces for molecular biology and cultivation of microorganisms. The laboratory also has access to a cold room and a utility room containing an autoclave, dishwasher, drying oven and ice machine.

Clinical:

The University at Buffalo, School of Dental Medicine houses a research clinic equipped with two operative units for participant sample collection and a sterilization room for cleaning, sterilizing and preparing equipment. The facility also includes a laboratory with the necessary equipment for sample processing.

Animal:

Not applicable.

Computer:

The Diaz lab has multiple dedicated workstations operating Windows, including Intel Core i7 2.9GHz processors, with 16GB RAM and 477GB hard drive storage space that is utilized for document processing, imaging processing, and bioinformatics data analysis. All are loaded with the major software required to conduct the proposed research, are connected to printers, and have reliable internet access. A lot of the work on bioinformatics is done by connecting to University at Buffalo Center for Computational Research (CCR)

Office:

Dr. Dam Soh has a desk in an office shared with other Diaz laboratory members on the 3rd floor of the Biomedical Research Building, adjacent to the laboratory facilities and next door to Dr. Diaz's office

Other:

Oral Biology equipment core: The Oral Biology Equipment Core, located within the Department of Oral Biology at the University at Buffalo (UB), provides access to essential instrumentation and technical support for molecular, microbiological, and biochemical research. The facility is equipped with a MagPix system for multiplexed immunoassays and nucleic acid analyses, a ChemiDoc Touch Imaging System for high-resolution gel and blot imaging, an XE-90 Ultracentrifuge for high-speed centrifugation, an Amaxa Nucleofector II and a Bio-Rad Electroporator for transfection and transformation applications, and a CFX Opus 96 Real-Time PCR System for quantitative gene expression and microbial detection assays. Additional instruments include a Spectrophotometer DU800 and a NanoDrop 2000 for nucleic acid and protein quantification, and a GelDoc XR+ Imaging System for visualization and documentation of gels. These instruments support all molecular and microbial components of the proposed work. The facility is maintained by trained technical staff and ensures high-quality, reproducible data in support of the project's overall aims.

Genomics & Bioinformatics Core Facility (GBC): Located in UB's Center of Excellence in Bioinformatics and Life Sciences (CBLS), the GBC is the only facility of its kind within UB that provides high-throughput massively parallel sequencing and bioinformatics expertise and services to university faculty, industry and other research institutions. The Core has been in existence since 2010 and offers a comprehensive suite of sequencing services that include exome, transcriptome, microbiome, and epigenome sequencing. The core has several Illumina next generation platforms including a NovaSeq6000, a NextSeq500, a Miseq and an Oxford Nanopore MinION sequencing platform for all library sequencing needs. In addition, the GBC has the 10X Genomics Chromium Platform for single cell transcriptomics, single cell ATACseq and immunogenomic identification from cultured cells or native tissues. Other on-site equipment for the generation of NGS libraries include the BluePippin gel purification system, Advanced Analytical's Fragment Analyzer, the BioRad QX100 and QX200 Droplet Digital PCR Machines, QIA Symphony SP robot for nucleic acid extraction, a Qiagen Q48 pyrosequencer and a Diagenode SX-8G IP-Star Compact system for performing automated ChIP. Staffed with three bench scientists and two computational scientists, the core has expertise in genomic sequencing and data analyses.

Optical Imaging and Analysis Facility (OIAF): Located on the 4th floor of the Biomedical Research Building, is equipped with a suite of advanced, high-performance instruments designed to support a vast range of biological and materials science research. OIAF offers a microscopy center on high-resolution and live-cell imaging, featuring the Andor Dragonfly Spinning Disk Confocal Microscope. This system is mounted on an inverted base and is highly valued for its speed and sensitivity, making it ideal for high-speed live-cell imaging with minimal phototoxicity and photobleaching. It utilizes solid-state laser lines (typically 405, 488, 561, and 635 nm) and a sensitive sCMOS camera, allowing for rapid acquisition of large, multi-channel volumetric images. Complementing this are general-purpose microscopes like an inverted Zeiss AxioObserver fluorescent microscope for routine and widefield imaging, and an upright fluorescent microscope for fixed samples. The facility may also house other specialized imaging platforms, such as an Olympus FV1000 Confocal/Multiphoton system. Also OIAF provides other essential lab tools like a NanoDrop One Spectrophotometer for quantifying nucleic acids and proteins, Bio-Rad ChemiDoc and GelDoc imagers for gel and Western blot analysis, a BioTek Synergy HT microplate reader, and qPCR thermocyclers for quantitative gene expression analysis. All systems are typically supported by dedicated Bioimage Analysis Workstations with powerful CPUs, substantial RAM (e.g., 512 GB), and dedicated GPUs to handle the computational demands of high-throughput image and data processing

Chemistry Instrumentation Center (CIC): A comprehensive core facility located at University at Buffalo, is a cutting-edge facility distinguished by its extensive array of high-resolution and multi-modal chemical analysis equipment. The center's instrumentation is configured to support the most challenging analytical needs in modern chemical, materials, and environmental research. CIC offers various chemical analytic instruments including Thermo Fisher Q-Exactive Liquid Chromatography Orbitrap Tandem Mass Spectrometers (QE-LCMSMS). These instruments are built for high-resolution and accurate mass analysis, handling non-targeted, targeted, and quantitative analyses, and are essential for identifying and tracking complex chemical species, such as environmental contaminants. A Thermo Scientific Q-Exactive Orbitrap Gas Chromatography/Tandem Mass Spectrometer is also available, providing high-resolution and accurate mass gas chromatography analysis for volatile and semi-volatile compounds. Thermo Fisher Linear Ion Trap (LTQ) mass spectrometer for routine project work and rapid sample testing. The CIC offers powerful elemental analysis through Inductively Coupled Plasma Mass Spectrometry (ICP-MS) systems, such as the Thermo Electron X Series 2 and Perkin Elmer Nexion 5000 ICP-MS/MS. These allow for highly sensitive trace elemental analysis in solid and liquid samples, with options for laser ablation for direct solid sample introduction. The CIC is staffed by Ph.D.-level chemical instrumentation specialists who function as technical collaborators, not just instrument custodians. This expert staff offers crucial support at every stage of a project

Dr. Alex Valm's lab in University at Albany: Dr. Valm's lab is equipped with a Zeiss LSM 980 confocal microscope with Airyscan 2, housed within the Biological Science Research Facilities, featuring an inverted stand, motorized stage, and high-NA objectives. Multiple excitation lasers (405–730 nm) and a ≈34-channel spectral detector enable spectral imaging and unmixing of overlapping fluorophores, while Airyscan 2 provides enhanced resolution and sensitivity. High-performance workstations support processing of large multidimensional datasets, 3D reconstruction, and quantitative analyses. These capabilities allow detailed study of microbial community structure, including multi-species imaging, spatial mapping, and reduced background interference in delicate samples. Dr. Soh will conduct imaging acquisition for Aim 2a in Dr. Valm's laboratory. Albany is driving distance from Buffalo.

University at Buffalo Center for Computational Research (CCR): The CCR is one of the top 10 academic supercomputing sites in the United States. This world-class computational research facility provides resources to faculty and students at UB, as well as to industrial and educational partners in Western New York. These resources are used to solve challenging problems in areas that include biology and medicine. The CCR has the capacity to support high-end computing, data storage, networking and visualization. With a current staff of 20 people, including 5 PhD level computational scientists, 6 system administrators, a database administrator and several programmers, they are able to provide the computational resources and support as needed. The CCR maintains a high-performance computing environment, high-end visualization laboratories, and support staff with expertise in computing, visualization, and networking. The Center's extensive computing facilities, which are housed in a state-of-the-art 4000 sq ft machine room, include a Linux cluster with more than 8000 processor cores and QDR Infiniband, a subset (32) of which contain (64) NVidia Tesla M2050 "Fermi" graphics processing units (GPUs). The Center also maintains several high-performance storage systems including Isilon-based

storage (325TB) as well as a parallel storage system from Panasas (170TB). The computer visualization laboratory features a tiled display wall, and a VisDuo passive stereo system. As a leading academic supercomputing facility, CCR has more than 70 Tflops of peak performance compute capacity (more than 100TFlop/s including GPUs).

Equipment

Core Equipment at Diaz Lab:

- The PI will have access to all essential laboratory equipment needed for microbial cultivation, molecular biology, and chemical analyses (supporting all Aims):
- Wet benches
- Fume hoods
- Laminar flow hood
- Two anaerobic chambers
- Refrigerators, -20°C and -80°C freezers
- Benchtop microcentrifuges
- Refrigerated tabletop centrifuge
- Analytical mass balance
- pH meter
- Incubators
- Drying oven
- Rotary evaporator
- Water baths
- PCR thermocycler
- Electrophoresis equipment and power supplies
- Flow cell chamber
- Spectrophotometer
- Sonicator
- A Labconco freeze dryer
- An Experion electrophoresis station
- A New Brunswick BioFlo®/CelliGen 115 Benchtop Fermentor & Bioreactor
- A Nikon phase/light and epifluorescence microscope

Shared Equipment and facilities:

- The PI will also have access to shared institutional equipment and core facilities for imaging (Aim 1, 2a), molecular analysis, sequencing (Aim 2b), and metabolomics (Aim 2b).
- A MagPix
- A ChemiDoc Touch
- XE-90 Ultracentrifuge
- Amaxa Nucleofactor II
- BioRad Electroporator
- CFX Opus 96 Real-Time PCR System
- Spectrophotometer DU800
- Nanodrop 2000
- GelDoc XR+
- Equipment in the Genomics & Bioinformatics Core Facility (GBC)
 - A NovaSeq6000 Illumina sequencer
 - A NextSeq500 Illumina sequencer
 - A Miseq Illumina sequencers
 - An Oxford Nanopore MinION sequencer
- UB Chemistry instrument Center
 - Thermo Fisher Q-Exactive Orbitrap Liquid Chromatography/Tandem Mass Spectrometer
 - Thermo Scientific Q-Exactive Orbitrap Gas Chromatography/Tandem Mass Spectrometer
 - Perkin Elmer Nexion 5000 Inductively Coupled Plasma Mass Spectrometer/Mass Spectrometer
- Optical Imaging and Analysis Facility
 - Andor Dragonfly confocal microscope
(Four solid-state laser lines: 405nm, 488nm, 561nm, and 635/637nm.)
 - Zeiss AxioObserver Microscope
 - Zeiss AxioSkop basic upright microscope
- Imaging equipment in Dr. Alex Valm's lab at University at Albany
 - Zeiss LSM 980 confocal microscope (Spectral Confocal microscope, Multiple wavelengths - 405 nm, 445 nm, 488 nm, 514 nm, 561 nm, 594 nm, 639 nm, and 730 nm)

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator				
Prefix: Dr.	First Name*: Dam	Middle Name	Last Name*: Soh	Suffix:
Position/Title*:				
Organization Name*:		State University of New York at Buffalo		
Department:		Department of Oral Biology		
Division:				
Street1*:		345 Biomedical Research Building		
Street2:				
City*:		Buffalo		
County:				
State*:		NY: New York		
Province:				
Country*:		USA: UNITED STATES		
Zip / Postal Code*:		14214-8024		
Phone Number*: 716-829-6284		Fax Number:		
E-Mail*: damsoh@buffalo.edu				
Credential, e.g., agency login: damsoh				
Project Role*: PD/PI		Other Project Role Category:		
Degree Type:		Degree Year:		
Attach Biographical Sketch*:		File Name:	1251-Biosketch_Soh_Final_editted.pdf	
Attach Current & Pending Support:		File Name:		

PROFILE - Senior/Key Person				
Prefix:	First Name*: Patricia	Middle Name	Last Name*: Diaz	Suffix: Ph.D.
Position/Title*:	Empire Innovation Professor of Oral Biology			
Organization Name*:	State University of New York at Buffalo			
Department:	Department of Oral Biology			
Division:	School of Dental Medicine			
Street1*:	347 Biomedical Research Building			
Street2:				
City*:	Buffalo			
County:	Erie			
State*:	NY: New York			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	14214-8024			
Phone Number*:	716-829-5822	Fax Number:		
E-Mail*:	pidiazmo@buffalo.edu			
Credential, e.g., agency login: diazmoreno				
Project Role*:	Other Professional	Other Project Role Category:	Mentor	
Degree Type:	Degree Year:			
Attach Biographical Sketch*:	File Name:	1252-Biosketch_Diaz_K08_final.pdf		
Attach Current & Pending Support:	File Name:	1253-NIH Other Support_Diaz_09_16_26_signed.pdf		

PROFILE - Senior/Key Person				
Prefix:	First Name*: Alex	Middle Name	Last Name*: Valm	Suffix: Ph.D.
Position/Title*:	Assistant Professor			
Organization Name*:	State University of New York at Albany			
Department:	Department of Biological Sciences			
Division:	The RNA Institute			
Street1*:	BIO 324			
Street2:	1400 Washington Avenue			
City*:	Albany			
County:	Albany			
State*:	NY: New York			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	12222-0100			
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Credential, e.g., agency login: A_Valm				
Project Role*:	Other Professional	Other Project Role Category:	Co-mentor	
Degree Type:	Degree Year:			
Attach Biographical Sketch*:	File Name:	1254-Valm-biosketch_final.pdf		
Attach Current & Pending Support:	File Name:	1255-other-support-format-page-rev-Valm.pdf		

PROFILE - Senior/Key Person				
Prefix:	First Name*: Hung	Middle Name	Last Name*: Ton-That	Suffix: Ph.D.
Position/Title*:	Professor			
Organization Name*:	University of California, Los Angeles			
Department:	Department of Dentistry			
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City*:	Los Angeles			
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State*:	CA: California			
Province:				
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Zip / Postal Code*:	90095-1668			
Phone Number*: (310) 267-5910	Fax Number:			
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Credential, e.g., agency login: tonthat				
Project Role*: Other Professional		Other Project Role Category: Co-mentor		
Degree Type:		Degree Year:		
Attach Biographical Sketch*:	File Name:	1256-Biosketch_Ton-That_2025_DS.pdf		
Attach Current & Pending Support:	File Name:	1257-Other_support_Ton-That_2025.pdf		

PROFILE - Senior/Key Person				
Prefix:	First Name*: Troy	Middle Name	Last Name*: Wood	Suffix: Ph.D.
Position/Title*:	Professor			
Organization Name*:	State University of New York at Buffalo			
Department:	Department of Chemistry			
Division:	College of Arts and Sciences			
Street1*:	417 Natural Sciences Complex			
Street2:				
City*:	Buffalo			
County:	Erie			
State*:	NY: New York			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	14260-3000			
Phone Number*: (716) 645-4144	Fax Number:			
E-Mail*: twood@buffalo.edu				
Credential, e.g., agency login: tdwood				
Project Role*: Other Professional		Other Project Role Category: Co-mentor		
Degree Type:		Degree Year:		
Attach Biographical Sketch*:	File Name:	1258-Biosketch_TWWood_Sep2025.pdf		
Attach Current & Pending Support:	File Name:	1259-OS Troy Wood_2025_Final.pdf		

BIOGRAPHICAL SKETCH

NAME: Soh, Dam

eRA COMMONS USER NAME (credential, e.g., agency login): damsoh

POSITION TITLE: PhD Candidate, Periodontology Graduate Resident

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
State University of New York at Binghamton, Binghamton, New York	BA	01/2010	Biology
Seoul National University, South Korea	MA	05/2014	Microbiology/Immunology
State University of New York at Buffalo, Buffalo, New York	DDS	05/2021	Dentistry

A. Personal Statement

I am a clinician-scientist in training, pursuing a graduate dual degree consisting of a specialization in periodontology and a PhD in oral biology with the career goal of becoming an academician and independent investigator to advance understanding of periodontal disease through translational research. My research interests focus on inter-species interactions and other ecological determinants that influence oral microbiome shifts associated with periodontal disease. Through my periodontology certificate program, I am developing comprehensive clinical expertise encompassing the full spectrum of periodontal care, from disease diagnosis to strategic treatment planning and clinical management of patients with varying degrees of periodontal disease. This clinical training provides essential insights into real-world periodontal pathology manifestations and therapeutic challenges. Additionally, these clinical skills have enabled me to participate in clinical research projects, which have resulted in one co-authored publication. I also directly perform oral examinations and sample acquisition for my own clinical research study. Concurrently, during my PhD in Oral Biology, my overarching research objective is to identify key microbial species and their functional capabilities related to environmental pH modification that influence the growth and pathogenicity of *Porphyromonas gingivalis*, a key periodontal pathogen in periodontitis progression. I have gained expertise in basic microbiology techniques, molecular biology, liquid chromatography-mass spectrometry (LC-MS), and I am currently developing proficiency in bioinformatic analysis of 16S rRNA gene amplicons and metagenomic microbiome datasets. Utilizing these skill sets, I have discovered a previously unrecognized three-species interaction among subgingival microbiome species in which inter-species metabolic activities modulate environmental pH and determine pathogen growth. I also participated on a project to investigate the effect of serum as a nutritional modifier of a subgingival model community. By integrating my clinical ability with mechanistic laboratory skills, I believe I can connect the gap between clinical periodontal practice and fundamental understanding of periodontal pathogenesis, ultimately contributing to more targeted and effective microbiome-based interventions for periodontal disease. With the support of this award, I will gain the essential experience and training necessary to accelerate my career development and achieve my goal of becoming a clinician-scientist and it will support me to obtain an academic position and establish an independent research group in the future.

B. Positions, Scientific Appointments, and Honors

Research and Professional Experiences

2021 – Present	Doctoral candidate (Dr. Patricia Diaz, Supervisor), University at Buffalo School of Dental Medicine
2015 – 2017	Research Technician (Dr. Stefan Ruhl, Supervisor) at University at Buffalo School of Dental Medicine
2012 – 2014	Graduate research under Dr. Gajin Jung in Seoul National University
2010 – 2012	Research Assistant, Severance Hospital Department of Radiology, South Korea

Awards and Honors

2025	9 th Annual Mini Symposium for Young Investigators Award <ul style="list-style-type: none"> • 2nd place for oral presentation for Post-doc category, presented at this Mini-Symposium at the 2025 AADOCR Annual Meeting in New York, NY. Symposium organized by the IADR Oral Microbiology and Immunology research group.
2025	Finger Lakes Microbiome Symposium (FiLMS) Student Award <ul style="list-style-type: none"> • 1st place for oral presentation for PhD category at the 2025 FiLMS in Rochester, NY
2025	James English Award for Advanced Education and/or M.S. Students <ul style="list-style-type: none"> • Awarded for poster presented at the 2025 Student Research Day at the University at Buffalo SDM
2024	James English Award for Advanced Education and/or M.S. Students <ul style="list-style-type: none"> • Awarded for poster presented at the 2024 Student Research Day at the University at Buffalo SDM
2021 – 2026	K12 Scholar (K12DE027827) Appointed as scholar on the University at Buffalo SDM K12 NIH Training grant
2021	AADOCR Bloc Travel Grant
2020	AADR Student Research Fellowship <ul style="list-style-type: none"> • Won a 2020 AADR Student Research Fellowship for the research proposal submitted
2020	Department of Oral Biology Award <ul style="list-style-type: none"> • Awarded for poster presented at the 2020 Student Research Day at the University at Buffalo SDM
2019	Department of Oral Biology Award <ul style="list-style-type: none"> • Awarded for poster presented at the 2019 Student Research Day at the University at Buffalo SDM
2019	AADR Student Competition for Advancing Dental Research Application (SCADA) Award <ul style="list-style-type: none"> • Selected as the representative SCADA competitor for University at Buffalo School of Dental Medicine (SDM)

C. Contributions to Science

1. Discovered that under anaerobiosis, the health-associated species *Actinomyces oris* modulates the environmental pH through lactate production to suppress the growth of *Porphyromonas gingivalis* (Pg), while the core species *Veillonella parvula* consumes acids thereby rescuing Pg. As a graduate student, I investigated the interactions within a model 6 species microbial community, discovering a 3-species interaction in which *A. oris* and *V. parvula* modulate the growth of the periodontal pathogen *Pg*. During this study, I discovered that only under anaerobic conditions mirroring the oxygen-limited subgingival environment, the health-associated early colonizer *A. oris* produces a range of organic acids, including lactate, which create an acidic environment that suppresses the growth of *P. gingivalis*. I made substantial progress in characterizing the molecular mechanisms underlying this pH-mediated inhibition by successfully identifying that lactate, produced under anaerobiosis by one of the lactate dehydrogenase genes encoded by *A. oris*, serves as the critical direct effector of *P. gingivalis* inhibition. Furthermore, I discovered that *V. parvula*, another prevalent core species in subgingival biofilms, plays a critical mediating role by consuming the organic acids produced by *A. oris* to levels sufficient for environmental neutralization. This acid consumption abrogates the inhibitory effect of the acidic environment created by *A. oris*, thereby creating conditions that permit *P. gingivalis* growth in a three-species consortium. I am currently preparing a first-author manuscript on this project for publication.

- a. **Soh D**, Parker C, Smardz M, Bhat AH, Ikonomou L, Tiede ER, Frerichs VA, Wood TD, Ton-That H, Diaz PI. Oral commensals modulate Porphyromonas gingivalis growth by niche-specific inter-species interactions. *In preparation*

2. Discovered that serum is a nutritional pressure that amplifies dysbiotic features in an oral microbiome synthetic community. During my PhD, I participated in a project investigating the effect of serum, as a surrogate for the inflammatory exudate, on a complex synthetic community model of the subgingival microbiome. In this study, we developed a 22-species synthetic community model of the subgingival microbiome maintained under continuous culture. Using this system, we interrogated the impact of serum, as a surrogate for the inflammatory exudate, on community structure and function. Through integrated 16S rRNA gene sequencing, metatranscriptomics, and metabolomics, we found that serum was not required for a community with a periodontitis-like configuration to establish, but its presence intensified features of dysbiosis. Serum increased total biomass, promoted polymicrobial aggregate formation, promoted nitrogen and protein metabolism thereby modifying the environmental pH towards alkalinity, and introduced nitrosative stress. Serum also modified the community metatranscriptome in ways that paralleled microbiome activities in human periodontitis. Serum, however, decreased community diversity by disproportionally conferring a competitive advantage to the pathogen *Porphyromonas gingivalis*. This synthetic community model revealed serum as a key nutritional pressure that modulates subgingival microbiome ecology and may perpetuate dysbiosis.

- a. Li L, Smardz M, **Soh D**, Marsh PD, Hoare A, Diaz, PI. Serum is a Nutritional Pressure that Amplifies Dysbiotic Features in an Oral Microbiome Synthetic Community. *bioRxiv*. 2025 Oct
doi: <https://doi.org/10.1101/2025.10.07.681017>. Published as a pre-print and currently under review.

3. Restorative artificial intelligence-driven implant dentistry for immediate implant placement with an interim crown: We have demonstrated for the first time how an artificial intelligence (AI)-assisted workflow can enhance the immediate implant placement with interim crowns in patients who have lost teeth in the esthetic zone due to oral disease. The AI-assisted process incorporates automated segmentation of bone and tooth structures from cone-beam computed tomography (CBCT) images, facilitating precise digital implant planning and the design of interim restorations that accurately replicate the natural tooth contour. During this study, I conducted virtual tooth extraction and implant planning via AI-assisted workflows and conducted the entire surgical procedure. This approach enables predictable implant positioning, thereby reducing surgical time and optimizing interim restoration delivery for superior clinical outcomes. This methodology is particularly valuable for achieving an optimal emergence profile, which is essential for guiding soft tissue healing and preserving gingival architecture for the definitive prosthesis. Ultimately, this AI-driven protocol provides a predictable solution for immediate implant placement in challenging esthetic cases, delivering favorable outcomes for both clinicians and patients.

- a. Marques VR, **Soh D**, Cerqueira G, Orgev A. Restorative artificial intelligence-driven implant dentistry for immediate implant placement with an interim crown: A clinical report. *J Prosthet Dent*. 2025 Aug 14:S0022-3913(25)00570-0. Epub ahead of print. PMID: 40817021.

BIOGRAPHICAL SKETCH

NAME: Diaz, Patricia I.

eRA COMMONS USER NAME: diazmoreno

POSITION TITLE: Empire Innovation Professor of Oral Biology; Director of UB Microbiome Center

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
Instituto de Ciencias de Salud CES, Colombia	DDS	12/1996	Dentistry
The University of Adelaide, Australia	PhD	08/2003	Microbiology
The University of North Carolina, Chapel Hill, NC	MS	05/2008	Periodontology
The University of North Carolina, Chapel Hill, NC	OTH	05/2008	Certificate in Periodontology

A. Personal Statement

Mentoring: During my career I have served as research mentor for dental and medical predoctoral students and Masters, PhD and specialty training graduate students. I co-direct the K12 Scholar program for dual degree clinician scientists at the University at Buffalo and co-direct the University at Buffalo-University of West Indies D43 training program to develop research capacity to study periodontitis. Five of my former trainees currently work as full-time faculty in Dental Schools. I currently serve as research advisor for 3 PhD students (Y2, Y3 and Y5) and 2 postdoctoral fellows. Under my mentorship, one of my former PhD trainees was awarded an F30 award, while a current postdoctoral fellow was awarded a K99 award. I therefore bring experience as a mentor to advice Dr. Soh and ensure the success of her training program and transition to an independent faculty position.

Research: I am a clinician scientist with training in dentistry/periodontology and molecular microbial ecology. My laboratory studies the ecology of oral microbiome communities and microbiome-host interactions in health and disease. My research group has led pioneer studies to define the ecological drivers and pathological consequences of dysbiotic shifts in the oral microbiome. We are particularly interested in defining inter-species interactions critical for microbiome community maturation and the outgrowth of pathobionts. Other areas of interest in my lab are defining the role of the oral microbiome as a modifier of mucosal responses to injury and the study of the relationship of the oral and gut microbiome and its effect on systemic disease. My laboratory utilizes an integrative approach closely coupling clinical studies, animal models, high throughput assays and computational methods.

Ongoing and recently completed projects that I would like to highlight include:

08/04/2022-08/03/2027

1R01DE032131-01, NIH/NIDCR

Diaz, Patricia (PI) and Schlecht, Nicolas (PI)

Host and microbial risk factors of oral thrush in cancer patients receiving chemotherapy

Role: PI

08/12/2022-08/11/2027

1R01DE032242-01

Burke, Robert (PI); Diaz, Patricia (MPI); Schlecht, Nicolas (MPI)

Impact of HIV, oral microbiome and mycobiome on oral HPV persistence

Role: MPI

04/01/25-03/31/27

R21DE034093-01A1; NIH/NIDCR

Diaz PI (PI)

Title: "Identification of diffusible small molecules that regulate replication of *Porphyromonas gingivalis*"

Role: PI

07/01/2020-06/30/2025

R01DE029034; NIH/NIDCR

Sfeir C (PI)

Title: "Treatment of periodontitis by homing M2 macrophages"

Role: Co-investigator

07/01/2022-06/30/2027

U01 DE031223-01A1; NIH,NIDCR

Shaddox (PI)

Title: "Susceptibility Patterns for Grade C Periodontitis in Young Individuals"

Role: Co-investigator

06/01/22-05/31/26

F30 GM146451; NIH/NIGMS

June (PI)

Title: "Oral to Gut Microbiome Transmission in Periodontitis and Type 2 Diabetes"

Role: Sponsor

08/01/25-07/31/30

K99 DE034829; NIH/NIDCR

Li (PI)

Title: "Using Novel Machine Learning Approaches to Understand Subgingival Microbiome Heterogeneity in Relation to Health and Disease"

Role: Sponsor

Citations:

1. Hoare A, Wang H, Meethil A, Abusleme L, Hong B-Y, Moutsopoulos NM, Marsh PD, Hajishengallis G, **Diaz PI**. A cross-species interaction with a symbiotic commensal enables cell-density-dependent growth and in vivo virulence of an oral pathogen. 2021. ISME J. 15(5):1490-1504. PMCID: PMC8115154.
2. Li L, Sohn J, Genco RJ, Wactawski-Wende J, Goodison S, **Diaz PI**^c, Sun Y^c. Computational approach to modeling microbiome landscapes associated with chronic human disease progression. PLoS Computational Biology. 2022. 18,8,e1010373.
3. Li L, Hayashi-Okada Y, Falkner KL, Cervi S, Andrusz S, Shimizu Y, Zambon JJ, Kirkwood KL, Schifferle RE, **Diaz PI**. Randomized Trial to Test a Chemo-Mechanical Antiplate Regimen as Adjunct to Periodontal Therapy. JDR Clin Trans Res. 2023 May 6:23800844231167065. doi: 10.1177/23800844231167065. PMID: 37148266.
4. Kim TS, Ikeuchi T, Theofilou VI, Williams DW, Greenwell-Wild T, June A, Adade EE, Li L, Abusleme L, Dutzan N, Yuan Y, Brechley L, Bouladoux N, Sakamachi Y; NIDCD/NIDCR Genomics and Computational Biology Core; Palmer RJ Jr, Iglesias-Bartolome R, Trinchieri G, Garantziotis S, Belkaid Y, Valm AM, **Diaz PI**, Holland SM, Moutsopoulos NM. Epithelial-derived interleukin-23 promotes oral mucosal immunopathology. Immunity. 2024 Apr 9;57(4):859-875.e11. PMID: 38513665; PMCID: PMC11058479.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2020 -	Professor of Empire Innovation, Department of Oral Biology, School of Dental Medicine, University at Buffalo, State University of New York, Buffalo, NY
2020-	Director, UB Microbiome Center, University at Buffalo, State University of New York, Buffalo, NY
2015 - 2020	Associate Professor, The University of Connecticut Health Center, School of Dental Medicine, Farmington, CT
2008 - 2014	Assistant Professor, The University of Connecticut Health Center, School of Dental Medicine, Farmington, CT

Scientific Appointments

2024 - 2026	President WNY branch of American Society of Microbiology
2023 -	Associate Editor, Journal of Periodontology
2022 -	President elect WNY branch of American Society of Microbiology
2021 -	Editorial Board, Journal of Clinical Periodontology
2019 -	Editorial Board, Journal of Dental Research
2017 -	Editorial Board, Molecular Oral Microbiology
2016 -	Member Task Force on Design and Analysis in Oral Health Research
2014 - 2020	Member Research Committee, American Academy of Periodontology
2011-	American Academy of Periodontology Board Certification

Honors

2025	IADR Distinguished Scientist Award in Research in Oral Biology
2024	Fellow of Cohort 7 of the 2024 Hispanic Leadership Institute, SUNY
2023 -	Sunstar Robert J. Genco Endowed Chair in Oral Biology
2018	Sunstar World Periodontal Research Award. Awarded to the paper "Clinical, Immune, and Microbiome Traits of Gingivitis and Peri-implant Mucositis. 2017. J Dent Res. 96(1): 47-55".
2013	Educator Award, American Academy of Periodontology
2012	Bud and Linda Tarrson Fellowship, American Academy of Periodontology
2006	Educator Scholarship, American Academy of Periodontology
2001	Unilever Travel Award, International Association of Dental Research

C. Contributions to Science

1. Defined dysbiotic shifts of the oral microbiome in periodontal disease: My laboratory was one of the first to develop and validate protocols and pipelines for oral microbiome characterization using high throughput sequencing. We defined the subgingival microbiome shifts associated with periodontitis, gingivitis and peri-implant mucositis. (Abusleme et al. ISME J 2013; Hong et al. PLoS 2015; Schincaglia et al. J Dent Res 2016). Our studies have provided a framework to understand periodontal dysbiosis. We showed distinct shifts occur in gingivitis and periodontitis and that shifts are due to an ecological process of microbial species succession without replacement. Our studies have also defined the specific species associated with health, gingivitis and periodontitis, and highlighted the existence of a group of prevalent core species, which do not change in terms of their proportions as shifts occur. We have hypothesized core species are essential metabolic anchors of subgingival communities, and have begun to test their role in model systems (see below). Our collaborative studies have also provided mechanistic evidence that oral dysbiosis plays a causative role in the pathophysiology of periodontitis (Dutzan et al. Sci Transl Med. 2018).

- a. Abusleme L, Dupuy AK, Dutzan N, Silva N, Burleson JA, Strausbaugh LD, Gamonal J, **Diaz PI**. The subgingival microbiome in health and periodontitis and its relationship with community biomass and inflammation. 2013. ISME J. 7(5):1016-25. PMID: PMC3635234.

- b. Hong BY, Araujo MVF, Strausbaugh LD, Terzi E, Ioannidou E, **Diaz PI**. Microbiome profiles in periodontitis in relation to host and disease characteristics. 2015. PLoS One. 10(5):e0127077. PMID: PMC4436126.
- c. Schincaglia GP, Hong BY, Rosania A, Barasz J, Thompson A, Sobue T, Panagakos F, Burleson JA, Dongari-Bagtzoglou A, **Diaz PI**. Clinical, Immune, and Microbiome Traits of Gingivitis and Peri-implant Mucositis. 2017. J Dent Res. 96(1): 47-55.
- d. Dutzan N, Kajikawa T, Abusleme L, Greenwell-Wild T, Zuazo CE, Ikeuchi T, Brenchley L, Abe T, Hurabielle C, Martin D, Morell RJ, Freeman AF, Lazarevic V, Trinchieri G, **Diaz PI**, Holland SM, Belkaid Y, Hajishengallis G, Moutsopoulos NM. A dysbiotic microbiome triggers T(H)17 cells to mediate oral mucosal immunopathology in mice and humans. 2018. Sci Transl Med. 10(463):eaat0797. PMID: PMC6330016.

2. Established that cell density is critical for the colonization of *Porphyromonas gingivalis* and defined inter-species interactions that promote the growth and in vivo virulence of this pathogen: A goal of my research program has been to define the key inter-species interactions in the oral microbiome that promote the outgrowth of pathogens. Our work with the periodontitis-associated anaerobe *P. gingivalis* has shown that although its ability to colonize and cause disease is limited when in low cell-density, its partnership with the commensal *Veillonella parvula* allows it to establish from a small inoculum (Hoare et al. 2021). This effect is mediated by a small diffusible molecule, the identity of which is currently being investigated. In addition, previous work revealed that the ubiquitous species *Fusobacterium nucleatum* is able to support the growth of *P. gingivalis* by protecting it from oxidative damage and supplying carbon dioxide (Diaz et al. Microbiol. 2002). Both *V. parvula* and *F. nucleatum* are core subgingival species, with these studies supporting the hypothesis that core species are critical for subgingival community maturation.

- a. Hoare A, Wang H, Meethil A, Abusleme L, Hong B-Y, Moutsopoulos NM, Marsh PD, Hajishengallis G, **Diaz PI**. A cross-species interaction with a symbiotic commensal enables cell-density-dependent growth and in vivo virulence of an oral pathogen. 2021. ISME J. 15(5):1490-1504. PMID: PMC8115154.
- b. **Diaz PI**, Zilm PS, Rogers AH. *Fusobacterium nucleatum* supports the growth of *Porphyromonas gingivalis* in oxygenated and carbon-dioxide-depleted environments. 2002. Microbiology. 148(Pt 2):467-72.
- c. **Diaz PI**, Valm AM. Microbial Interactions in Oral Communities Mediate Emergent Biofilm Properties. 2020. J Dent Res. 99(1):18-25. PMID: PMC6927214.

3. Defined drivers of susceptibility to oral comorbidities of cancer chemotherapy: One of our interests has been to define the pathophysiology of oral toxicities during cancer treatment. The factors that underline susceptibility to develop side effects of chemotherapy and radiation therapy are not completely understood and no effective treatments exist to prevent these complications. Through clinical studies we have shown that chemotherapy induces oral bacteriome dysbiosis, independent of concomitant antibiotic intake, and that these dysbiotic shifts may contribute to increase the severity of oral mucositis (Hong et al. Microbiome 2019). In current studies, we are using a mouse model of oral mucositis to define the cellular events associated with lesion development and the potential for the microbiome to affect lesion severity. Our studies have also focused on establishing predisposing factors to oral candidiasis in cancer patients. Our work shows that certain bacteria act as risk factors for oral candidiasis (Diaz et al. J Fungi 2019). Mechanistic experiments in my lab and collaborative studies with the Dongari-Bagtzoglou lab support the concept that bacteria may play a role in oral candidiasis through inter-kingdom interactions that affect *Candida albicans* virulence.

- a. **Diaz PI**, Xie Z, Sobue T, Thompson A, Biyikoglu B, Ricker A, Ikonou L, Dongari-Bagtzoglou A. Synergistic interaction between *Candida albicans* and commensal oral streptococci in a novel in vitro mucosal model. 2012. Infect Immun. 80(2):620-32. PMID: PMC3264323.
- b. Bertolini M, Ranjan A, Thompson, A, **Diaz, PI**, Sobue T, Maas K, Dongari-Bagtzoglou A. *Candida albicans* induces mucosal bacterial dysbiosis that promotes invasive infection. 2019. PLoS Pathog. 15(4): e1007717. PMID: PMC6497318.
- c. Hong BY, Sobue T, Choquette LC, Dupuy AK, Thompson A, Burleson JA, Salner A, Schauer PK, Joshi P, Fox E, Shin D-G, Weinstock GM, Strausbaugh LD, Dongari-Bagtzoglou A, Peterson DE, **Diaz PI**.

Chemotherapy-induced oral mucositis is associated with detrimental bacterial dysbiosis. 2019. Microbiome. 7(1):66.

- d. **Diaz PI**^c, Hong BY, Dupuy AK, Choquette L, Thompson A, Salner AL, Schauer PK, Hegde U, Burleson JA, Strausbaugh LD, Peterson DE, Dongari-Bagtzoglou A^c. Integrated Analysis of Clinical and Microbiome Risk Factors Associated with the Development of Oral Candidiasis during Cancer Chemotherapy. 2019. J Fungi (Basel). 5 (2). pii: E49. doi: 10.3390/jof5020049.

4. Pioneered studies on the oral mycobiome and discovered the existence of two oral mycotypes: Our lab optimized methods for oral mycobiome characterization. We were the first to report that by using adequate lysis protocols, *Malassezia* are a genus frequently detected in the oral cavity (Dupuy et al 2014). We later published an analysis of the salivary mycobiome in a large cohort, showing that two fungal community types exist, each dominated by either *Malassezia* or *Candida*, and defined the clinical and ecological associations of these communities (Hong et al 2020).

- a. Dupuy AK, David MS, Li L, Heider TN, Peterson JD, Montano EA, Dongari-Bagtzoglou A, **Diaz PI**, Strausbaugh LD. Redefining the human oral mycobiome with improved practices in amplicon-based taxonomy: discovery of *Malassezia* as a prominent commensal. 2014. PLoS One. 9(3):e90899. PMCID: PMC3948697.
- b. Abusleme L, **Diaz PI**, Freeman AF, Greenwell-Wild T, Brenchley L, Desai JV, Ng WI, Holland SM, Lionakis MS, Segre JA, Kong HH, Moutsopoulos NM. Human defects in STAT3 promote oral mucosal fungal and bacterial dysbiosis. 2018. JCI Insight. 3(17):e122061. PMCID: PMC617814.
- c. **Diaz PI**, Hong BY, Dupuy AK, Choquette L, Thompson A, Salner AL, Schauer PK, Hegde U, Burleson JA, Strausbaugh LD, Peterson DE, Dongari-Bagtzoglou A. Integrated Analysis of Clinical and Microbiome Risk Factors Associated with the Development of Oral Candidiasis during Cancer Chemotherapy. 2019. J Fungi (Basel). 5(2):49. PMCID: PMC6617088.
- d. Hong BY, Hoare A, Cardenas A, Dupuy AK, Choquette L, Salner AL, Schauer PK, Hegde U, Peterson DE, Dongari-Bagtzoglou A, Strausbaugh LD, **Diaz PI**. The Salivary Mycobiome Contains 2 Ecologically Distinct Mycotypes. 2020. J Dent Res. 99(6):730-738. PMCID: PMC7243416.

Complete List of Published Work in MyBibliography:

<https://www.ncbi.nlm.nih.gov/myncbi/patricia.diaz.1/bibliography/public/>

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Alex M. Valm

eRA COMMONS USER NAME (credential, e.g., agency login): A_Valm

POSITION TITLE: Associate Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Bradley University, Peoria, IL	B.S.	05/1996	Biology
University of North Carolina, Chapel Hill, NC	M.S.	12/2001	Cell Biology
Analytical and Quantitative Light Microscopy Course; MBL, Woods Hole, MA	Certificate	05/2007	Quantitative Microscopy
Brown University, Providence, RI	Ph.D.	05/2012	Pathobiology
NIH: NICHD, Bethesda, MD	PostDoc	08/2017	Cell Biology and Microbiome

A. Personal Statement

I am Associate Professor of biology at The State University of New York at Albany. I have broad training in microbiology, biophysics and quantitative imaging. Relevant to the training and mentoring K08 proposal here, I have expertise in conceptualizing and realizing technologies to transform the capacity for multispectral fluorescence imaging of the oral microbiome. With my NIH F-31 supported PhD work in the lab of Dr. Gary Borisy at the MBL, I developed CLASI-FISH for Combinatorial Labeling and Spectral Imaging FISH, which allowed the first labeling and imaging of 15 different taxa of microbes in a single image. As a PI at SUNY Albany I have established a robust research program to map the systems level biogeography of the dental plaque microbiome in animal and in vitro models, as well as human tissues. Our aims are to understand the community level structural features that contribute to health and disease. I direct a robust laboratory with 12 trainees including 3 postdocs, 4 PhD and Master's students, and undergraduates. I have graduated several PhD students in the MCDN program at SUNY Albany and currently train 3 postdocs. With 15+ years in the field and as a pioneer of combinatorial 16S FISH labeling of microbiomes, I am well suited to serve as Co-Mentor on this K08 proposal to identify the role those metabolic activities, especially pH modulation, by local microbiome members plays in establishing the biofilm niche for *Porphyromonas gingivalis*, in model biofilms using multi-omics and spectral imaging.

Ongoing projects that I would like to highlight include:

R01 DE030927

Alex Valm (PI)

07/06/2021-06/30/2026

Oral microbial community structure and assembly: from molecule to microbiome

R01 DE031213

Alex Valm (PI)

08/01/2022-7/31/2027

Biofilm Spatial Structure in the Transition from Health to Periodontal Disease

Citations:

- a. Wang R, Feng Y, **Valm AM**. A Framework of Multi-View Machine Learning for Biological Spectral Unmixing of Fluorophores with Overlapping Excitation and Emission Spectra. *Briefings in Bioinformatics* 2024 Nov 22;26(1):bbaf005. doi: 10.1093/bib/bbaf005 PMID: PMC11726699
- b. Wang R, Lemus AA, Henneberry CM, Ying Y, Feng Y, and **Valm AM**. Unmixing Biological Fluorescence Image Data with Sparse and Low-Rank Poisson Regression. *Bioinformatics* Apr. 2023 39(4):btad159. doi: 10.1093/bioinformatics/btad159 PMC10081874
- c. Diaz PI, **Valm AM**. Microbial Interactions in Oral Communities Mediate Emergent Biofilm Properties. *J Dent Res*. 2020 Jan;99(1):18-25. PubMed Central PMID: PMC6927214.
- d. **A.M. Valm**, S. Cohen, W. Legant, J. Melunis, U. Hershberg, E. Wait, A.R. Cohen, M. Davidson, E. Betzig, J. Lippincott-Schwartz. 2017. Applying systems-level spectral imaging and analysis to reveal the organelle interactome. *Nature* 546:162-167. doi: 10.1038/nature22369. PMC5536967

B. Positions, Scientific Appointments, and Honors**Positions of Employment**

2025-	Associate Professor, Department of Biology	University at Albany, SUNY, Albany, NY
2025-	Coordinator of Imaging and AI in Biology	University at Albany, SUNY, Albany, NY
2019-	Associate Faculty Member, RNA Institute	University at Albany, SUNY, Albany, NY
2017-2025	Assistant Professor, Department of Biology	University at Albany, SUNY, Albany, NY
2017	Course Facilitator, Physiology Course	MBL, Woods Hole, MA
2016	Teaching Assistant, Optical Microscopy Course	MBL, Woods Hole, MA

Other Positions: Grant Reviewerships

2025	NIH: Cellular Molecular Technologies (CMT) Reviewer, <i>ad hoc</i>
2024	NIH: Macromolecular Biophysics & Biological Chemistry Review Branch (MBBC) ZRG1 MBBC G (50) Collaborative Program Grant for Multidisciplinary Teams (RM1) Mail Reviewer, <i>ad hoc</i>
2023	NIH: Office of the Director, ZRG1 CDB-J (30) I S10 Shared Instrumentation Program Reviewer, <i>ad hoc</i>
2023	NIH: ODCS Study Section (Oral, Dental and Craniofacial Science), Mail Reviewer, <i>ad hoc</i>
2022	NIH: Office of the Director, ZRG1CBJ30 S10 Shared Instrumentation Program Reviewer, <i>ad hoc</i>
2022	U.K. Biotechnology and Biological Sciences Research Council (BBSRC), <i>ad hoc</i>
2022	NIH: GVE Study Section (Genomic Variation and Evolution), Center for Scientific Review, <i>ad hoc</i>
2020	NIH: ODCS Study Section (Oral, Dental and Craniofacial Science), <i>ad hoc</i>
2020	NSF: Division of Integrative Organismal Systems (IOS), <i>ad hoc</i>
2020	Swiss National Science Foundation, Division of Biology and Medicine, <i>ad hoc</i>

Other Positions: Journal Reviewerships

2020-	<i>Microorganisms</i> (Review Board Member)
2016-	<i>Ad hoc</i> reviewer for 28+ scientific journals

Memberships:

2006-	Biophysical Society
2006-	New England Society for Microscopy
2007-	American Society of Microbiology
2017-	International Association for Dental Research
2022-	Microscopy Society of America

Awards and Honors

1994	Phi Eta Sigma
1996	BS with Honors, Bradley University
2008-2011	Ruth L. Kirschstein Predoctoral National Research Service Award (NIH F31)
2011	Best Abstract Award, Meeting of the Royal Microscopical Society, Belfast, UK.
2011	ASCB Pre-doctoral Travel Award.
2011	ASCB New and Noteworthy Abstract Award.
2013-2016	Intramural NIH PRAT Postdoctoral Fellowship.

2017	Honorable Mention, Nikon Small World in Motion Contest
2020	3M Non-tenured Faculty Award
2023	SUNY Albany Excellence in Research Award

C. Contributions to Science

1. Development of transformative tools for multispectral biological imaging.

Throughout my training and career, I have dedicated myself to the development of new technologies for systems level biological imaging. During my PhD work, I developed Combinatorial Labeling and Spectral Imaging (CLASI)-FISH, a new labeling and image technique which allowed the discrimination of 28 different strains of bacteria. During my PhD, I received advanced training in biological imaging at the Analytical and Quantitative Light Microscopy Course at MBL in 2007 and training in scientific computing by attending the Advanced Mathematica Summer School programming workshop in 2008. This workshop was a turning point in my career—here I learned the language of scientific computing and how to communicate with computer scientists by way of developing novel linear unmixing algorithms that use a priori information about our labeled samples to improve the unmixing result. We applied this algorithm to identify 120 differently labeled bacteria in a single specimen. During my postdoc, I assembled a highly collaborative team of engineers, physicists and biologists in the lab of my mentor, Dr. Jennifer Lippincott Schwartz and our collaborator Dr. Eric Betzig. I was able to bring physicists and biologists together to achieve an extraordinary goal of full 3-D time lapse imaging of 6 organelles in live cells with multispectral lattice light sheet imaging. With this body of work, I have carved a comfortable niche at the interface of biology and imaging. Since joining the faculty at University at Albany, I have developed a robust collaboration with mathematicians and experts in machine learning including Dr. Yunlong Feng, MPI on this proposal. Together we co-mentor a postdoctoral scientist, Dr. Ruogu Wang who is a PhD Mathematician in my laboratory. Our work aims to transform the ability to distinguish hundreds of fluorophores in biological fluorescence images using cutting edge machine learning.

- a. Wang R, Feng Y, **Valm AM**. A Framework of Multi-View Machine Learning for Biological Spectral Unmixing of Fluorophores with Overlapping Excitation and Emission Spectra. *Briefings in Bioinformatics* 2024 Nov 22;26(1):bbaf005. doi: 10.1093/bib/bbaf005 PMID: PMC11726699
- b. Wang R, Lemus AA, Henneberry CM, Ying Y, Feng Y, and **Valm AM**. Unmixing Biological Fluorescence Image Data with Sparse and Low-Rank Poisson Regression. *Bioinformatics* Apr. 2023 39(4):btad159. doi: 10.1093/bioinformatics/btad159 PMID10081874
- c. **Valm, AM** & Feng, Y, inventors; Research Foundation for the State University of New York, assignee. Unmixing image data. United State patent pending US20240273678A1. 2024 Aug 15
- d. **Valm, AM**; Feng, Y; Wang, R, inventors; Research Foundation for the State University of New York, assignee. Multi-view machine learning for biological spectral unmixing of fluorophores with overlapping emission and excitation spectra. United State provisional patent 63/647,671. 2024 Apr 17

2. Systems level imaging and spatial biology of oral microbial communities.

Throughout my career, I have desired to study the systems level spatial structure of the human microbiome. Molecular sequencing approaches have revolutionized the study of the human microbiome and provide exhaustive information on the functional and taxonomic compositions of human associated microbial communities but provide little information on the spatial arrangement of organisms on the micron scale. As a graduate student, I developed a novel imaging technology that I called CLASI for Combinatorial Labeling and Spectral Imaging and combined this with fluorescence in situ hybridization. This technology overcame limitations in fluorescence microscopy to greatly expand the number of distinguishable different types of bacteria in a specimen. I used the newly created Human Oral Microbiome Database (HOMD) and bioinformatics tools to identify or design taxon-specific probes for 15 different genera and families of microbes in the oral microbiome. I designed a human subjects protocol to recruit volunteers to donate dental plaque samples, then I applied the CLASI-FISH probes and imaging approach to map the spatial distribution of 15 different taxa of oral microbes in semi-dispersed dental plaque. This work has laid the foundation for the first CLASI-FISH imaging of intact dental plaque. Most recently I have been collaborating with researchers in the Division of Intramural Research at NIH

to map the spatial distribution of early colonizing bacteria on a removable enamel chip worn by healthy volunteers. With antibody and FISH labeling, we discovered a repeatable yet previously undescribed microbial structure with cells of the genus *Rothia* centrally located in spherical clusters of cells of the genera *Streptococcus* and *Haemophilus*.

- a. Krishnamoorthy AL, Lemus AA, Solomon AP, **Valm AM**, Neelakantan P. Interactions between *Candida albicans* and *Enterococcus faecalis* in an Organotypic Oral Epithelial Model. Microorganisms. 2020 Nov 11;8(11) PubMed Central PMCID: PMC7696566.
- b. Palmer RJ Jr, Shah N, **Valm A**, Paster B, Dewhirst F, Inui T, Cisar JO. Interbacterial Adhesion Networks within Early Oral Biofilms of Single Human Hosts. Appl Environ Microbiol. 2017 Jun 1;83(11) PubMed Central PMCID: PMC5440702.
- c. **Valm AM**, Mark Welch JL, Borisy GG. CLASI-FISH: principles of combinatorial labeling and spectral imaging. Syst Appl Microbiol. 2012 Dec;35(8):496-502. PubMed Central PMCID: PMC3407316.
- d. **Valm AM**, Mark Welch JL, Rieken CW, Hasegawa Y, Sogin ML, Oldenbourg R, Dewhirst FE, Borisy GG. Systems-level analysis of microbial community organization through combinatorial labeling and spectral imaging. Proc Natl Acad Sci U S A. 2011 Mar 8;108(10):4152-7. PubMed Central PMCID: PMC3054005.

2. Spectral imaging and analysis to reveal the dynamic organelle interactome.

Like the genetically distinct cells in a microbial biofilm that individually perform specific metabolic functions, the different membrane bound compartments within a single eukaryotic cell, the organelles, perform specialized biochemical functions. However, these different functions must be coordinated for the cell to function as a system. Organelle contact sites are recognized as important, dynamic structures within cells where inter-organelle communication occurs, mainly in the form of lipid and Ca⁺⁺ exchange. With this postdoctoral project, I led a team of highly interdisciplinary scientists made up of cell biologists, engineers and physicists, to map all of the organelle contacts between 6 different organelles in eukaryotic cells. I developed an imaging informatics pipeline to map the distribution of contacts revealed by spectral imaging of cells labeled with 6 different fluorescent proteins conjugated to genetically encoded organelle markers for lysosomes, peroxisomes, mitochondria, the ER, the Golgi and lipid droplets. I further adapted a novel lattice light-sheet microscope for spectral imaging and designed an excitation-side linear unmixing algorithm. Our imaging approach revealed for the first time the ubiquity of organelle-organelle contacts within cells, especially those involving the ER. Further, we discovered that although individual organelles and their contacts with others are highly dynamic, the global frequency of organelle contacts remains remarkably constant over long timescales. Going forward, the computational tools that I developed for excitation-side linear unmixing are directly adaptable for the development of novel algorithms and in silico microscope models proposed here.

- a. Cohen S, **Valm AM**, Lippincott-Schwartz J. Interacting organelles. Curr Opin Cell Biol. 2018 Aug;53:84-91. PubMed Central PMCID: PMC6241252.
- b. Cohen S, **Valm AM**, Lippincott-Schwartz J. Multispectral Live-Cell Imaging. Curr Protoc Cell Biol. 2018 Jun;79(1):e46. PubMed Central PMCID: PMC6283277.
- c. Stefan CJ, Trimble WS, Grinstein S, Drin G, Reinisch K, De Camilli P, Cohen S, **Valm AM**, Lippincott-Schwartz J, Levine TP, Iaea DB, Maxfield FR, Futter CE, Eden ER, Judith D, van Vliet AR, Agostinis P, Tooze SA, Sugiura A, McBride HM. Membrane dynamics and organelle biogenesis-lipid pipelines and vesicular carriers. BMC Biol. 2017 Oct 31;15(1):102. PubMed Central PMCID: PMC5663033.
- d. **Valm AM**, Cohen S, Legant WR, Melunis J, Hersberg U, Wait E, Cohen AR, Davidson MW, Betzig E, Lippincott-Schwartz J. Applying systems-level spectral imaging and analysis to reveal the organelle interactome. Nature. 2017 Jun 1;546(7656):162-167. PubMed Central PMCID: PMC5536967.

3. Dysbiotic microbiome communities induce intra- and intercellular host tissue invasion and pathology.

The tumor microenvironment has been demonstrated to harbor a specific microbiome, especially in the case of colorectal carcinomas. Microbes have been localized both inter- and intracellularly in tumor and immune cells and the microbial community composition varies with tumor type. Here we asked the question whether cigarette smoking and somatic mutations generate a dysbiotic microbiota associated with lung carcinogenesis. We found

lower alpha diversity in normal lung tissue than in tumor or tumor-adjacent tissue and a significant association between *Acidovorax* sp. and carcinoma cases with TP53 mutations. Sequencing results were corroborated with FISH imaging of *Acidovorax* probes. In a separate line of work, we identified a synergistic, community invasive phenotype when *C. albicans* and *Enterococcus faecalis* were incubated together in an organotypic human oral mucosa epithelial tissue model. With FISH and combined immunofluorescence, we identified increased tissue disruption and invasion of *E. faecalis* when biofilms were formed in the presence of *C. albicans*.

- a. Krishnamoorthy AL, Lemus AA, Solomon AP, **Valm AM**, Neelakantan P. Interactions between *Candida albicans* and *Enterococcus faecalis* in an Organotypic Oral Epithelial Model. Microorganisms. 2020 Nov 11;8(11) PubMed Central PMCID: PMC7696566.
- b. Greathouse KL, White JR, Vargas AJ, Bliskovsky VV, Beck JA, von Muhlinen N, Polley EC, Bowman ED, Khan MA, Robles AI, Cooks T, Ryan BM, Padgett N, Dzutsev AH, Trinchieri G, Pineda MA, Bilke S, Meltzer PS, Hokenstad AN, Stickrod TM, Walther-Antonio MR, Earl JP, Mell JC, Krol JE, Balashov SV, Bhat AS, Ehrlich GD, **Valm A**, Deming C, Conlan S, Oh J, Segre JA, Harris CC. Interaction between the microbiome and TP53 in human lung cancer. Genome Biol. 2018 Aug 24;19(1):123. PubMed Central PMCID: PMC6109311.

4. Spatial structure mediates health and disease in the human oral microbiome.

With my early work developing CLASI-FISH, I received training in microbial infection and pathobiology. Neither my PhD nor postdoc training was performed in an oral microbiology setting. To position myself at the forefront of dental and oral microbiology, I have established collaborations with dental clinician researchers and oral microbiologists. Together we have investigated the spatial architecture of oral communities in states of health and disease. I have further collaborated with these experts in the field to publish timely review articles that summarize our understanding of the role of biofilm spatial structure in mediating the transition from health to disease in the oral cavity. Through systematic review of the recent literature, my expert coauthors and I have identified an emerging paradigm in oral diseases mediated by dysbiotic microbial communities, namely that the physical structures of biofilms contribute to emergent properties that promote health and disease. These literature reviews comprise a significant body of work for me as I establish myself as an engaged member of the human oral microbiology scientific community.

- a. Borisy GG, **Valm AM**. Spatial scale in analysis of the dental plaque microbiome. Periodontol 2000. 2021 Mar 10; PubMed PMID: 33690940.
- b. Diaz PI, **Valm AM**. Microbial Interactions in Oral Communities Mediate Emergent Biofilm Properties. J Dent Res. 2020 Jan;99(1):18-25. PubMed Central PMCID: PMC6927214.
- c. **Valm AM**. The Structure of Dental Plaque Microbial Communities in the Transition from Health to Dental Caries and Periodontal Disease. J Mol Biol. 2019 Jul 26;431(16):2957-2969. PubMed Central PMCID: PMC6646062.
- d. Palmer RJ Jr, Shah N, **Valm A**, Paster B, Dewhirst F, Inui T, Cisar JO. Interbacterial Adhesion Networks within Early Oral Biofilms of Single Human Hosts. Appl Environ Microbiol. 2017 Jun 1;83(11) PubMed Central PMCID: PMC5440702.

Complete List of Published Work in MyBibliography:

<https://www.ncbi.nlm.nih.gov/sites/myncbi/1NWn7vcykptkn/bibliography/53423586/public/?sort=date&direction=ascending>

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: **Ton-That, Hung**

eRA COMMONS USER NAME (credential, e.g., agency login): tonthat

POSITION TITLE: Professor and Chair of Oral and Systemic Health Sciences

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of California, Los Angeles	B.S.	06/1996	Chemistry
University of California, Los Angeles	Ph.D.	09/2000	Microbiology
University of California, Los Angeles	Postdoc	12/2000	Microbiology
University of Chicago, IL	Postdoc	06/2003	Microbiology

A. Personal Statement

I am a well-trained investigator with a broad range of expertise, including bacterial genetics, molecular biology, biochemistry, and bacterial pathogenesis. My laboratory employs a multidisciplinary approach combining classical and modern techniques that include bacterial genetics, various biochemical methods, electron microscopy, electron cryo-tomography, X-ray crystallography, biophysics, mass spectrometry, cell-based assays, and rodent models of infection. I have significantly contributed to the discovery of *Staphylococcus aureus* sortase – a conserved transpeptidase enzyme that catalyzes cell wall anchoring of virulence factors – and subsequently, the elucidation of the sortase-mediated cell wall anchoring pathway in Gram-positive bacteria. Early work as a postdoc and later work in my laboratory have pioneered the field of sortase-mediated pilus assembly in Gram-positive bacteria. We made key discoveries elucidating the assembly mechanisms of Gram-positive pili, a major virulence factor involved in adhesion, biofilm formation and bacterial pathogenesis, establishing the current model of biphasic pilus assembly in Gram-positive bacteria. Furthermore, we defined the major pathway of MdbA-mediated post-translocational protein folding in Gram-positive actinobacteria, including oral colonizers *Actinomyces oris*, *Corynebacterium diphtheriae*, and *Corynebacterium matruchotii*. One of the laboratory's strengths is our development of genetic tools for bacterial research; for example, we have developed allelic exchange and Tn5 transposon systems for *C. diphtheriae* and *A. oris*. Later, we also developed a genetic toolbox for *Fusobacterium nucleatum*, an oral pathobiont that has been linked to promotion of periodontitis, preterm birth, and colorectal cancer. With *F. nucleatum* as a subject of this renewal grant application, our long-term goal is to understand the pathophysiology of *F. nucleatum* by elucidating molecular mechanisms of virulence, aiming to identify key players of fusobacterial virulence that may be attractive targets for future development of effective therapies that combat oral and extraoral diseases promoted by this pathobiont.

Thus, all trainees in my laboratory have ample opportunity to learn advanced technology and to learn how to tackle problems with multidisciplinary approaches. I have mentored more than 33 pre-doctoral and postdoctoral trainees (currently mentoring 5 graduate students and 1 postdoctoral fellow), many of whom have gone on to obtain postdoctoral positions in excellent institutions, as well as pursuing an independent career in academia and industry. In addition, I have also mentored more than 22 undergraduate trainees (currently mentoring 3 undergraduates), many of them continuing on graduate studies. My pre-doctoral trainees were well trained and highly productive; several of them graduated with distinction, including Outstanding PhD Thesis Awards and Dean's and President's research scholarships. Many of trainees also received NIH fellowships including the Ruth L. Kirschstein National Research Service Awards. Furthermore, I have helped

reorganizing the research training programs at the UCLA School of Dentistry, serving as Director of the NIH-funded research training program for predoctoral and postdoctoral trainees (domestic and foreign) to conduct basic, translational, and clinical research with a dental, oral, and craniofacial focus. This training program provides 5 training tracks, including Dentist-Scientist Trainee Program (DSTP), Dentist-PhD Program (D-PhD), Dentist-Scientists Postdoctoral Fellow (DSPF), Predoctoral PhD Trainee (PhD), and Oral Health Postdoctoral Fellow (OHPF).

In summary, with my track record of leadership, mentoring, research productivity, and funding, I am qualified to serve as a mentor for Dr. Dam Soh.

Ongoing projects that I would like to highlight include:

T90-DE030860, (Ton-That and Wong, MPI)

NIH/NIDCR; 07/01/2021 – 06/30/2026

UCLA Dentist-Scientist and Oral Health-Researcher Training Program

R01DE033900, Ton-That (PI)

NIH/NIDCR; 05/01/2023– 04/30/2029

Assembly and function of outer membrane tubules in *Fusobacterium nucleatum*

R01-R01DE017382, Ton-That (PI)

NIH/NIDCR; 02/19/2008– 08/31/2029

Molecular Assembly on the Cell Surface of Actinomyces

Relevant Citations: Below are publications by my former and current trainees (underlined).

- 1) Wu C, Chen YW, Scheible M, Chang C, Wittchen M, Lee JH, Luong TT, Tiner BL, Tauch A, Das A, and Ton-That H (2021). Genetic and molecular determinants of polymicrobial interactions in *Fusobacterium nucleatum*. **Proc Natl Acad Sci U S A**, 118(23):e2006482118. PMC8201914
- 2) Chen YW, Camacho MI, Chen Y, Bhat AH, Chang C, Peluso EA, Wittchen M, Tauch A, Wu C, Das A, and Ton-That H (2022). Hydrogen sulfide biosynthetic enzymes are required for *Fusobacterium nucleatum* fitness, antibiotic sensitivity, and virulence. **mBio**, 13(5):e0193622. doi: 10.1128/mbio.01936-22. PMC9600241
- 3) Reardon-Robinson ME, Nguyen MT, Sanchez BC, Osipiuk J, Rückert C, Chang C, Chen B, Nagvekar R, Joachimiak A, Tauch A, Das A, Ton-That H (2023). A cryptic oxidoreductase safeguards oxidative protein folding in *Corynebacterium diphtheriae*. **Proc Natl Acad Sci U S A**, 120(8):e2208675120. doi: 10.1073/pnas.2208675120. PMC9974433
- 4) Britton TA, Wu C, Chen YW, Franklin D, Chen Y, Camacho MI, Luong TT, Das A, and Ton-That H (2024). The respiratory enzyme complex Rnf is vital for metabolic adaptation and virulence in *Fusobacterium nucleatum*. **mBio**, 15(1):e0175123; DOI: 10.1128/mbio.01751-23. PMC10790702

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2025-present	Chair, Division of Oral and Systemic Health Sciences, School of Dentistry, University of California at Los Angeles (UCLA), CA
2018-present	Professor, Division of Oral and Systemic Health Sciences, School of Dentistry, UCLA
2018-present	Professor (Joint Appointment), Department of Microbiology, Immunology & Molecular Genetics, School of Medicine, UCLA
2015-2018	Professor, Department of Microbiology & Molecular Genetics, University of Texas Health Science Center at Houston, TX
2009-2015	Associate Professor, Department of Microbiology & Molecular Genetics, University of Texas Health Science Center at Houston, TX
2004-2008	Assistant Professor, Department of Molecular, Microbial, & Structural Biology, University of Connecticut Health Center, CT
2003-2004	Research Assistant Professor, Department of Molecular Genetics & Cell Biology, University of Chicago, IL
1995-1997	Research Assistant, Department of Microbiology & Immunology UCLA School of Medicine, CA

Other Experience and Professional Memberships

2018 –	Member, Molecular Biology Institute, UCLA
2018 –	Member, California NanoSystems Institute (CNSI), UCLA
2015 –	Member, American Society for Biochemistry and Molecular Biology
1998 –	Member, American Society for Microbiology
2017-2023	Standing member, the NIH ODCS Study Section
2023	Mail in reviewer, Ohio Cancer Research, Columbus, OH
2019	Mail in reviewer, Mitacs Accelerate, Canada
2009-2016	Reviewer, NIH Infectious Diseases and Microbiology Fellowship F13 Study Section
2015	<i>Ad hoc</i> reviewer, the CRFS study section
2015 (2x)	<i>Ad hoc</i> reviewer, the NIH ODCS Study Section
2014	Mail in reviewer, The Wellcome Trust for Pathogen Biology and Disease Transmission, London, UK
2013	<i>Ad hoc</i> reviewer, the NIH Bacterial Pathogenesis (BACP) Study Section
2013	<i>Ad hoc</i> reviewer, the NIH Oral, Dental and Craniofacial Sciences (ODCS) Study Section
2013	<i>Ad hoc</i> Reviewer, The Oklahoma Center for the Advancement of Science and Technology's (OCAST) Health Research program
2012	Mail in reviewer for The Wellcome Trust/ DBT India Alliance, India
2012	Reviewer for the R15 Infectious Diseases and Microbiology Fellowship Panel, ZRG1 IDM-C
2010	Mail in reviewer for the Medical Research Council, South Africa
2009	Mail in reviewer for the Medical Research Council New Investigator Grant, UK
2009	<i>Ad hoc</i> reviewer, the NIH ARRA AREA Special Emphasis R15 Study Section
2007	<i>Ad hoc</i> reviewer, Phillip Morris External Research

Honors/Awards

2025	Dr. No-Hee Park Endowed Chair
2018	Elected Fellow, American Academy of Microbiology
2013	Dean's Teaching Excellence Award, UT Medical School at Houston
2007	ICAAC Young Investigator Award (American Society for Microbiology)
2000	Arnold Ravin-Muriel Rogers Fellowship
2000-2001	Post-doctoral Microbial Pathogenesis Training Grant, NIH, Department of Microbiology & Immunology, UCLA School of Medicine
1999-2000	Pre-doctoral Microbial Pathogenesis Training Grant, NIH, Department of Microbiology & Immunology, UCLA School of Medicine

C. Contributions to Science

1. **Sortase-catalyzed cell wall anchoring in Gram-positive bacteria:** Work by Sjöquist and colleagues in early 1970s suggested that *S. aureus* protein A, an immunomodulatory molecule, is anchored to the bacterial cell wall. However, the mechanism of cell wall anchoring in Gram-positive bacteria was not known until early 2000s, whereby my early contributions include the discovery of sortase SrtA in *S. aureus* and the elucidation of sortase-catalyzed cell wall anchoring of Gram-positive surface proteins using *S. aureus* as an experimental model.
 - a) Mazmanian SK, Liu G, Ton-That H, and Schneewind O (1999) Identification of Sortase, the Transpeptidase that Anchors Surface Proteins to the Cell Wall Envelope of *Staphylococcal aureus*. **Science**, 285: 760-763. PMID: 10427003
 - b) Ton-That H, Liu G, Mazmanian SK, Faull KF, and Schneewind O (1999) Purification and Characterization of Sortase, the Transpeptidase that Cleaves Surface Proteins of *Staphylococcal aureus* at LPXTG Motif. **Proc Natl Acad Sci U S A**, 96: 12424-12429. PMC22937
 - c) Ton-That H, Mazmanian SK, Faull KF, and Schneewind O (2000) Anchoring of Surface Proteins to the Cell Wall of *Staphylococcus aureus*. I. Sortase Catalyzed Transpeptidation Reaction Using LPETG-Peptide and NH₂-Gly₃ Substrates. **Journal of Biological Chemistry**, 275: 9876-9881. PMID: 10734144

- d) Ilangovan U*, Ton-That H*, Iwahara J, Schneewind O, and Clubb RT (2001) Structure of Sortase, the Transpeptidase that Anchors Proteins to the Cell Wall of *Staphylococcus aureus*. **Proc Natl Acad Sci U S A**, 98: 6056-6061. (*These authors contributed equally to this work) PMC33421

2. Pilus assembly in Gram-positive bacteria: Like Gram-negative bacteria, Gram-positive counterparts produce adhesive pili or fimbriae. However, an assembly mechanism of covalently linked pili was not known until my discovery in 2003 that in *Corynebacterium diphtheriae* – a causative agent of diphtheria – a pilus-specific sortase catalyzes pilus polymerization followed by cell wall anchoring of the resulting pilus polymers mediated by the housekeeping sortase. Since then, we have developed the general experimental approaches and methodologies, such as immuno-electron microscopy and structural biology, which helped to identify and study pili in many other Gram-positive pathogens, including *Actinomyces oris*, *Enterococcus faecalis*, and many species of streptococci. Subsequent work in my laboratory, using *A. oris* and *C. diphtheriae* as experimental models, has elucidated the conserved biphasic mechanism of sortase-mediated pilus assembly in Gram-positive bacteria.

- a) Reardon-Robinson ME*, Wu C*, Mishra A*, Chang C, Bier N, Das A, and Ton-That H (2014). Pilus Hijacking by a Bacterial Coaggregation Factor Critical for Oral Biofilm Development. **Proc Natl Acad Sci U S A**, 111(10):3835–3840. PMC3956193
- b) Chang C*, Amer BR*, Osipiuk J, McConnell SA, Huang IH, Hsieh V, Fu J, Nguyen HH, Muroski J, Flores E, Loo R, Loo J, Putkey JA, Joachimiak A, Das A, Clubb RT[†], and Ton-That H[†] (2018). *In vitro* reconstitution of sortase-catalyzed pilus polymerization reveals structural elements involved in pilin crosslinking. **Proc Natl Acad Sci U S A**, 115(24):E5477-E5486. PMC6004493
- c) Chang C, Wu C, Osipiuk J, Siegel SD, Zhu S, Liu X, Joachimiak A, Clubb RT, Das A, and Ton-That H (2019). Cell-to-cell interaction requires optimal positioning of a pilus tip adhesin modulated by Gram-positive transpeptidase enzymes. **Proc Natl Acad Sci U S A**, 116(36):18041-18049. PMC6731673
- d) Ramirez NA, Wu C, Chang C, Siegel SD, Das A, and Ton-That H (2022). A conserved signal-peptidase antagonist modulates membrane homeostasis of actinobacterial sortase critical for surface morphogenesis. **Proc Natl Acad Sci U S A**, 119(28): e2203114119. PMC9282373

3. Oxidative protein folding in Gram-positive bacteria: Disulfide bond formation contributes to the overall protein folding process, stabilizing structures and protecting against degradation. In Gram-negative bacteria, this process occurs in the oxidizing periplasmic space and is required a pair of oxidoreductase enzymes DsbA and DsbB. However, the mechanism of oxidative protein folding in single-membrane Gram-positive bacteria, which are not considered to have periplasms, was not well understood until my laboratory's findings in 2015. We revealed that an oxidative folding pathway for pilus proteins in *A. oris* that is comprised of the membrane-bound thiol-disulfide oxidoreductases MdbA and VKOR. This disulfide bond-forming machine is essential for cell growth as deletion of *mdbA* is lethal to cells. Significantly, a similar pathway is present in *C. diphtheriae* and *C. matruchotii*. Because over 60% of signal peptide-containing proteins encoded by these bacteria harbor two or more Cys residues, we posit that intramolecular disulfide bond formation constitutes a major folding pathway for Actinobacteria including *Mycobacterium tuberculosis*, and as such, is an important target for therapeutic development.

- a) Reardon-Robinson ME, Osipiuk J, Jooya N, Chang C, Joachimiak A, Das A, and Ton-That H (2015). A thiol-disulfide oxidoreductase of the Gram-positive pathogen *Corynebacterium diphtheriae* is essential for viability, pilus assembly, toxin production and virulence. **Molecular Microbiology**, 98(6):1037-50. PMC4981772
- b) Sanchez B, Chang C, Wu C, Tran B, and Ton-That H (2017). Electron transport chain is biochemically linked to pilus assembly required for polymicrobial interactions and biofilm formation in the Gram-positive actinobacterium *Actinomyces oris*. **mBio**, 8(3). pii: e00399-17. PMC5478893.
- c) Luong TT, Tirgar R, Reardon-Robinson ME, Joachimiak A, Osipiuk J, and Ton-That H (2018). Structural basis of a thiol-disulfide oxidoreductase in the hedgehog-forming actinobacterium *Corynebacterium matruchotii*. **Journal of Bacteriology**, 200(9):e00783-17. PMC5892113
- d) Reardon-Robinson M, Nguyen MT, Sanchez BC, Osipiuk J, Rückert C, Chang C, Chen B, Nagvekar R, Joachimiak A, Tauch A, Das A, and Ton-That H (2023). A cryptic thiol-disulfide oxidoreductase

safeguards oxidative protein folding in *Corynebacterium diphtheriae*. **Proc Natl Acad Sci U S A**, 120(8):e2208675120. PMC9974433

4. Virulence determinants of *F. nucleatum*: *F. nucleatum* is a Gram-negative colonizer that plays a key role in the development of oral biofilms. This anaerobic pathobiont is also associated with preterm birth and colorectal cancer. However, little is known about virulence mechanisms and factors associated with these processes. A major obstacle limiting progress has been the lack of robust genetic tools and systematic investigations. My lab began to tackle this problem, successfully developing a facile gene deletion method and a robust transposon system for *F. nucleatum*. Using these tools, we revealed several important findings, linking cell division and surface dynamics to biofilm development, and cell metabolism to polymicrobial interactions and bacterial virulence. Collectively, our work lays a foundation for future molecular, genetic and biochemical investigations of cellular processes in this clinically significant pathogen.

- a) Wu C, Al Mamun AA, Luong TT, Hu B, Gu J, Lee JH, D'Amore M, Das A, and Ton-That H (2018). Forward Genetic Dissection of Biofilm Development by *Fusobacterium nucleatum*: Novel Functions of Cell Division Proteins FtsX and EnvC. **mBio**, 9(2):e00360-18. PMC5915739
- b) Peluso EA, Scheible M, Ton-That H[†], and Wu C[†] (2020). Genetic manipulation and virulence assessment of *Fusobacterium nucleatum*. **Current Protocols in Microbiology**, 57(1):e104. doi: 10.1002/cpmc.104. PMC7398570. [†]Corresponding authors
- c) Wu C, Chen YW, Scheible M, Chang C, Wittchen M, Lee JH, Luong TT, Tiner BL, Tauch A, Das A, and Ton-That H (2021). Genetic and molecular determinants of polymicrobial interactions in *Fusobacterium nucleatum*. **Proc Natl Acad Sci U S A**, 118(23):e2006482118. PMC8201914
- d) Scheible M, Nguyen CT, Luong TT, Lee JH, Chen YW, Chang C, Wittchen M, Camacho MI, Tiner BL, Wu C, Tauch A, Das A, and Ton-That H (2022). The fused methionine sulfoxide reductase MsrAB promotes oxidative stress defense and bacterial virulence in *Fusobacterium nucleatum*. **mBio**, 23(3):e0302221. PMC9239216

5. Development of genetic tools for Gram-negative and Gram-positive bacteria: Facile genetic manipulation is critical to bacterial research. When we began to investigate the molecular assembly on the cell surface of Gram-positive oral colonizers *A. oris*, *C. diphtheriae*, and *C. mantruchotii*, no robust genetic tools were available for these bacteria. Therefore, we made strong efforts to develop corresponding robust gene deletion methods and transposon mutagenesis systems. Later, we also developed a genetic toolbox – gene deletion and transposon mutagenesis – for the Gram-negative pathobiont *F. nucleatum*. Our developed genetic tools for these microbes have helped move the respective fields forward and have been greatly beneficial to other investigators.

- a) Chenggang Wu, Melissa E. Reardon-Robinson, and Hung Ton-That (2016). Analysis of cell morphology and viability in the *srtA* mutant of *Actinomyces oris*. In *Bacterial Cell Wall Homeostasis: Methods and Protocols, Methods in Molecular Biology*, Ed. Hee-Jeon Hong (Springer), 1440:109-22
- b) Chang C, Nguyen MT, and Ton-That H (2020). Genetic manipulations of *Corynebacterium diphtheriae* and other *Corynebacterium* species. **Current Protocols in Microbiology**, 58(1):e111; doi: 10.1002/cpmc.111. PMC7939059
- c) Peluso EA, Scheible M, Ton-That H[†], and Wu C[†] (2020). Genetic manipulation and virulence assessment of *Fusobacterium nucleatum*. **Current Protocols in Microbiology**, 57(1):e104. doi: 10.1002/cpmc.104. PMC7398570. [†]Corresponding authors
- d) Bhat AH and Ton-That H (2024). Single-copy gene editing of a cell wall-anchored pilin in *Actinomyces oris*. *Methods in Molecular Biology*, 2727:125-134. doi: 10.1007/978-1-0716-3491-2_10. PMID: 37815713

Complete List of Published Work in My Bibliography (NCBI)

<https://www.ncbi.nlm.nih.gov/myncbi/hung.ton-that.1/bibliography/public/>

BIOGRAPHICAL SKETCH**DO NOT EXCEED FIVE PAGES.**

NAME: Troy D. Wood

eRA COMMONS USER NAME (credential, e.g., agency login): tdwood

POSITION TITLE: Professor, Laboratory Director

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Indiana University, Bloomington, IN	BS (Honors)	05/1989	Chemistry
The Ohio State University, Columbus, OH	PhD	09/1993	Chemistry
Cornell University, Ithaca, NY	Postdoc	07/1995	Chemistry

A. Personal Statement

I have 30+ years of training in mass spectrometry as an analytical chemist. My graduate and post-doctoral work centered on utility of Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry. As a graduate student, I worked in the area of laser desorption ionization for use in materials science and biochemical research. As a post-doc, I was one of the earliest practitioners of “top-down” proteomics to gain structural insight into primary sequence of proteins using electrospray ionization coupled to tandem FT-ICR. I have been a faculty member in the Dept. of Chemistry at the University at Buffalo (UB) since 1995, having supervised 30 Ph.D. and 11 Masters students. I have been serving in the NIAID contracted Clinical Pharmacology Quality Assurance Program (CPQA) at UB since 2008 and am now its Laboratory Director. I have been a Co-Investigator (Co-I) or collaborator on several previous and current NIH projects and contracts (75N93022C00009, 272201500006C, R21-CA185841, 272200800019C-5-0-1, R01-MH078009, R01-AI177997, R21-OH12389, S10RR029517-01). Our research today is focused in the areas of proteomics and metabolomics. In collaboration with CPQA, we are adapting enzyme microreactors developed in our lab to map and quantify broadly neutralizing antibody (bNab) formulations used in HIV pharmacotherapy. We are also using this technology in paleoproteomics to identify ancient proteins in fossilized eggshells, and in neurobiology to identify a protein in amniotic fluid and placenta that enhances hypoalgesia (with A. C. Thompson, M. B. Kristal, and J. M. DiPirro). In a series of papers, we have identified bilins, a group of compounds produced by microbiome action upon bilirubin diglucuronide, as potential biomarkers of autism and have demonstrated that the metabolite stercobilin is depleted approximately 45% in the fecal matter of an autism mouse model. With H. Nguyen (U. Missouri), metabolomics is used to understand the molecular mechanisms by which different cultivars of soybeans use to adapt to drought stress. My group is also involved in developing mass spectrometry imaging (MSI), which has been applied to plant and animal tissues, most recently in the examination of demyelination/remyelination in lysolecithin-induced spinal cord injury (with F. Sim) and multimodal MSI (with A. C. Thompson). My role in this project will be to provide advice and guidance to Dam Soh on liquid-chromatography tandem mass spectrometry (LC-MS/MS) method development for targeted metabolomic analysis of gingival crevicular fluid (GCF).

Citations

1. Gould, C. E.; Ma, Q.; Cha, R.; Zemaitis, K. J.; DiFrancesco, R.; Morse, G. D.; **Wood, T. D.** “LC-MS/MS Method for Quantifying the HIV-1 Broadly Neutralizing Antibody PGT 121.414.LS in Human Serum,” *J. Chromatogr. B*, 2025, 1266, 124774. PMID: 40886420.
2. Sekera, E. R.; Saraswat, D.; Zemaitis, K. J.; Sim, F.; **Wood, T. D.** “MALDI Mass Spectrometry Imaging in a Primary Demyelination Model of Murine Spinal Cord,” *J. Am. Soc. Mass Spectrom.* 2020, 31, 2462-

2468. PMID: 32926612.

3. Zemaitis, K. J.; Izydorczak, A. M.; Thompson, A. C.; **Wood, T. D.** "Streamlined Multimodal DESI and MALDI Mass Spectrometry Imaging on a Singular Dual-Source FT-ICR Mass Spectrometer," *Metabolites* 2021, 11, 253. PMID: 33923908, PMCID: PMC8073082.
4. Sekera, E. R.; Rudolph, H. L.; Carro, S. D.; Morales, M. J.; Bett, G. C. L.; Rasmusson, R. L.; **Wood, T. D.** "Depletion of Stercobilin in Fecal Matter from a Mouse Model of Autism Spectrum Disorders," *Metabolomics* 2017, 13, 132. PMID: 29147105. PMCID: PMC5685184.

B. Positions, Scientific Appointments, and Honors

Employment History:

- **Professor** (2023-present), Dept. of Chemistry, University at Buffalo, State University of New York, Buffalo, NY. Also serves as Director of Undergraduate Studies.
- **Laboratory Director** (2023-present), Clinical Pharmacology Quality Assurance (CPQA) Program and Center for Integrated Global Biomedical Sciences (CIGBS), University at Buffalo, Buffalo, NY.
- **Associate Professor** (2001-2023), Dept. of Chemistry, University at Buffalo, State University of New York, Buffalo, NY. Also served as Director of Undergraduate Studies (2020-2023).
- **Associate Director** (2022-2023), Proficiency Testing Unit, Clinical Pharmacology Quality Assurance (CPQA) Program, University at Buffalo, Buffalo, NY.
- **Adjunct Assistant Research Professor of Molecular & Cellular Biophysics** (1998-2006).
- **Vice President** (2000-2025), Nanogenesys, Inc., Kenmore, NY.
- **Adjunct Assistant Research Professor of Chemistry** (1996-98), Roswell Park Cancer Institute, Buffalo, NY.
- **Assistant Professor** (1995-2001), Dept. of Chemistry, University at Buffalo, State University of New York, Buffalo, NY.
- **Postdoctoral Associate** (1993-95), Dept. of Chemistry, Cornell University, Ithaca, NY.

Honors/Awards:

- | | |
|---------|---|
| 2018 | Student-selected Commencement Speaker, College of Arts & Sciences, University at Buffalo |
| 2016 | Scientific Congress of Guatemala, Citation |
| 2014 | Milton Plesur Award for Excellence in Teaching, Student Association, University at Buffalo |
| 2008 | Chancellor's Award for Excellence in Teaching, State University of New York |
| 2006 | Niagara Frontier Intellectual Property Law Association, Third Place, Physical Sciences Division |
| 2004 | Patent Award, the State University of New York Research Foundation |
| 2003 | Niagara Frontier Intellectual Property Law Association Inventor of the Year, Nominee. |
| 2002 | Entrepreneur Award, the State University of New York Research Foundation |
| 2000-01 | DuPont Office of Education (OOE) Science and Engineering Grant, Analytical Sciences Division. |
| 1998 | American Society for Mass Spectrometry Research Award (Exxon Education Foundation) |
| 1989-92 | Department of Education National Needs Fellowship, The Ohio State University |
| 1988 | Harry G. Day Summer Research Scholarship, Indiana University. |
| 1989 | The Waldo Semon National Undergraduate Research Symposium, Honorable Mention |

C. Contributions to Science

- a. As an independent investigator, my group has been involved in developing stable nanoESI emitters for mass spectrometry. We overcame the limitations of early commercial emitters made with gold films by developing stable coatings using polyaniline and graphite. This led to two US Patents in nanoESI and the formation of a start-up company, Nanogenesys, Inc., which developed them into a product line. Other manufacturers increased the stability of their metal films by incorporating chromium, reducing Nanogenesys' market share, and driving it from the market (though it still exists as a consulting firm); nevertheless, our innovations changed the nature of the marketplace and now stable nanoESI emitters are ubiquitous. We have extended the capabilities of such emitters by integrating them with enzyme microreactors. This area of research is active, and is being used to map broadly neutralizing antibodies used for pharmacological HIV therapy as well as to identify ancient proteins from fossilized egg shells.

- i. Maziarz, E. P., III; Lorenz, S. A.; White, T. P.; **Wood, T. D.** "Polyaniline: A Conductive Polymer Coating for Durable Nanospray Emitters," *J. Am. Soc. Mass Spectrom.* 2000, 11, 659-663. PMID: 10883822.
 - ii. Zhao, C.; Jiang, H.; Smith, D. R.; Bruckenstein, S.; **Wood, T. D.** "Integration of an On-Line Protein Digestion Microreactor and a Nanoelectrospray Emitter for Rapid Peptide Mapping," *Anal. Biochem.* 2006, 359, 167-175. PMID: 17078919.
 - iii. Long, Y.; **Wood, T. D.** "Activity of the Integrated On-line Trypsin Microreactor and Nanoelectrospray Emitter in Acetonitrile-Water Co-solvent Mixtures," *Microfluid Nanofluid* 2013, 15, 57-64.
 - iv. Long, Y.; **Wood, T. D.** "Immobilized Pepsin Microreactor for Rapid Peptide Mapping with Nanoelectrospray Ionization Mass Spectrometry," *J. Am. Soc. Mass Spectrom.* 2015, 26, 194-197. PMID: 25374334.
- b. A major emphasis of my research has centered on using mass spectrometry to identify biological markers of disease. In collaboration with Medical School at the University of Sassari (Italy) were the first to identify gluten exorphin peptides in human biofluids (plasma), and our studies of the instability of exorphins in biofluids strongly suggests they are not likely to be important in the etiology of autism. In collaboration with others, we also used our low-flow electrospray emitters to perform peptide mapping to identify that superoxide dismutase (SOD) was a common molecular marker in CSF from those with both familial AND sporadic amyotrophic lateral sclerosis (ALS) and not other neurodegenerative diseases. This was a very significant result because it showed that variation in SOD sequence alone could not be responsible for ALS, yet indicated that elevated levels of SOD in spinal fluid could be a viable diagnostic for ALS. Most recently, we have integrated mass spectrometry imaging (MSI) using MALDI and DESI to profile biological tissues, including tracking molecular changes that occur upon spinal cord injury.
- i. Gruzman, A.; Wood, W. L.; Alpert, E.; Prasad, M. D.; Miller, R. G.; Rothstein, J. D.; Bowser, R.; Hamilton, R.; **Wood, T. D.**; Cleveland, D. W.; Lingappa, V. R.; Liu, J. "A Common Molecular Signature in SOD1 for both Sporadic and Familial Amyotrophic Lateral Sclerosis," *Proc. Natl. Acad. Sci.* 2007, 104, 12524-12529. PMID: 17636119. PMCID: PMC1941502.
 - ii. Sekera, E. R.; Saraswat, D.; Zemaitis, K. J.; Sim, F.; **Wood, T. D.** "MALDI Mass Spectrometry Imaging in a Primary Demyelination Model of Murine Spinal Cord," *J. Am. Soc. Mass Spectrom.* 2020, 31, 2462-2468. PMID: 32926612.
 - iii. Friesen, W. L.; Schultz, B. J.; Destino, J. F.; Alivio T. E. G.; Steet, J. R.; Banerjee, S.; **Wood, T. D.** "Two-dimensional Graphene as a Matrix for Imaging Mass Spectrometry," *J. Am. Soc. Mass Spectrom.* 2015, 26, 1963-1966. PMID: 26323616. PMCID: PMC4607658.
 - iv. Zemaitis, K. J.; Izydorczak, A. M.; Thompson, A. C.; **Wood, T. D.** "Streamlined Multimodal DESI and MALDI Mass Spectrometry Imaging on a Singular Dual-Source FT-ICR Mass Spectrometer," *Metabolites* 2021, 11, 253. PMID: 33923908, PMCID: PMC8073082.
- c. During the past decade, much of our effort has been dedicated toward metabolomics investigations. In one area of our metabolomics research, we have focused on understanding the role of tetrapyrrole bilins, produced by microbial action upon metabolites of bilirubin in the gut, as a diagnostic for autism spectrum disorders. A second area of activity has been dedicated toward understanding the metabolomic changes in soybeans as a function of season and under drought-stress conditions.
- i. Sekera, E. R.; Rudolph, H. L.; Carro, S. D.; Morales, M. J.; Bett, G. C. L.; Rasmuson, R. L.; **Wood, T. D.** "Depletion of Stercobilin in Fecal Matter from a Mouse Model of Autism Spectrum Disorders," *Metabolomics* 2017, 13, 132. PMID: 29147105. PMCID: PMC5685184.
 - ii. **Wood, T. D.**; Tiede, E. R.; Izydorczak, A. M.; Zemaitis, K. J.; Ye, H.; Nguyen, H. T. "Chemical Informatics Combined with Kendrick Mass Analysis to Enhance Annotation and Identify Pathways in Soybean Metabolomics," *Metabolites* 2025, 15(2), 73. PMID: 39997698. PMCID: PMC11857611.
 - iii. Zemaitis, K. J.; Ye, H.; Nguyen, H. T.; **Wood, T. D.** "Direct Infusion Metabolomics of the Photosystem and Chlorophyll Related Metabolites within a Drought Tolerant Plant Introduction of *Glycine max*," *Metabolites* 2021, 11, 843. PMID: 34940601, PMCID: PMC8706244.
 - iv. Yilmaz, A.; Rudolph, H. L.; Hurst, J. J.; **Wood, T. D.** "High-Throughput Metabolic Profiling of Soybean Leaves by Fourier Transform Ion Cyclotron Resonance Mass Spectrometry," *Anal. Chem.* 2016, 88, 1188-94. PMCID: PMC4817222.

- d. During my postdoctoral research and early independent academic career, I was involved in using mass spectrometry to study conformational properties of gas-phase protein ions and distinguishing between solution-phase and gas-phase formed small molecule oligomers using hydrogen-deuterium exchange (HDX). HDX of cytochrome c showed that it could be induced to fold in the vacuum state in the absence of water, and that while tertiary contacts may be lost in going to the gas-phase, secondary structural elements are still present. This work proved that intrinsic in the amino acid sequence is information necessary for adopting protein conformation. In my early independent career, we used HDX to distinguish that dimers of paclitaxel and certain oligoamides were formed in solution, while other complexes were formed only through the process of ionization; this showed HDX could have inherent value in screening for potential drug-drug interactions in solution.
 - i. **Wood, T. D.**; Chorush, R. A.; Wampler, F. M., III; Little, D. P.; O'Connor, P. B.; McLafferty, F. W. "Gas Phase Folding and Unfolding of Cytochrome c Cations," *Proc. Natl. Acad. Sci. USA* 1995, 92, 2451-2454. PMID: 7708663. PMCID: PMC42235.
 - ii. McLafferty, F. W.; Guan, Z.; Haupts, U.; **Wood, T. D.**; Kelleher, N. L. "Gaseous Conformational Structures of Cytochrome c," *J. Am. Chem. Soc.* 1998, 120, 4732-4740.
 - iii. Lorenz, S. A.; Maziarz, E. P., III; **Wood, T. D.** "Using Solution-Phase Hydrogen/Deuterium (H/D) Exchange to Determine the Origin of Non-covalent Complexes Observed by Electrospray Ionization Mass Spectrometry: in Solution or in Vacuo?" *J. Am. Soc. Mass Spectrom.* 2001, 12, 795-804. PMID: 11444601.
 - iv. Jiang, H.; Li, M.; Moy, M. A.; Gong, B.; **Wood, T. D.** "Mechanistic Information on the Disulfide-bond Reaction and the Role of Hydrogen Bonds by Nanoelectrospray Mass Spectrometry," *J. Mass Spectrom.* 2008, 43, 664-673. PMID: 18172858.
- e. Over much of my career, I have been involved in using mass spectrometry to elucidate protein structure. This involved some "top-down" proteomics in my postdoctoral and independent career on enzymes that lacked active site mapping, such as creatine kinase and glucokinase. It has also involved "bottom-up" approaches to identify proteins, including studies with collaborators to identify novel phosphorylation sites induced by interleukin-17 on an oligonucleotide-binding protein, heterogeneity of an antibody used in HIV-1 therapy, and differences induced by ethanol on protein expression in zebrafish.
 - i. Gould, C. E.; Ma, Q.; Cha, R.; Frerichs, V. A.; Friedman, A. E.; Difrancesco, R.; Morse, G. D.; **Wood, T. D.** "Proteoform Characterization of HIV-1 Broadly Neutralizing Antibody PGT 121.414.LS Product Through Middle-up and Bottom-up Proteomics for Clinical Support," *J. Am. Soc. Mass Spectrom.* Accepted.
 - ii. Damodaran, S.; Dlugos, C. A.; **Wood, T. D.**; Rabin, R. A. "Effects of Chronic Ethanol Administration on Brain Protein Levels: A Proteomic Investigation Using 2-D DIGE System," *Eur. J. Pharmacol.* 2006, 547, 75-82. PMID: 16978605.
 - iii. Sekera, E. R.; **Wood, T. D.** "Sequencing Proteins from Bottom to Top: Combining Techniques for Full Sequence Analysis of Glucokinase," *Adv. Exp. Med. Biol.* 2019, 1140, 111-119. PMID: 31347044.
 - iv. Shen, F.; Li, N.; Gade, P.; Kalvakolanu, D. V.; Weibley, T.; Doble, B.; Woodgett, J. R.; **Wood, T. D.**; Gaffen, S. L. "IL-17 Receptor Signaling Negatively Regulates C/EBP β by Sequential Phosphorylation of the Regulatory 2 Domain," *Sci. Signal.*, 2009, 2, ra8. PMID: 19244213. PMCID: PMC2754870.

Complete List of Published Work in My Bibliography:

<http://www.ncbi.nlm.nih.gov/sites/myncbi/troy.wood.1/bibliography/47891595/public/?sort=date&direction=ascending>

PHS OTHER SUPPORT
For All Application Types – DO NOT SUBMIT UNLESS REQUESTED

There is no "form page" for reporting Other Support. Information on Other Support should be provided in the format shown below.

*Name of Individual: Diaz, Patricia I.
 Commons ID: diazmoreno

Other Support – Project/Proposal

AWARDED

Title: "Host and microbial risk factors of oral thrush in cancer patients receiving chemotherapy"
 Major goals: The goal of this proposal is to determine the factors that underline susceptibility to oropharyngeal candidiasis in chemotherapy recipients through clinical and in vitro mechanistic studies.

Status of Support: Awarded

Project number: R01 DE032131-01

Name of PD/PI: Diaz, PI and Schlecht, N

Source of Support: NIH; NIDCR

Primary Place of Performance: University at Buffalo

Project/Proposal Start and End Date: 08/2022-05/2027

Total Award Amount (including Indirect Costs): \$3,664,558

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
2025	2.4 calendar months
2026	2.4 calendar months
2027	2.4 calendar months

There is no scientific or budgetary overlap

Title: "Impact of HIV, oral microbiome and mycobiome on oral HPV persistence"

Major goals: This study will leverage repeat oral saliva samples collected from individuals enrolled in the MACS-WIHS Combined Cohort Study (MWCSS) to investigate the associations between the oral microbiome and mycobiome and risk of acquisition and persistence of high-risk *alpha*, *beta*, and *gamma* oral HPV.

Status of Support: Awarded

Project number: 312207PO903922 (Prime: R01DE032242-01)

Name of PD/PI: Burk, R, Diaz, P and Schlecht, N

Source of Support: Albert Einstein College of Medicine (Prime: NIH/NIDCR)

Primary Place of Performance: University at Buffalo

Project/Proposal Start and End Date: 09/2022-08/2027

Total Award Amount (including Indirect Costs): \$788,459

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
2025	1.2 calendar months
2026	1.2 calendar months
2027	1.2 calendar months

Name of Individual:
Commons ID:

There is no scientific or budgetary overlap

Title: "Susceptibility Patterns for Grade C Periodontitis in Young Individuals"

Major goals: This multi-center study will evaluate clearly defined cases of localized aggressive periodontitis, in different regions of the globe, and within families, to identify genetic, host response and microbial factors associated with susceptibility.

Status of Support: Awarded

Project number: 320000537123245 (Prime: U01 DE031223-01A1)

Name of PD/PI: Shaddox, L

Source of Support: University of Kentucky (Prime: NIH; NIDCR)

Primary Place of Performance: University at Buffalo

Project/Proposal Start and End Date: 09/2022-08/2027

Total Award Amount (including Indirect Costs): \$634,248

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
2025	0.96 calendar months
2026	0.96 calendar months
2027	0.96 calendar months

There is no scientific or budgetary overlap

Title: Buffalo Oral-Research and Specialty Training Program (BORST)

Major Goals: The goal of this training program is to provide dentists specializing in the areas of periodontics, orthodontics, or oral pathology with research training in oral biology leading to a PhD.

Status of Support: Awarded

Project Number: 2 K12 DE027827-06

Name of PD/PI: Kirkwood, K

Source of Support: NIH; NIDCR

Primary Place of Performance: University at Buffalo

Project/Proposal Start and End Date: 09/2023- 08/2028

Total Award Amount (including Indirect Costs): \$1,585,459

Person Months (Calendar) per budget period.

Year (YYYY)	Person Months (##.##)
2025	0.6 calendar months
2026	0.6 calendar months
2027	0.6 calendar months
2028	0.6 calendar months

There is no scientific or budgetary overlap

Title: Building Research Capacity to Study Periodontitis and Associated Systemic Comorbidities in the Caribbean

Major Goals: This D43 training program aims to expand the inter-disciplinary research capacity of the Faculty of Medical Sciences at the University of West Indies, in collaboration with the University at Buffalo and Rush University, by developing a program to study the determinants of periodontitis and associated non-communicable chronic diseases in Jamaica.

Name of Individual:
Commons ID:

Status of Support: Awarded
Project Number: D43 TW012454
Name of PD/PI: Diaz, P.; Brown, P.; Landay A. (MPI)
Source of Support: NIH; Fogarty
Primary Place of Performance: Buffalo, NY; Chicago, IL; and Kingston, Jamaica
Project/Proposal Start and End Date: 04/2023-05/2028
Total Award Amount (including Indirect Costs): \$1,083,183
*Person Months (Calendar) per budget period.

Year (YYYY)	Person Months (##.##)
2025	0.6 calendar months
2026	0.6 calendar months
2027	0.6 calendar months
2028	0.6 calendar months

There is no scientific or budgetary overlap

Title: "Identifying the multiscale dynamics of oral pathobiome colonization (Dynobiome)"
Major goals: The aim of this project is to evaluate biophysical determinants and develop mathematical models of *Porphyromonas gingivalis* oral colonization.

Status of support: awarded
Project number: 20230871067(Prime: OISE-9531011)
Name of PD/PI: Hatzikirou, H; Diaz, PI; Quasimeh, M
Source of Support: CRDF Global (Prime: National Science Foundation)
Primary Place of Performance: University at Buffalo
Project/Proposal Start and End Date: 06/2023-08/2026
Total Award Amount (including Indirect Costs): \$104,998 (UB subcontract)
Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
2. 2025	0.6 calendar months
3. 2026	0.6 calendar months

There is no scientific or budgetary overlap

Title: "Identification of diffusible small molecules that regulate replication of *Porphyromonas gingivalis*"

Major goals: The aim of this project is to identify small molecules produced by *Porphyromonas gingivalis* when in high density or by the commensal *Veillonella parvula* and allow *P. gingivalis* to initiate replication from a low density inoculum.

Status of support: Awarded
Project number: 1R21DE034093-01A1
Name of PD/PI: Diaz, PI
Source of Support: NIH; NIDCR
Primary Place of Performance: University at Buffalo
Project/Proposal Start and End Date: 04/2025-03/2027
Total Award Amount (including Indirect Costs): \$442,750
Person Months (Calendar/Academic/Summer) per budget period.

Name of Individual:
Commons ID:

Year (YYYY)	Person Months (##.##)
1. 2026	1.2 calendar months
2. 2027	1.2 calendar months

There is no scientific or budgetary overlap

Title: "Influence of Scaling and Root Planing with Minocycline Microspheres on the Composition and Functional Characteristics of Subgingival Microbiome Communities"

Major goals: This project evaluates the effect of Arrestin on the subgingival microbiome

Project number: 20624RFSUNY (Prime 2020-5280)

Name of PD/PI: Salman, A and Diaz, P

Source of Support: Bausch Health

Primary Place of Performance: University at Buffalo (Prime: University of West Virginia)

Project/Proposal Start and End Date: 07/2020-12/2025 (NCE)

Total Award Amount (including Indirect Costs): \$273,664 (UB subcontract)

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
2025	0 calendar months

Title: "Testing of anti-plaque agents in subject-specific, clinically-relevant, subgingival biofilm models"

Major goals: This project will develop patient-specific models of oral biofilms to test the effectiveness of different antimicrobials

Project number: SBMC002

Name of PD/PI: Diaz, P

Source of Support: Sunstar Inc.

Primary Place of Performance: University at Buffalo

Project/Proposal Start and End Date: 12/2021-11/2026 (NCE)

Total Award Amount (including Indirect Costs): \$483,756

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
2025	0 calendar months

PENDING

Title: Precision Microbiome Sequencing: A Targeted Approach to Identify and Quantify Bacterial Strains

Major Goals: The goal of this project is to develop an approach that can accurately measure bacterial strain presence and abundance from all microbiome sample types, including difficult samples with low biomass and high levels of host contamination.

Status of Support: Pending

Project Number: N/A

Name of PD/PI: Buck M (PI)

Source of Support: NIH

Primary Place of Performance: Buffalo, NY

Project/Proposal Start and End Date: 12/2025-11/2027

Total Award Amount (including Indirect Costs): \$439,085

*Person Months (Calendar) per budget period.

Name of Individual:
Commons ID:

Year (YYYY)	Person Months (##.##)
2026	0.6 calendar months
2027	0.6 calendar months

There is no scientific or budgetary overlap

Title: Treatment for Peri-implantitis via Local Immunomodulation and Effect on the Microbiome
Major Goals: This study will evaluate the effect of immunomodulation via local delivery on peri-implantitis assessed via bone, immune and microbiome outcomes. The Diaz laboratory will be in charge of performing shotgun metagenomic sequencing and bioinformatic analysis of oral samples derived from mice.

Status of Support: Pending

Project Number: N/A

Name of PD/PI: Sfeir C (PI)

Source of Support: NIH

Primary Place of Performance: University of Pittsburgh

Project/Proposal Start and End Date: 12/2025-11/2030

Total Award Amount (including Indirect Costs): \$717,195 (UB subcontract)

*Person Months (Calendar) per budget period.

Year (YYYY)	Person Months (##.##)
2026	1.2 calendar months
2027	1.2 calendar months
2028	1.2 calendar months
2029	1.2 calendar months
2030	1.2 calendar months

There is no scientific or budgetary overlap

Title: "Treatment of periodontitis by homing M2 macrophages"

Major goals: This renewal application will evaluate the manner in which periodontal immunomodulation via local delivery of CCL2 modulates macrophage phenotype/function, ameliorating bone loss and promoting a homeostatic microbiome using a ligature-induced periodontitis mouse model.

Project number: N/A

Status of Support: Pending

Name of PD/PI: Sfeir, C

Source of Support: University of Pittsburgh (Prime: NIH/NIDCR)

Primary Place of Performance: University of Pittsburgh

Project/Proposal Start and End Date: 04/2026-03/2031

Total Award Amount (including Indirect Costs): \$977,553 (UB subcontract)

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
2026	1.2 calendar months
2027	1.2 calendar months
2028	1.2 calendar months
2029	1.2 calendar months
2030	1.2 calendar months

OMB No. 0925-0001 and 0925-0002 (Rev. 10/2021 Approved Through 01/31/2026)

Name of Individual:
Commons ID:


There is no scientific or budgetary overlap

IN-KIND

No in-kind support

***Overlap** (summarized for each individual): There is no scientific or budgetary overlap.

I, PD/PI or other senior/key personnel, certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

*Signature:  Digitally signed by
Patricia Diaz
Date: 2025.09.16 15:17:36
-04'00'

Date: _____

PHS OTHER SUPPORT
For All Application Types – DO NOT SUBMIT UNLESS REQUESTED

There is no "form page" for reporting Other Support. Information on Other Support should be provided in the format shown below.

*Name of Individual: Alex M. Valm
Commons ID: A_Valm

Other Support – Project/Proposal

*Title: Oral microbial community structure and assembly: from molecule to microbiome

*Major Goals: The major goals of this project are to map the spatial structure of model microbial dental plaque communities and to identify novel genes involved in structure-function relationships in oral biofilms.

*Status of Support: Active

Project Number: 1 R01 DE 030927-05

Name of PD/PI: Alex Valm

*Source of Support: NIH

*Primary Place of Performance: State University of New York at Albany

Project/Proposal Start and End Date: (MM/YYYY) (if available): 07/2021-06/2026

* Total Award Amount (including Indirect Costs): 1,796,963

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2021	3.0
2. 2022	3.0
3. 2023	3.0
4. 2024	3.0
5. 2025	3.0

*Title: Biofilm Spatial Structure in the Transition from Health to Periodontal Disease

*Major Goals: The major goals of this project are to map the spatial structure of dental plaque in intact biofilms on teeth from human and canine patients with periodontal disease and to identify changes in gene expression that occur when co-localized species in dental plaque co-aggregate with each other.

*Status of Support: Active

Project Number: 1 R01 DE 031213-04

Name of PD/PI: Alex Valm

*Source of Support: NIH

Name of Individual:
Commons ID:

*Primary Place of Performance: State University of New York at Albany

Project/Proposal Start and End Date: (MM/YYYY) (if available): 08/2022-07/2027

* Total Award Amount (including Indirect Costs): 2,315,730

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2022	2.0
2. 2023	2.0
3. 2024	2.0
4. 2025	2.0
5. 2026	2.0

*Title: MRI: Acquisition of a Trapped Ion Mobility Spectrometer Quadrupole Time-of-Flight (TIMS-QTOF) with MALDI for Multidisciplinary Research and Education

*Major Goals: The major goal of this Major Research Instrumentation Grant project is to acquire a Bruker TIMS-QTOF with MALDI instrument for the University at Albany for multidisciplinary research and education across departments and colleges in the region.

*Status of Support: Active

Project Number: 2216089

Name of PD/PI: Yanna Liang

*Source of Support: NSF

*Primary Place of Performance: State University of New York at Albany

Project/Proposal Start and End Date: (MM/YYYY) (if available): 10/2022-09/2025

* Total Award Amount (including Indirect Costs): 1,099,000

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2023	0.25
2. 2024	0.25
3. 2025	0.25

*Title: Machine Learning Enabled Spatial Biology of Microbiomes

*Major Goals: The major goals of this project are to develop A.I. technologies to greatly expand the number of differentiable species of microbes in images of human microbiome samples, to generate a multi-laboratory use E. coli data set for benchmarking new spectral imaging technologies, and to design a software tool to design probes for closely related species of microbes that have highly homologous 16S rRNA sequences.

*Status of Support: Pending

Name of Individual:
Commons ID:

Project Number: 1R01GM163219-01

Name of PD/PI: Alex Valm & Yunlong Feng

*Source of Support: NIH

*Primary Place of Performance: State University of New York at Albany

Project/Proposal Start and End Date: (MM/YYYY) (if available): 12/2025-11/2030

* Total Award Amount (including Indirect Costs): \$3,607,126

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2026	2.0
2. 2027	2.0
3. 2028	2.0
4. 2029	2.0
5. 2030	2.0

*Title: Tools of a Pioneer Colonizer

*Major Goals: The major goals of this project are to identify the response of Streptococcus gordonii to different mucins, through structural biology of the muc5 binding surface protein and DNA/RNA sequencing and imaging of synthetic dental plaque communities.

*Status of Support: Pending

Project Number: Pending

Name of PD/PI: Mark Herzberg

*Source of Support: NIH

*Primary Place of Performance: University of Minnesota

Project/Proposal Start and End Date: (MM/YYYY) (if available): 4/2026-03/2031

* Total Award Amount (including Indirect Costs): \$397,861

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2026	1.0
2. 2027	1.0
3. 2028	1.0
4. 2029	1.0
5. 2030	1.0

IN-KIND

Name of Individual:
Commons ID:

*Summary of In-Kind Contribution: 12 strains of mutant *Streptococcus gordonii* from University of Minnesota

*Status of Support: Active

*Primary Place of Performance: State University of New York at Albany

Project/Proposal Start and End Date (MM/YYYY) (if available): 01/2024-12/2025

*Person Months (Calendar/Academic/Summer) per budget period

Year (YYYY)	Person Months (##.##)
1. [enter year 1]	0.25
2. [enter year 2]	0.25

*Estimated Dollar Value of In-Kind Information: \$120

*Summary of In-Kind Contribution: 10 strains of bacteria from the zebrafish gut microbiome from the Pasteur Institute

*Status of Support: Active

*Primary Place of Performance: State University of New York at Albany

Project/Proposal Start and End Date (MM/YYYY) (if available): 04/2025-03/2026

*Person Months (Calendar/Academic/Summer) per budget period

Year (YYYY)	Person Months (##.##)
1. 2025	0.25

*Estimated Dollar Value of In-Kind Information: \$1,000

*Summary of In-Kind Contribution: 1 competent *Streptococcus* cell line from University of Florida

*Status of Support: Active

*Primary Place of Performance: State University of New York at Albany

Project/Proposal Start and End Date (MM/YYYY) (if available): 05/2025-04/2028

*Person Months (Calendar/Academic/Summer) per budget period

Year (YYYY)	Person Months (##.##)
1. 2025	0.25
2. 2026	0.25
3. 2027	0.25

*Estimated Dollar Value of In-Kind Information: \$100

OMB No. 0925-0001 and 0925-0002 (Rev. 10/2021 Approved Through 09/30/2024)

Name of Individual:
Commons ID:

***Overlap** (summarized for each individual):
None

I, PD/PI or other senior/key personnel, certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

*Signature: Alex Valm

Date: September 13, 2025

Name: Ton-That, Hung
E-Commons ID: tonthat

OTHER SUPPORT – Project/Proposal

1. ACTIVE

Project/Proposal Title	Molecular Assembly on the Cell Surface of Actinomyces				
Major Goals	In this project, we continue to examine novel aspects of molecular assembly on the cell surface of <i>Actinomyces</i> spp. and the role of fimbrial and non-fimbrial proteins in <i>Actinomyces</i> interactions with other bacteria and host cells and in the formation of dental plaque or biofilms.				
Status of Support	Active				
Project Number	R01DE017382				
Name of PD/PI	Ton-That, Hung				
Source of Support	NIH-NIDCR National Institute of Dental and Craniofacial Research				
Primary Place of Performance	University of California, Los Angeles				
Project/Proposal Support Start Date	09/12/2024				
Project/Proposal Support End Date	08/31/2029				
Total Project Award Amount (including Indirect Costs)	\$2,142,640.00				
Person Months Per Budget Period	Year	Cal	Acad	Sum	
	2025	3.0			
	2026	3.0			
	2027	3.0			
	2028	3.0			
	2029	3.0			

Project/Proposal Title	Cell Surface Polymer Display in Gram-Positive Bacteria				
Major Goals	Research studies how Gram-positive bacteria assemble polymeric virulence factors, with a focus on sortase-mediated synthesis of pili, and glycosyltransferase-mediated assembly of wall teichoic acids. Both processes are potential targets for antibiotic development.				
Status of Support	Active				
Project Number	R01AI052217				
Name of PD/PI	Clubb, Robert Thompson				
Source of Support	NIH-NIAID National Institute of Allergy and Infectious Diseases				
Primary Place of Performance	University of California, Los Angeles				

Name: Ton-That, Hung
E-Commons ID: tonthat

Project/Proposal Support Start Date	07/15/2024				
Project/Proposal Support End Date	06/30/2029				
Total Project Award Amount (including Indirect Costs)	\$2,840,535.00				
Person Months Per Budget Period	Year	Cal	Acad	Sum	
	2025	0.6			
	2026	0.6			
	2027	0.6			
	2028	0.6			
	2029	0.6			

Project/Proposal Title	Assembly and Function of Outer Membrane Tubules in <i>Fusobacterium Nucleatum</i>				
Major Goals	This study aims to define the mechanism of outer membrane tubule (OMT) biogenesis and its role in metabolic adaptation and virulence of <i>Fusobacterium nucleatum</i> .				
Status of Support	Active				
Project Number	R01DE033900				
Name of PD/PI	Ton-That, Hung				
Source of Support	NIH-NIDCR National Institute of Dental and Craniofacial Research				
Primary Place of Performance	University of California, Los Angeles				
Project/Proposal Support Start Date	05/01/2024				
Project/Proposal Support End Date	04/30/2029				
Total Project Award Amount (including Indirect Costs)	\$2,313,094.00				
Person Months Per Budget Period	Year	Cal	Acad	Sum	
	2025	2.4			
	2026	2.4			
	2027	2.4			
	2028	2.4			
	2029	2.4			

Project/Proposal Title	Molecular Basis of Heme Scavenging by Gram-positive Bacteria				
Major Goals	The major goal of this project is to determine how Actinobacteria, using the human pathogen <i>Corynebacterium diphtheriae</i> as an experimental model, forage heme-iron from human hemoglobin via a				

Name: Ton-That, Hung
E-Commons ID: tonthat

	combinational approach of microbiology, proteomics, biochemistry, and structural biology.			
Status of Support	Active			
Project Number	R01AI161828			
Name of PD/PI	Clubb, Robert Thompson			
Source of Support	NIH-NIAID National Institute of Allergy and Infectious Diseases			
Primary Place of Performance	University of California, Los Angeles			
Project/Proposal Support Start Date	01/19/2021			
Project/Proposal Support End Date	12/31/2026			
Total Project Award Amount (including Indirect Costs)	\$3,016,482.00			
Person Months Per Budget Period (Annual Average)	Year	Cal	Acad	Sum
	2021 - 2025	0.6		
	2026	0.6		

Project/Proposal Title	UCLA Dentist-Scientist and Oral Health-Researcher Training Program
Major Goals	This R90 component of the T90/R90 research training program aims to provide PhD and postdoctoral training to non-US-citizen dentists with valid dental degrees from accredited institutions, who are currently in the U.S. The T90/R90 program seeks to bolster a vigorous and diverse dental, oral and craniofacial research workforce via a rigorous and interdisciplinary training program that offers trainees novel and innovative research training experiences in a highly supportive institutional environment. The training program will train the next generation of dentist-scientists and oral health scientists to conduct basic, translational, and clinical research to improve dental, oral, and craniofacial health.
Status of Support	Active
Project Number	R90DE031531
Name of PD/PI	Ton-That, Hung
Source of Support	NIH-NIDCR National Institute of Dental and Craniofacial Research
Primary Place of Performance	University of California, Los Angeles
Project/Proposal Support Start Date	07/01/2021
Project/Proposal Support End Date	06/30/2026

Name: Ton-That, Hung
E-Commons ID: tonthat

Total Project Award Amount (including Indirect Costs)	\$858,082.00				
Person Months Per Budget Period	Year	Cal	Acad	Sum	
	2026	0.6			

Project/Proposal Title	Metabolic Modulation of Fusobacterium Nucleatum Virulence				
Major Goals	This study aims to examine how ethanolamine metabolism by <i>F. nucleatum</i> modulates its virulence potential.				
Status of Support	NCE				
Project Number	R21DE032906				
Name of PD/PI	Ton-That, Hung				
Source of Support	NIH-NIDCR National Institute of Dental and Craniofacial Research				
Primary Place of Performance	University of California, Los Angeles				
Project/Proposal Support Start Date	03/01/2023				
Project/Proposal Support End Date	02/28/2026				
Total Project Award Amount (including Indirect Costs)	\$604,857.00				
Person Months Per Budget Period (Annual Average)	Year	Cal	Acad	Sum	
	2024 - 2026				

2. PENDING

Project/Proposal Title	Fusobacterium Nucleatum-derived Hydrogen Sulfide as a Fundamental Driver of CIMP Colorectal Cancer				
Major Goals	This study aims to determine how particular bacterial-derived H ₂ S can contribute to oncogenesis by rewiring host cell metabolism and epigenetic actions.				
Status of Support	Pending				
Project Number	Not Applicable				
Name of PD/PI	Andrew Intlekofer				
Source of Support	NIH - National Institutes of Health				
Primary Place of Performance	University of California, Los Angeles				
Project/Proposal Support Start Date	04/01/2026				
Project/Proposal Support End Date	03/31/2031				

Name: Ton-That, Hung
E-Commons ID: tonthat

Total Project Award Amount (including Indirect Costs)	\$480,375.00			
Person Months Per Budget Period	Year	Cal	Acad	Sum
	2029	0.6000	0.0000	0.0000
	2031	0.6000	0.0000	0.0000
	2027	0.6000	0.0000	0.0000
	2030	0.6000	0.0000	0.0000
	2028	0.6000	0.0000	0.0000

Project/Proposal Title	Genetic and Molecular Basis of LPS-associated Motility and Virulence in <i>Fusobacterium Nucleatum</i>			
Major Goals	This study aims to examine the role of LPS in the pathophysiology of <i>F. nucleatum</i> .			
Status of Support	Pending			
Project Number	R01			
Name of PD/PI	Ton-That, Hung			
Source of Support	NIH - National Institutes of Health			
Primary Place of Performance	University of California, Los Angeles			
Project/Proposal Support Start Date	04/01/2026			
Project/Proposal Support End Date	03/31/2031			
Total Project Award Amount (including Indirect Costs)	\$2,980,198.00			
Person Months Per Budget Period	Year	Cal	Acad	Sum
	2031	3.6000	0.0000	0.0000
	2029	3.6000	0.0000	0.0000
	2027	3.6000	0.0000	0.0000
	2028	3.6000	0.0000	0.0000
	2030	3.6000	0.0000	0.0000

Project/Proposal Title	Engineered <i>Veillonella parvula</i> for Tumor-Targeted Immunotherapy and Precision Elimination in Head and Neck Squamous Cell Carcinoma			
Major Goals	This study aims to engineer <i>V. parvula</i> for immunotherapy			
Status of Support	Pending			
Project Number	R21			
Name of PD/PI	Wen, Jing			
Source of Support	NIH - National Institutes of Health			
Primary Place of Performance	University of California, Los Angeles			

Name: Ton-That, Hung
E-Commons ID: tonthat

Project/Proposal Support Start Date	12/01/2025				
Project/Proposal Support End Date	11/30/2027				
Total Project Award Amount (including Indirect Costs)	\$433,125.00				
Person Months Per Budget Period	Year	Cal	Acad	Sum	
	2026	0.6000	0.0000	0.0000	
	2027	0.6000	0.0000	0.0000	

Project/Proposal Title	Genome-wide Identification of OSCC-associated Factors in Fusobacterium Nucleatum				
Major Goals	This study aims to identify <i>F. nucleatum</i> virulence factors associated with OSCC at the genome-wide level.				
Status of Support	Pending				
Project Number	R21				
Name of PD/PI	Ton-That, Hung				
Source of Support	NIH - National Institutes of Health				
Primary Place of Performance	University of California, Los Angeles				
Project/Proposal Support Start Date	07/01/2025				
Project/Proposal Support End Date	06/30/2027				
Total Project Award Amount (including Indirect Costs)	\$433,125.00				
Person Months Per Budget Period	Year	Cal	Acad	Sum	
	2027	1.2000	0.0000	0.0000	
	2026	1.2000	0.0000	0.0000	

3. IN-KIND CONTRIBUTIONS

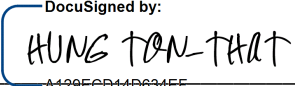
Summary of In Kind Contribution	NONE				
Status of Support					
Primary Place of Performance					
Person Months Per Budget Period	Year	Cal	Acad	Sum	
Estimated Dollar Value of In-Kind Information	\$				

4. OVERLAP

Name: Ton-That, Hung
E-Commons ID: tonthat

5. PI Certification

I, PD/PI or other senior/key personnel, certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

DocuSigned by:

A129ECD14D634EF...

Signature

9/15/2025

Date

PHS OTHER SUPPORT
For All Application Types – DO NOT SUBMIT UNLESS REQUESTED

There is no "form page" for reporting Other Support. Information on Other Support should be provided in the format shown below.

*Name of Individual: Troy D. Wood
 Commons ID: tdwood

Other Support – Project/Proposal

*Title: Clinical Pharmacology Quality Assurance (CPQA)

*Major Goals: The purpose of the NIAID Clinical Pharmacology Quality Assurance (CPQA) program is to provide a comprehensive quality assessment program for clinical pharmacologic laboratories testing samples from subjects enrolled in NIAID supported clinical studies.

*Status of Support: Active

Project Number: 75N93022C00009

Name of PD/PI: Morse, Gene D.

*Source of Support: NIH/NIAID

*Primary Place of Performance: University at Buffalo

Project/Proposal Start and End Date: (MM/YYYY) (if available): 05/2022-05/2029

* Total Award Amount (including Indirect Costs): \$14,561,135

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2025	1.20
2. 2026	1.20
3. 2027	1.20
4. 2028	1.20
5. 2029	1.20

*Title: Pharmacokinetics and Pharmacodynamics of Mechanistically Aware Phage Cocktails

*Major Goals: Cocktails of individually efficacious phages have been proposed as a strategy to create clinically viable phage therapeutics with sufficient spectrum of activity to combat highly resistant *Pseudomonas aeruginosa*. As such, this project will generate a highly optimized N-phage cocktail using a hybrid machine learning-pharmacokinetic/pharmacodynamic paradigm to strategically select N-phage components, dose, and administration schedule to maximize the killing of multi- and extensively-drug resistant *Pseudomonas aeruginosa*. This project will leverage phage whole-body distribution from our murine pneumonia model to establish a human, physiologically based pharmacokinetic model, which will allow detailed assessment of expected human exposures to phage and describe key aspects of target-mediated phage distribution.

*Status of Support: Active

OMB No. 0925-0001 and 0925-0002 (Rev. 10/2021 Approved Through 01/31/2026)

Name of Individual: Troy D. Wood
 Commons ID: tdwood

Project Number: R01AI177997-01

Name of PD/PI: Smith, Nicholas

*Source of Support: NIH/NIAID

*Primary Place of Performance: University at Buffalo

Project/Proposal Start and End Date: (MM/YYYY) (if available): 07/2024-07/2029

* Total Award Amount (including Indirect Costs): \$3,647,260

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2025	1.20
2. 2026	1.20
3. 2027	1.20
4. 2028	1.20
5. 2029	1.20

*Title: Characterization of the POEF Molecule

*Major Goals: The purpose of this award is to identify the molecule(s) present in amniotic fluid and placenta (POEF, placental opioid enhancing factor) whose activity seems to be specific to opioid-induced analgesia as it does not modify analgesia produced by non-opioid mediated noxious stimuli or non-opioid drug induced hypoalgesia.

*Status of Support: Active

Project Number: None

Name of PD/PI: Thompson, A.C.

*Source of Support: Nathan Goldin Research Fund, Community Foundation of Greater Buffalo

*Primary Place of Performance: University at Buffalo and Buffalo State University

Project/Proposal Start and End Date: (MM/YYYY) (if available): 08/2021-until funds spent (est. 08/26)

* Total Award Amount (including Indirect Costs): \$100,000

Year (YYYY)	Person Months (##.##)
1. 2025	0.60
2. 2026	0.60

*Title: Hearing Loss by the Synergistic Effect of Chronic Inhalation of Exposure of Manganese Fumes with Occupational Noise Exposure

*Major Goals: To determine if chronic inhalation of Mn fumes induces hearing loss and if it has a synergistic effect with concurrent occupational noise exposure.

*Status of Support: Active

OMB No. 0925-0001 and 0925-0002 (Rev. 10/2021 Approved Through 01/31/2026)

Name of Individual: Troy D. Wood
Commons ID: tdwood

Project Number: R21OH012389-01

Name of PD/PI: Krishnan Muthaiah, Vijaya Prakash

*Source of Support: CDC/NIOSH

*Primary Place of Performance: University at Buffalo

Project/Proposal Start and End Date: (MM/YYYY) (if available): 09/2024-09/2026

* Total Award Amount (including Indirect Costs): \$429,792

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2025	1.20 (1 Academic mo. and 0.2 summer mo.)
2. 2026	1.20 (1 Academic mo. and 0.2 summer mo.)

*Title: Identification of diffusible small molecules that regulate replication of *Porphyromonas gingivalis*

*Major Goals: *Porphyromonas gingivalis* (Pg) is a late-colonizer with a demonstrated role in the etiology of periodontitis and implicated as a risk factor in several systemic conditions. Our previous work showed that population size, which determines the availability of a still unidentified endogenous diffusible small molecule(s), limits the in vitro growth and the in vivo colonization and virulence of Pg. Our work also showed that the inability of Pg to grow in vitro and colonize in vivo from a small inoculum was overcome by forming a partnership with the ubiquitous Gramnegative early colonizer *Veillonella parvula* (Vp). In preliminary studies we show that intermediates of menaquinone biosynthesis may play a role stimulating growth of low cell-density Pg and may be produced by certain strains of Pg and by Vp. However, other strains of Pg capable of supporting their own growth via small diffusible cues present in their spent media may utilize a different strategy as they do not have the machinery for menaquinone biosynthesis. Accordingly, the overall goal of this application is to establish the identity of the growth-promoting cues produced by different strains of Pg and by Vp that support Pg replication from a low cell density population. Therefore, in this proposal we will utilize chemical and molecular genetic strategies to identify the soluble cues produced by Pg 381, Pg W83 and Vp that stimulate growth of Pg from low cell density inocula. We anticipate this work will elucidate the mechanisms regulating cell density-dependent replication in different strains of Pg and the manner in which inter-species interactions with early biofilm colonizers, such as Vp, can help Pg overcome the growth barrier and allow its replication and establishment in the oral cavity.

*Status of Support: ACTIVE

Project Number: R21DE034093-01A1

Name of PD/PI: Diaz-Moreno, Patricia

*Source of Support: NIH/NIDCR

*Primary Place of Performance: University at Buffalo

Project/Proposal Start and End Date: (MM/YYYY) (if available): 04/2025-03/2027

* Total Award Amount (including Indirect Costs): \$442,750

Name of Individual: Troy D. Wood
 Commons ID: tdwood

Year (YYYY)	Person Months (##.##)
1. 2026	0.60
2. 2027	0.60

*Title: Nutritional Metabolomics in Age-Related Macular Degeneration and Retinal Integrity

*Major Goals: Age-related macular degeneration (AMD) is a leading cause of vision loss in older adults; the burden of which is increasing. Existing research indicates that foods and nutrients influence AMD progression and may influence the structural integrity of the neurosensory retina. Several studies have shown that there are differentiating metabolomic profiles between individuals with and without AMD. *However, existing metabolomic studies of AMD have not examined metabolites using a hypothesis-driven approach focused on diet, and all but one was cross-sectional in design. Cross-sectional designs are limited by their inability to determine if metabolomic signatures are biomarkers for disease progression or biomarkers of a current disease state. To establish the temporality of these associations, prospective studies must be conducted. Furthermore, metabolomic biomarkers of dietary intake are not prone to the same measurement error and social desirability bias as with self-reported dietary intake.* The Carotenoids in Age-Related Eye Disease Study (CAREDS), an ancillary study of the Women's Health Initiative (WHI) Observational Study, was designed to study the role of nutrition in age-related eye disease in postmenopausal women with a baseline (2001-2004) and 15-year follow-up exam (2016-2019). CAREDS has a meticulously curated data set describing the full spectrum of the type and stage of AMD phenotypes, other indicators of retinal health, dietary intake, and genetic risk factors for AMD. We proposed to use mass spectrometry to analyze ~2,000 metabolites from targeted aqueous and lipid panels using stored participant serum from WHI onset (1993-1998). We aim to examine prospective associations between (a) derived metabolomic dietary pattern scores (MetDietPS), (b) individual metabolites (n~2,000), and (c) metabolic pathways, with **(Aim 1)** prevalent AMD assessed at CAREDS baseline (n=1,874) and the incidence of AMD over 15 years (n=1,296), **(Aim 2)** macular pigment optical density (MPOD) at CAREDS baseline (n=1,805) and changes in the MPOD over 15 years (n=427), and **(Aim 3)** the structural integrity of the neurosensory retina as indicated by the thickness of the retinal nerve fiber layer and ganglion cell complex of the macula measured at the CAREDS 15-year follow-up exam (n=454). MPOD is a measure of the concentration of retinal carotenoid pigments (lutein and zeaxanthin) in the macula and is hypothesized to be associated with a reduced risk of AMD. The MetDietPS will circumvent limitations present with self-reported diet. This study will deepen the understanding of the metabolic processes and pathways that link dietary intake to retinal health. Findings could lead to the identification of novel metabolomic biomarkers that would identify people most at risk for the development or progression of AMD and retinal degeneration with aging as well as targets for therapeutic interventions of AMD in drug/supplementation trials. Such work has the potential to help personalize dietary recommendations to maintain the integrity of the retina, moving us beyond the current nutritional treatment of a high-dose antioxidant supplementation.

*Status of Support: Active

Project Number: R01-EY036600-01A1

Name of PD/PI: Millen, Amy

*Source of Support: NIH/NEI

OMB No. 0925-0001 and 0925-0002 (Rev. 10/2021 Approved Through 01/31/2026)

Name of Individual: Troy D. Wood
Commons ID: tdwood

*Primary Place of Performance: University at Buffalo

Project/Proposal Start and End Date: (MM/YYYY) (if available): 08/2025-03/2029

* Total Award Amount (including Indirect Costs): \$3,182,065

Year (YYYY)	Person Months (##.##)
1. 2026	0.60
2. 2027	0.60
3. 2028	0.60
4. 2029	0.60

I, PD/PI or other senior/key personnel, certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

*Signature: _____ Date: _____

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

UEI*: LMCJKRFW5R81

Budget Type*: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: The Research Foundation for SUNY on behalf of U. at Buffalo

Start Date*: 07-01-2026 End Date*: 06-30-2027 Budget Period: 1

A. Senior/Key Person												
Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary (\$)	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits (\$)*	Funds Requested (\$)*
1 . Dr.	Dam		Soh		PD/PI	81,229.00	12.00			81,229.00	20,307.00	101,536.00
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons:		File Name:									Total Senior/Key Person	101,536.00

B. Other Personnel							
Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
Total Number Other Personnel						Total Other Personnel	
						Total Salary, Wages and Fringe Benefits (A+B)	101,536.00

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

UEI*: LMCJKRFW5R81
Budget Type*: ☒ Project ☐ Subaward/Consortium
Organization: The Research Foundation for SUNY on behalf of U. at Buffalo

Start Date*: 07-01-2026 End Date*: 06-30-2027 Budget Period: 1

C. Equipment Description	
List items and dollar amount for each item exceeding \$5,000	
Equipment Item	Funds Requested (\$)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	Funds Requested (\$)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,300.00
2. Foreign Travel Costs	
Total Travel Cost	3,300.00

E. Participant/Trainee Support Costs	Funds Requested (\$)*
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	Total Participant Trainee Support Costs

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

UEI*: LMCJKRFW5R81

Budget Type*: ☒ Project ☐ Subaward/Consortium

Organization: The Research Foundation for SUNY on behalf of U. at Buffalo

Start Date*: 07-01-2026

End Date*: 06-30-2027

Budget Period: 1

F. Other Direct Costs	Funds Requested (\$)*
1. Materials and Supplies	7,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Data Management and Sharing Plan Costs	144.00
9. Services Fee	1,000.00
10. Subject Recruitment	400.00
11. Sequencing Core Services - Aim 2A	550.00
12. Sequencing Core Services - Aim 2B	8,000.00
13. Course Tuition, Room, and Board	4,500.00
Total Other Direct Costs	21,594.00

G. Direct Costs	Funds Requested (\$)*
Total Direct Costs (A thru F)	126,430.00

H. Indirect Costs			
Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
1 . MTDC	8.00	126,430.00	10,114.00
Total Indirect Costs			10,114.00
Cognizant Federal Agency	DHHS Mike Leonard 212-264-4310		
(Agency Name, POC Name, and POC Phone Number)			

I. Total Direct and Indirect Costs	Funds Requested (\$)*
Total Direct and Indirect Institutional Costs (G + H)	136,544.00

J. Fee	Funds Requested (\$)*
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K. Total Costs and Fee	Funds Requested (\$)*
	136,544.00

L. Budget Justification*	File Name: 1234-BUDGET JUSTIFICATION_Dam.pdf
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RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

UEI*: LMCJKRFW5R81

Budget Type*: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: The Research Foundation for SUNY on behalf of U. at Buffalo

Start Date*: 07-01-2027 End Date*: 06-30-2028 Budget Period: 2

A. Senior/Key Person												
Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary (\$)	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits (\$)*	Funds Requested (\$)*
1 . Dr.	Dam		Soh	DDS	PD/PI	83,666.00	12.00			83,666.00	20,080.00	103,746.00
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons:		File Name:									Total Senior/Key Person	103,746.00

B. Other Personnel							
Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
Total Number Other Personnel						Total Other Personnel	
						Total Salary, Wages and Fringe Benefits (A+B)	103,746.00

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

UEI*: LMCJKRFW5R81
Budget Type*: ☒ Project ☐ Subaward/Consortium
Organization: The Research Foundation for SUNY on behalf of U. at Buffalo

Start Date*: 07-01-2027 End Date*: 06-30-2028 Budget Period: 2

C. Equipment Description	
List items and dollar amount for each item exceeding \$5,000	
Equipment Item	Funds Requested (\$)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	Funds Requested (\$)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	5,000.00
2. Foreign Travel Costs	
Total Travel Cost	5,000.00

E. Participant/Trainee Support Costs	Funds Requested (\$)*
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	Total Participant Trainee Support Costs

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

UEI*: LMCJKRFW5R81

Budget Type*: ☒ Project ☐ Subaward/Consortium

Organization: The Research Foundation for SUNY on behalf of U. at Buffalo

Start Date*: 07-01-2027

End Date*: 06-30-2028

Budget Period: 2

F. Other Direct Costs	Funds Requested (\$)*
1. Materials and Supplies	9,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Data Management and Sharing Plan Costs	0.00
9. Services Fee	1,000.00
10. Subject Recruitment	400.00
11. Sequencing Core Services - Aim 2A	550.00
12. Sequencing Core Services - Aim 2B	9,000.00
13. Course Tuition, Room, and Board	0.00
Total Other Direct Costs	19,950.00

G. Direct Costs	Funds Requested (\$)*
Total Direct Costs (A thru F)	128,696.00

H. Indirect Costs			
Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
1 . MTDC	8.00	128,696.00	10,296.00
Total Indirect Costs			10,296.00
Cognizant Federal Agency	DHHS Mike Leonard 212-264-4310		
(Agency Name, POC Name, and POC Phone Number)			

I. Total Direct and Indirect Costs	Funds Requested (\$)*
Total Direct and Indirect Institutional Costs (G + H)	138,992.00

J. Fee	Funds Requested (\$)*
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K. Total Costs and Fee	Funds Requested (\$)*
	138,992.00

L. Budget Justification*	File Name: 1234-BUDGET JUSTIFICATION_Dam.pdf
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RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

UEI*: LMCJKRFW5R81

Budget Type*: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: The Research Foundation for SUNY on behalf of U. at Buffalo

Start Date*: 07-01-2028 End Date*: 06-30-2029 Budget Period: 3

A. Senior/Key Person												
Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary (\$)	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits (\$)*	Funds Requested (\$)*
1 . Dr.	Dam		Soh	DDS	PD/PI	86,176.00	12.00			86,176.00	20,682.00	106,858.00
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons:		File Name:									Total Senior/Key Person	106,858.00

B. Other Personnel							
Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
Total Number Other Personnel						Total Other Personnel	
						Total Salary, Wages and Fringe Benefits (A+B)	106,858.00

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

UEI*: LMCJKRFW5R81
Budget Type*: ☒ Project ☐ Subaward/Consortium
Organization: The Research Foundation for SUNY on behalf of U. at Buffalo

Start Date*: 07-01-2028 End Date*: 06-30-2029 Budget Period: 3

C. Equipment Description	
List items and dollar amount for each item exceeding \$5,000	
Equipment Item	Funds Requested (\$)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	Funds Requested (\$)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,500.00
2. Foreign Travel Costs	
Total Travel Cost	3,500.00

E. Participant/Trainee Support Costs	Funds Requested (\$)*
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	Total Participant Trainee Support Costs

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

UEI*: LMCJKRFW5R81

Budget Type*: ☒ Project ☐ Subaward/Consortium

Organization: The Research Foundation for SUNY on behalf of U. at Buffalo

Start Date*: 07-01-2028

End Date*: 06-30-2029

Budget Period: 3

F. Other Direct Costs	Funds Requested (\$)*
1. Materials and Supplies	8,500.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Data Management and Sharing Plan Costs	0.00
9. Services Fee	1,000.00
10. Subject Recruitment	400.00
11. Sequencing Core Services - Aim 2A	550.00
12. Sequencing Core Services - Aim 2B	9,000.00
13. Course Tuition, Room, and Board	0.00
Total Other Direct Costs	21,450.00

G. Direct Costs	Funds Requested (\$)*
Total Direct Costs (A thru F)	131,808.00

H. Indirect Costs			
Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
1 . MTDC	8.00	131,808.00	10,545.00
Total Indirect Costs			10,545.00
Cognizant Federal Agency		DHHS Mike Leonard 212-264-4310	
(Agency Name, POC Name, and POC Phone Number)			

I. Total Direct and Indirect Costs	Funds Requested (\$)*
Total Direct and Indirect Institutional Costs (G + H)	142,353.00

J. Fee	Funds Requested (\$)*
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K. Total Costs and Fee	Funds Requested (\$)*
	142,353.00

L. Budget Justification*	File Name: 1234-BUDGET JUSTIFICATION_Dam.pdf
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RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

UEI*: LMCJKRFW5R81

Budget Type*: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: The Research Foundation for SUNY on behalf of U. at Buffalo

Start Date*: 07-01-2029 End Date*: 06-30-2030 Budget Period: 4

A. Senior/Key Person												
Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary (\$)	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits (\$)*	Funds Requested (\$)*
1 . Dr.	Dam		Soh	DDS	PD/PI	88,761.00	12.00			88,761.00	21,303.00	110,064.00
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons:		File Name:									Total Senior/Key Person	110,064.00

B. Other Personnel							
Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
Total Number Other Personnel						Total Other Personnel	
						Total Salary, Wages and Fringe Benefits (A+B)	110,064.00

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

UEI*: LMCJKRFW5R81
Budget Type*: ☒ Project ☐ Subaward/Consortium
Organization: The Research Foundation for SUNY on behalf of U. at Buffalo

Start Date*: 07-01-2029 End Date*: 06-30-2030 Budget Period: 4

C. Equipment Description	
List items and dollar amount for each item exceeding \$5,000	
Equipment Item	Funds Requested (\$)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	Funds Requested (\$)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	
Total Travel Cost	4,000.00

E. Participant/Trainee Support Costs	Funds Requested (\$)*
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	Total Participant Trainee Support Costs

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

UEI*: LMCJKRFW5R81

Budget Type*: ☒ Project ☐ Subaward/Consortium

Organization: The Research Foundation for SUNY on behalf of U. at Buffalo

Start Date*: 07-01-2029

End Date*: 06-30-2030

Budget Period: 4

F. Other Direct Costs	Funds Requested (\$)*
1. Materials and Supplies	10,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Data Management and Sharing Plan Costs	0.00
9. Services Fee	1,000.00
10. Subject Recruitment	400.00
11. Sequencing Core Services - Aim 2A	550.00
12. Sequencing Core Services - Aim 2B	9,000.00
13. Course Tuition, Room, and Board	0.00
Total Other Direct Costs	20,950.00

G. Direct Costs	Funds Requested (\$)*
Total Direct Costs (A thru F)	135,014.00

H. Indirect Costs			
Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
1 . MTDC	8.00	135,014.00	10,801.00
Total Indirect Costs			10,801.00
Cognizant Federal Agency	DHHS Mike Leonard 212-264-4310		
(Agency Name, POC Name, and POC Phone Number)			

I. Total Direct and Indirect Costs	Funds Requested (\$)*
Total Direct and Indirect Institutional Costs (G + H)	145,815.00

J. Fee	Funds Requested (\$)*
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K. Total Costs and Fee	Funds Requested (\$)*
	145,815.00

L. Budget Justification*	File Name: 1234-BUDGET JUSTIFICATION_Dam.pdf
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RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

UEI*: LMCJKRFW5R81

Budget Type*: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: The Research Foundation for SUNY on behalf of U. at Buffalo

Start Date*: 07-01-2030 End Date*: 06-30-2031 Budget Period: 5

A. Senior/Key Person												
Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary (\$)	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits (\$)*	Funds Requested (\$)*
1 . Dr.	Dam		Soh	DDS	PD/PI	91,424.00	12.00			91,424.00	21,942.00	113,366.00
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons:		File Name:									Total Senior/Key Person	113,366.00

B. Other Personnel							
Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
Total Number Other Personnel						Total Other Personnel	
						Total Salary, Wages and Fringe Benefits (A+B)	113,366.00

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

UEI*: LMCJKRFW5R81
Budget Type*: ☒ Project ☐ Subaward/Consortium
Organization: The Research Foundation for SUNY on behalf of U. at Buffalo

Start Date*: 07-01-2030 End Date*: 06-30-2031 Budget Period: 5

C. Equipment Description	
List items and dollar amount for each item exceeding \$5,000	
Equipment Item	Funds Requested (\$)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	Funds Requested (\$)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	5,000.00
2. Foreign Travel Costs	
Total Travel Cost	5,000.00

E. Participant/Trainee Support Costs	Funds Requested (\$)*
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	Total Participant Trainee Support Costs

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

UEI*: LMCJKRFW5R81

Budget Type*: ☒ Project ☐ Subaward/Consortium

Organization: The Research Foundation for SUNY on behalf of U. at Buffalo

Start Date*: 07-01-2030

End Date*: 06-30-2031

Budget Period: 5

F. Other Direct Costs	Funds Requested (\$)*
1. Materials and Supplies	6,000.00
2. Publication Costs	5,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Data Management and Sharing Plan Costs	0.00
9. Services Fee	0.00
10. Subject Recruitment	0.00
11. Sequencing Core Services - Aim 2A	0.00
12. Sequencing Core Services - Aim 2B	9,000.00
13. Course Tuition, Room, and Board	0.00
Total Other Direct Costs	20,000.00

G. Direct Costs	Funds Requested (\$)*
Total Direct Costs (A thru F)	138,366.00

H. Indirect Costs			
Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
1 . MTDC	8.00	138,366.00	11,069.00
Total Indirect Costs			11,069.00
Cognizant Federal Agency	DHHS Mike Leonard 212-264-4310		
(Agency Name, POC Name, and POC Phone Number)			

I. Total Direct and Indirect Costs	Funds Requested (\$)*
Total Direct and Indirect Institutional Costs (G + H)	149,435.00

J. Fee	Funds Requested (\$)*
---------------	------------------------------

K. Total Costs and Fee	Funds Requested (\$)*
	149,435.00

L. Budget Justification*	File Name: 1234-BUDGET JUSTIFICATION_Dam.pdf
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RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION

A. Senior Personnel

Dam Soh, MS, DDS, Principal Investigator (PI), will receive 12 calendar months of support for each year of the mentored K08 study. The PI will oversee the proposed project and be responsible for all components, including data analysis, sample collections and communication of findings in peer-reviewed publications and professional conferences. The PI will devote 100% effort to the proposed research and training activities over five years.

Fringe Benefits

Fringe benefit rates are based on the current state and federally negotiated agreements.

B. Other Direct Costs

Materials and Supplies

- **Disposables:** for pipette tips, plates, centrifuge tubes, conical tubes, microbial cultivation supplies, metabolomic supplies and related consumables.
 - \$3,000/year in Year 1
 - \$4000/year in Year 2
 - \$3500/year in Year 3
 - \$5000/year in Year 4 and 5
- **Reagents:** \$3,000/year in Years 1–4 for DNA and RNA extraction kits, probes, and dyes.
- **Miscellaneous Supplies / Maintenance:** \$1,000/year in Years 2–5 for general laboratory needs.

Services Fee

\$1000/year in Years 1-4 are requested for use of the core equipment including confocal microscopy (Aim 1 and 2)

Clinical supplies related to subject recruitment

\$1000/year in Years 1-4 are requested for use of the research clinic (Aim 2a)

Subject Recruitment

\$400/year in Years 1–4 is requested to support recruitment (Aim 2a). Each subject will complete three visits (initial exam, membrane insertion, and membrane collection). Subjects will be compensated \$50 for visit 2 and visit 3. Each year, there will be 3 participants recruited. Requested cost will cover visit 2 and 3 for three subjects plus additional 1 subject in case of subject compliance does not met.

Sequencing Core Services

Library preparation and 16S rRNA sequencing (Aim 2a) and metatranscriptomic sequencing (Aim 2b) will be performed at the UB Genomics Core Facility using the Illumina NovaSeq platform.

- **Aim 2a:** We will recruit 10 subjects and collect plaque samples from the deepest site in each quadrant (4 sites/subject; 40 total samples) across Years 1–4. To account for cases in which *P. gingivalis* is present at relative abundance >1%, an additional 10% (4 samples) is included for testing alternate sites, for a total of 44 samples. Sequencing costs are \$50 per sample. The total request is \$2,200 (\$550/year in Years 1–4). DNA extraction will be performed by the PI; extraction kits are included under Reagents.
- **Aim 2b:** We will recruit 80 subjects (40 Pg-positive and 40 Pg-negative) and collect plaque samples from the deepest site in each side (2 sites/subject; 160 total samples) across Years 1–5. To allow for repeats, an additional 10% (16 samples) is included, for a total of 88 samples. Sequencing costs are \$250 per sample. The total request is \$44,000 (\$8,000/year in Year 1 and \$9000/year in Years 2–5). RNA extraction will be performed by the PI; extraction kits are included under Reagents.

Data Management and Sharing Justification:

To support data management and storage costs, these funds are requested under Other Direct Costs. Metatranscriptomic sequencing data to access the community function of subgingival plaque will be generated. Sequencing data and metadata will be available in the GenBank and Sequence Read Archive (SRA) of NCBI, which are NIH approved repositories for genomic studies (No cost). The PI, Dr. Dam Soh, will be responsible for curating data and developing supporting documentation (No cost). High-performance Computing (HPC) Resources at The University at Buffalo will be used for data analysis and local data storage for 2TB (\$72/TB). Estimated total direct costs: \$144.

Publication Costs

Publication fees of \$2,000 are requested for Year 3 and \$5,000 for Year 5. These funds will support open-access publication of research findings in peer-reviewed journals

Travel

Funds are requested for scientific meetings to present research data findings and to collaborate with peers. The travel costs will include conference/workshop registration, air and local travel, lodging and meals per diem.

- \$3,300 in Years 1 (1 domestic conference and travel for Analytical and Quantitative Light Microscopy course)
- \$5,000 in Year 2 (1 domestic and 1 international conference)
- \$3,500 in Year 3 (2 domestic conferences)
- \$4,000 in Year 4 (1 International conference)
- \$5,000 in Year 5 (1 domestic and 1 international conference)

Planned Domestic meetings:

- Year 1: Penn Perio
- Year 2: American Academy of Periodontology Conference (AAP)
- Year 3: Penn Perio, AADOCR
- Year 5: American Society of Microbiology Conference

Planned International meetings:

- Year 2: International Association for Dental Research (IADR)
- Year 4: International Conference on Oral Mucosal Immunity and Microbiome
- Year 5: International Association for Dental Research (IADR)

Course Tuition, Room, and Board

In Year 1, \$3,600 is requested for tuition and \$900 for room and board to attend the Analytical and Quantitative Light Microscopy course by Marine Biological Laboratory at Woods Hole, MA.

Indirect Costs

The indirect costs are calculated at the University at buffalo predetermined Facilities and Administrative (F&A) cost rate of 8% TDC per Sponsor Guidelines.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals (\$)	
Section A, Senior/Key Person		535,570.00
Section B, Other Personnel		
Total Number Other Personnel		
Total Salary, Wages and Fringe Benefits (A+B)		535,570.00
Section C, Equipment		
Section D, Travel		20,800.00
1. Domestic	20,800.00	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		103,944.00
1. Materials and Supplies	40,500.00	
2. Publication Costs	7,000.00	
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	144.00	
9. Other 2	4,000.00	
10. Other 3	1,600.00	
11. Other 4	2,200.00	
12. Other 5	44,000.00	
13. Other 6	4,500.00	
14. Other 7		
15. Other 8		
16. Other 9		
17. Other 10		
Section G, Direct Costs (A thru F)		660,314.00
Section H, Indirect Costs		52,825.00
Section I, Total Direct and Indirect Costs (G + H)		713,139.00
Section J, Fee		
Section K, Total Costs and Fee (I + J)		713,139.00

PHS 398 Cover Page Supplement**1. Vertebrate Animals Section**

Are vertebrate animals euthanized? ☐ Yes ☐ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

☐ Yes ☐ No

If "No" to AVMA guidelines, describe method and provide scientific justification

.....

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

☐ Yes ☒ No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount (\$)	*Source(s)
----------------	--------------------------	------------

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ☐ Yes ☒ No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

☐ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Human Fetal Tissue Section

*Does the proposed project involve human fetal tissue obtained from elective abortions? ☐ Yes ☒ No

If "yes" then provide the HFT Compliance Assurance

If "yes" then provide the HFT Sample IRB Consent Form

5. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ☐ Yes ☐ No

If the answer is "Yes" then please answer the following:

*Previously Reported: ☐ Yes ☐ No

6. Change of Investigator/Change of Recipient Organization Section

☐ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

☐ Change of Recipient Organization

*Name of former organization:

PHS 398 Career Development Award Supplemental Form

OMB Number: 0925-0001

Expiration Date: 12/31/2027

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	
Candidate Section	
2. Candidate Information and Goals for Career Development	1240-Cand info.pdf
Research Plan Section	
3. Specific Aims	1241-Specific Aims_1 page.pdf
4. Research Strategy*	1242-Strategy.pdf
5. Progress Report Publication List (for Renewal applications)	
6. Training in the Responsible Conduct of Research	1243-Training_Responsible Conduct of Research.pdf
Other Candidate Information Section	
7. Candidate's Plan to Provide Mentoring	
Mentor, Co-Mentor, Consultant, Collaborators Section	
8. Plans and Statements of Mentor and Co-Mentor(s)	1244-Mentor statements final.pdf
9. Letters of Support from Collaborators, Contributors, and Consultants	
Environment and Institutional Commitment to Candidate Section	
10. Description of Institutional Environment	1245-Description of Institutional Environment.pdf
11. Institutional Commitment to Candidate's Research Career Development	1246-Institutional Committment.pdf
12. Description of Candidate's Contribution to Program Goals	
Other Research Plan Section	
13. Vertebrate Animals	
14. Select Agent Research	
15. Consortium/Contractual Arrangements	
16. Resource Sharing	1247-Resource Sharing Plan.pdf
17. Other Plan(s)	1248-DMSP_Soh_Final.pdf
18. Authentication of Key Biological and/or Chemical Resources	1249-AKBCR_Final.pdf
Appendix	
19. Appendix	

PHS 398 Career Development Award Supplemental Form

Citizenship*:

20. U.S. Citizen or Non-Citizen National?* ☐ Yes ☒ No

If no, select most appropriate Non-U.S. Citizen option

- ☒ With a Permanent U.S. Resident Visa
- ☐ With a Temporary U.S. Visa
- ☐ Not Residing in the U.S.

If you are a non-U.S. citizen with a temporary visa applying for an award that requires permanent residency status, and expect to be granted a permanent resident visa by the start date of the award, check here: ☐

Candidate Information and Goals for Career Development

CANDIDATE BACKGROUND

My research journey began as an undergraduate in Molecular Ecology, where I studied microbial ecosystems in wetlands, sparking my passion for microbiology. During my Master's degree at Seoul National University, I conducted health-focused projects testing natural compounds in *in vitro* and animal models through collaborations with the Gajin Jung Immunology Institute, gaining expertise in microbial, molecular, and animal model techniques. These experiences broadened my laboratory skills, deepened my understanding of microbiology and immunology, and taught me to manage complex research projects while highlighting the importance of translating laboratory discoveries into clinical applications. After completing my master's degree, I returned to the United States to pursue dental training at the University at Buffalo. Throughout dental school, I remained actively engaged in research, presenting findings at institutional and national conferences while fulfilling rigorous clinical requirements. This commitment to integrating research and clinical training led me to the NIH K12 Institutional Career Development Program, where I am pursuing dual degrees in a Periodontology certification and a PhD in Oral Biology. Over the past four years, I have gained comprehensive clinical expertise in periodontal diagnosis, treatment planning, and disease management across the full spectrum of periodontal conditions. Working closely with multidisciplinary teams has broadened my perspective beyond periodontics to overall oral health. I have also developed refined clinical dexterity, enabling not only precise patient treatment but also thorough oral examinations and reliable sample acquisition from research participants, skills that directly enhance the quality and rigor of my translational research.

My PhD research under the mentorship of Dr. Patricia Diaz investigates fundamental questions regarding how subgingival microbial species modulate pathogen colonization through inter-species interactions and environmental modulation, with the goal of identifying strategies to prevent dysbiotic shifts. I have uncovered a previously unrecognized three-species interaction within the subgingival microbiome, in which metabolic cross-feeding shapes environmental pH and directly influences pathogen establishment. Mechanistically, I identified the gene product in *Actinomyces oris* responsible for inhibiting *Porphyromonas gingivalis* by constructing mutants in collaboration with the laboratory of Dr. Ton-That (mentoring committee). In parallel, I collaborated in the Diaz lab on a project examining the effects of serum, as a surrogate for inflammatory exudate, on a complex synthetic community model of the subgingival microbiome, providing insights into how host-derived factors influence subgingival microbiome dynamics. Additionally, I developed a liquid chromatography–mass spectrometry (LC-MS) method in Dr. Wood's lab (mentoring committee) to identify and quantify trace amounts of organic acids in biological samples. Through these studies, I have developed advanced expertise across multiple domains critical for independent research success, including microbial cultivation methodologies, biofilm model development, metabolomics using LC-MS, and molecular biology techniques. Beyond technical training, I have attended grant-writing workshops and initiated training in bioinformatics for 16S rRNA gene sequencing and metagenomic datasets. I have also gained extensive experience presenting to different audiences through weekly departmental Journal Club participation and presentation of my work at institutional and national conferences. To date, I have co-authored two publications, with additional manuscripts in preparation based on my PhD research.

Together, my academic, clinical, and research experiences provide a strong foundation for my trajectory toward becoming an independent clinician-scientist, uniquely positioned to conduct research in oral microbiology and periodontal pathogenesis.

CAREER GOALS AND OBJECTIVES

My research objective is to advance understanding of microbial pathogenesis in periodontitis by defining how inter-species interactions and environmental factors shape biofilm architecture and enable or antagonize pathogen colonization. My long-term goal is to establish an independent career as a clinician-scientist conducting translational research that bridges mechanistic discoveries in oral microbiology with therapeutic innovations in periodontology. Building on my current work, this project will characterize the spatial organization of subgingival plaque biofilms and investigate how metabolically distinct microenvironments emerge from inter-species metabolic interactions. These studies will provide mechanistic insight into the drivers of community dysbiosis and lay the foundation for developing novel strategies to prevent or disrupt pathogenic biofilm states. Through this research, I aim to advance fundamental understanding of disease initiation and progression.

To achieve my career objectives, I have identified critical areas requiring additional mentored training:

Advanced imaging techniques: including confocal microscopy and spectral imaging, will provide unprecedented insights into dynamic microbial interactions within biofilms. (In collaboration with Dr. Alex Valm, I will be appointed at U. Albany as a visiting scholar to be able to access Dr. Valm's lab and resources and receive hands-on mentorship for Aim 2a)

Bioinformatic analysis: proficiency in analyzing 16S rRNA amplicon data and metagenomic datasets is crucial for comprehensive characterization of microbial community functions and identification of key species and functional pathways associated with disease. (In the Diaz lab)

Liquid chromatography-mass spectrometry (LC-MS): I aim to gain further expertise in LC-MS, applying this technique to the analysis of GCF samples from research participants. (In collaboration with Dr. Troy Wood)

Molecular genetics: I will continue to seek mentorship for creation of bacterial mutants as required by my project.

This multidisciplinary expertise will provide powerful tools for investigating microbial pathogenesis and broaden my capabilities across complementary research areas. I have assembled a strong mentorship team who will guide me in developing the skills to conceptualize, design, and execute independent research. Their mentorship will extend beyond technical training to include career development, grant writing, manuscript preparation, and professional networking, ensuring comprehensive preparation for independent academic research and clinical practice. In my third training year, coinciding with PhD completion, I plan to pursue a tenure-track faculty appointment at a leading research university. My goal is to secure a position that maintains the translational focus defining my research vision, providing access to clinical populations for translational studies and state-of-the-art facilities for mechanistic investigations. This positioning will enable establishment of an independent research laboratory while maintaining essential clinical engagement. The proposed K08 research represents the logical next step in my career trajectory, providing the resources and mentorship needed to establish independence as a clinician-scientist. My current K12 training has successfully demonstrated my ability to balance clinical responsibilities with research productivity, establishing a solid foundation for a successful independent career conducting translational research.

CANDIDATE'S PLAN FOR CAREER DEVELOPMENT

For this K08 proposal, I have assembled a distinguished mentoring committee consisting of my primary mentor, Dr. Patricia I. Diaz (Professor of Empire Innovation and Director of the UB Microbiome Center, University at Buffalo), and co-mentors Dr. Hung Ton-That (Professor, Division of Oral and Systemic Health Sciences Chair, UCLA), Dr. Alex Valm (Assistant Professor, Department of Biological Sciences, University at Albany), and Dr. Troy D. Wood (Professor, Department of Chemistry, University at Buffalo). This exceptional team will provide comprehensive guidance throughout this training period, ensuring my successful development as an independent clinician-scientist.

Scientific Research Training:

- Dr. Diaz will provide ongoing feedback and guidance through weekly one-on-one meetings and group discussions. Having worked closely with her during my PhD, I gained experience in biofilm models and contributed to the lab's bioinformatics projects. I have basic coding skills and have gained proficiency in MATLAB. Under her mentorship, I will continue to gain expertise in periodontal microbiome ecology, including inter-species interaction analysis, and further develop expertise in bioinformatic analysis and interpretation.

- Dr. Ton-That will provide expertise in bacterial genetics, with a focus on mutant strain construction. He will continue to train me in designing and generating targeted gene knockouts as needed by the project. He will also assist in interpreting experimental results and troubleshooting technical challenges, ensuring that I gain a strong foundation in microbial genetics.

- Dr. Valm will mentor me in advanced imaging and computational analysis of microbial communities, providing hands-on guidance in image acquisition, processing, and quantitative analysis. He will provide access to state-of-the-art imaging resources and advise on experimental design, data interpretation, and integration with other datasets, supporting my development as an independent researcher in microbial spatial ecology. Also, I will enroll in the **Analytical and Quantitative Light Microscopy course** at the Marine Biological Laboratory to advance my imaging expertise. This intensive course, which Dr. Valm and his students have previously attended, provides

a strong theoretical and practical foundation that directly complements my training. The program covers microscopy theory and quantitative analysis, including fluorescence and label-free imaging, live-cell imaging and digital image processing. Through lectures and hands-on exercises, I will acquire high-quality imaging and quantitative analysis skills essential for studying biofilm spatial architecture and microbial dynamics.

- Dr. Wood and I have worked closely to develop a targeted LC-MS method for detecting and quantifying trace amounts of organic acids produced by microbial communities. He will provide expert guidance in advanced analytical chemistry techniques, including method optimization, calibration, validation, and troubleshooting of LC-MS workflows. Additionally, Dr. Wood will advise on experimental design to ensure robust and reproducible metabolite identification and quantification and offer further technical guidance as needed to support the successful completion of my research aims.

Lastly, I will review data and progress with my mentoring team during bi-monthly meetings and actively participate in professional meetings to present findings, foster collaborations, and stay current in oral microbiology, supporting my growth as an independent researcher.

Career Development Training: I will participate in career development and training activities strategically designed to address knowledge gaps, develop essential skills, and prepare for an independent research career. To enhance grant-writing and scientific communication, I will prepare annual K08 progress reports, participate in the Office of Research and Academic (ORA) yearly grant-writing workshops for proposal development and compliance, and write manuscripts under Dr. Diaz's guidance to improve communication of complex research findings for high-impact journals. I plan to apply for AADOCR's *Mind the Future* program to develop research and grant-writing competence, career development, personal growth, and interpersonal and communication skills. Building on my clinical and didactic teaching experience with dental students, I will participate in UB's Preparing for Academic Careers Seminar Series to learn effective teaching strategies and mentor undergraduate and dental students in the Diaz Laboratory to gain experience guiding junior researchers. For research ethics, I will complete the Responsible Conduct of Research (RCR) program through UB CTSI to uphold research integrity standards. To prepare for the academic job market, I will attend the Job Search Bootcamp workshop at UB's Career Design Center for interview preparation and salary negotiation training. Throughout this period, I will utilize personalized career counseling services and ongoing guidance from my mentoring committee for strategic professional decision-making and successful transition to an independent position.

These career development activities complement my research training and will provide the multifaceted skill set necessary to succeed as an independent clinician-scientist conducting translational research in oral microbiology.

Table 1: Proposed Activities of Training Objectives

Training Objective	Modality	Specific Developmental Activities	Period of Activity during Award				
			Y1	Y2	Y3	Y4	Y5
Objective 1: Research • Extend knowledge of Microbial ecology, advanced imaging and bioinformatics • Receive training from mentor and co-mentors • Examine proposed specific aims • Present research findings	Training from Mentors	Primary Mentor: Dr. Diaz, Weekly meetings	X	X	X	X	X
		Visit Dr. Alex Valm's laboratory (U. Albany) as required for hands-on mentoring	X	X	X	X	X
		Project discussion with mentor committee: bi-monthly as a team, monthly one-on-one	X	X	X	X	X
	Project Activity	Research on Specific Aims	X	X	X	X	X
		Paper Writing and Submission	X	X	X	X	X
Meetings/Conferences	Presentation at research meetings	X	X	X	X	X	
Objective 2: Career Development • Learn skills to effectively publish and communicate research findings • Develop ability to be independence • Collaborate with interdisciplinary experts	Coursework	Responsible Conduct of Research (RCR)	X				
		Analytical and Quantitative Light Microscopy	X				
	Seminars/Workshops	Professional development Workshop series	X	X			
		ORA grant writing workshop	X	X			
		Job Search Bootcamp Workshop		X			
		AADOCR Mind the Future				X	
	Meetings/Conferences	University at Buffalo /University of Rochester Microbiome Group Meeting, monthly	X	X	X	X	X
	Skills training from mentoring team	Manuscript writing skills	X	X	X	X	X
		Grant writing skills	X	X	X	X	X
		Lab and research budget management	X	X	X	X	X
		Public presentation skills	X	X	X	X	X
		Career Development	X	X	X	X	X
	Service	Mentor graduate / dental students	X	X	X	X	X

SPECIFIC AIMS Periodontitis is a prevalent chronic inflammatory disease affecting nearly half of US adults¹, and is driven by a dysbiotic subgingival microbiome that triggers and perpetuates periodontal tissue destruction. The shifts in the subgingival microbiome composition, structure, and function that occur with periodontitis are governed by a range of still incompletely understood ecological factors^{2,3}. Among these, environmental pH is a critical ecological factor that shapes microbial communities, as bacterial metabolism alters local pH, creating niches that select for species with specific pH tolerances^{4,5}. Within biofilms, where bacteria exist in close proximity, these local pH variations may influence the spatial and temporal organization of species. Yet, the role of the pH modifying activities of bacteria and local pH gradients as determinants of oral biofilm architecture and pathogen colonization remains unclear. Although the relationship of pH and biofilm composition and architecture has been studied in the context of supragingival plaque and caries^{6,7}, the role of pH as a determinant of polymicrobial biofilm assembly and pathogen establishment in the subgingival niche is unknown.

Porphyromonas gingivalis (*Pg*) is a Gram-negative, anaerobic, asaccharolytic bacterium strongly associated with periodontitis progression and a risk factor in several systemic conditions⁸⁻¹⁴. While *Pg* colonizes some individuals at low abundance in periodontal health, it becomes highly enriched in periodontitis^{15,16}. However, *Pg* colonization is highly variable, with 20 to 40% of subjects with periodontitis showing no signs of *Pg* colonization. Factors that influence *Pg* establishment in subgingival plaque may include availability of iron as heme^{17,18}, protein-rich nutritional substrates derived from the inflammatory exudate¹⁹⁻²² and a reduced atmosphere^{23,24}. The role of pH as a determinant of *Pg* colonization has been overlooked, although *Pg* is intolerant to acidic pH conditions²⁵⁻²⁷. *Pg* outgrowth in periodontitis may be facilitated by the alkaline environment in periodontal pockets²⁸. However, how *Pg* overcomes the less favorable pH conditions formed by acid-producing early colonizers during plaque establishment remains unknown. **In this proposal we will evaluate the hypothesis that the establishment of *Pg* in dental biofilms is influenced by the metabolic activities of other microbiome species which modify the local pH selecting out or supporting *Pg*'s growth.**

Previously, we found under batch growth that the health-associated commensal *Actinomyces oris* (*Ao*) inhibits *Pg* by producing organic acids and reducing the medium pH to levels *Pg* cannot tolerate. However, this inhibitory effect is abrogated by another commensal, *Veillonella parvula* (*Vp*), which metabolizes *Ao*-derived acids thereby neutralizing the pH and restoring a favorable environment for *Pg*. These findings highlighted a previously unrecognized three-species interaction among subgingival plaque colonizers in which metabolic activities modulate environmental pH and determine pathogen growth. However, critical gaps remain in understanding these interactions in biofilm growth mode, where pH micro gradients may establish and determine spatial species organization. Here, we will investigate whether local pH modulation and spatial organization determine *Pg* growth in model biofilms and evaluate the relevance of micro-scale biofilm pH and, macro-scale subgingival microbiome metabolic activities as determinants of *Pg* establishment. These studies will provide novel insights into the role of pH within subgingival biofilms as a determinant of the colonization of an important human pathogen.

Aim 1. Evaluate, in a 3-species biofilm model consortium, whether the local pH modulating activities of *Ao* and *Vp* determine the spatial organization and biomass accumulation of the acid-intolerant pathobiont *Pg*. Aim 1a: Investigate correlations between local pH gradients and spatial species distributions in dual or three species biofilms of *Ao*, *Vp* and *Pg* grown under continuous flow. Biofilms grown for different time periods will be imaged to spatially measure pH and localize species, evaluating whether pH gradients correlate with the biomass and positioning of *Pg* relative to other species. Aim 1b: Mechanistically evaluate the role of acid production by *Ao* in governing biofilm spatial organization and *Pg* growth. *Ao* mutants deficient in lactate will be evaluated to determine whether loss of acidification capacity disrupts pH gradients and biofilm architecture, altering the establishment and positioning of *Pg* within the community.

Aim 2: Evaluate the relationship of local plaque pH and community-wide pH-related metabolic activities with *Pg* colonization of human subgingival biofilms. Aim 2a: Assess whether local plaque pH correlates with the spatial distribution of *Pg* in subgingival plaque. In situ subgingival biofilms will be collected using a biofilm carrier system and imaged to determine spatially-resolved pH gradients and distribution of *Pg* and other bacterial taxa. Aim 2b: Evaluate community-wide pH-modulating metabolic activities of subgingival plaque and their relationship to *Pg* colonization status. Metatranscriptomic profiling will compare *Pg*-positive and negative subjects with comparable periodontal health to identify differentially expressed pH-modifying gene functions. Concurrent targeted metabolomic analysis of gingival crevicular fluid (GCF) using LC-MS will quantify select organic acids in matched samples.

These studies are part of the training of Dr. Soh as a clinician-scientist (Periodontology specialty/Ph.D in Oral Biology) under the guidance of an experienced mentoring team. Through complementary training in clinical research, biofilm models, microscopic imaging, mass spectrometry and bioinformatics, together with a well-developed plan to strengthen scientific communication skills, this award will provide her with a strong foundation to launch her independent research career.

A. SIGNIFICANCE

Relevance of understanding dental biofilm maturation and microbiome shifts: Although knowledge of the oral microbiome has rapidly advanced through DNA sequencing-based community characterizations, the ecological pressures that shape the microbiome under health and disease states are incompletely understood. Dental plaque is a polymicrobial community that assembles on tooth surfaces and in which microbes closely interact. This evolving communities are shaped by their surrounding environment with their taxonomic and functional configuration shifting from states of homeostasis to disease. The chronic inflammatory disease periodontitis is intricately linked to profound compositional and functional shifts in the subgingival microbiome residing at the gingival crevice^{9,29}. The relationship between microbial dysbiosis and destructive inflammation of the periodontal tissues is thought to be part of a self-perpetuating cycle in which enrichment of pathobionts at the gingiva triggers a dysregulated inflammatory host response causing tissue inflammation and damage, which in turns alters the subgingival environment promoting further pathobiont enrichment^{22,30,31}. Understanding of the ecological drivers of subgingival community dysbiosis remain incomplete, hindering the development of successful therapies to restore communities to a health-compatible configuration. Not only it is critical to identify pathogens but also understanding which partner species are essential for their establishment and outgrowth is essential. Similarly, there is little knowledge on species with the potential to antagonize pathogens. A better understanding of these interactions is needed to develop approaches for microbiome manipulation.

***Porphyromonas gingivalis* (Pg) is a critical subgingival pathogen:** *Pg* is a Gram-negative anaerobic and asaccharolytic bacterium strongly associated with periodontitis^{34,35}. Recent studies have shown *Pg* can also be a risk factor for other systemic diseases^{13,36}. *Pg* has been recognized as a causative pathogen critical for initiation and progression of periodontitis^{34,35}, and plaque collected from deep periodontal pockets has shown that its abundance correlates with severity of disease^{15,29,37}. *Pg* has numerous virulence factors including cysteine proteases (gingipains), lipopolysaccharide, fimbriae, and hemagglutinins that enable the bacterium to colonize periodontal pockets and counteract host defense mechanisms^{20,38-40}. It's ability to disable local immune

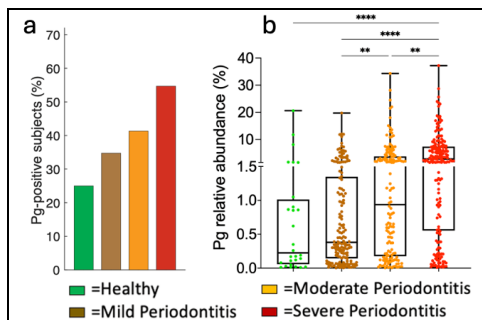


Fig. 1. Prevalence and relative abundance of *Pg* in subgingival microbiome samples of 1255 subjects characterized via 16S rRNA gene sequencing. Periodontal status was determined according to the CDC/AAP classification^{32,33}. **a.** *Pg* prevalence in subjects with different severity of periodontitis. **b.** *Pg* relative abundance in *Pg*-positive subjects.

responses, including complement and neutrophil chemotaxis, as shown in animal models, is thought to contribute to disease progression by enabling microbiome biomass to increase and dysbiotic shifts to occur^{10,11,31,41}. While *Pg* can exist as a transient oral commensal in children and at low abundance in early dental biofilms of certain individuals, its prevalence increases with disease progression. *Pg* is detected in over 70% of individuals with periodontitis, whereas it is detected in less than 25% of healthy subjects^{15,16}. Furthermore, our preliminary cross-sectional analysis of 1,255 adults with varying periodontal conditions revealed that both the prevalence and relative abundance of *Pg* increase with disease severity (Fig. 1a, b). However, in *Pg*-positive subjects, substantial inter-individual variation in *Pg* levels was observed within each severity group, highlighting the complex nature of bacterial colonization and suggesting that multiple hosts and/or environmental factors might influence *Pg* establishment and growth in subgingival plaque. Several environmental factors may favor *Pg* outgrowth including a reduced atmosphere²³, increased flow of protein-rich gingival crevicular fluid (GCF)¹⁹⁻²¹, availability of heme^{17,18}, and optimal pH range²⁷. In vitro experiments have shown that *Pg* can only grow in conditions with pH between 6.7 to 8.0^{25,26}. Environmental pH is not only critical for *Pg* growth but also for its virulence as the enzymatic activity of gingipains is optimal at 7.5 to 8.0 pH²⁷. Importantly, once *Pg* grows reaching a high cell density it raises the pH through ammonia production establishing an environment that benefits itself²⁰. Therefore, *Pg* requires an optimal environmental pH during initial establishment, but once it grows reaching a high biomass it creates a favorable pH for its own continued replication. However, it remains unclear whether these assumptions derived from in vitro batch culture experiments translate to complex biofilms and the human subgingival environment. A better understanding of the factors that control *Pg* colonization is essential for the development of therapies to prevent its outgrowth.

Inter-species interactions in dental plaque may modulate *Pg* colonization: Direct and indirect inter-species interactions shape the growth of species within polymicrobial communities. Beyond physical interactions, multiple environmental factors affect species arrangements in a microbial community, such as local nutrient availability or chemical gradients created by metabolites of heterogeneously-distributed species. Investigations in model systems have revealed oral microbiome species that positively or negatively interact with *Pg*. For example, *Pg* synergizes with other periodontitis-associated taxa, including *Treponema denticola*⁴². *Fusobacterium nucleatum*,

an abundant core species in dental plaque, has been shown to benefit *Pg* by creating reduced conditions, supplying CO₂ and facilitating multi-species aggregates^{43,44}. *Pg* also benefits from metabolic cooperation with the early-colonizer *Streptococcus gordonii*, which provides *Pg* with 4-aminobenzoate/para-amino benzoic acid (pABA)⁴⁵. Although these studies represent progress towards understanding determinants of *Pg* colonization, more work is needed to identify critical species for early establishment of *Pg* within the complex human oral biofilm milieu. Since *Pg* is sensitive to low pH, it is possible that the acid-producing and acid-neutralizing activities of other dental plaque species determine its ability to colonize and to flourish at a particular site.

Inter-species metabolic interactions that affect local pH may determine *Pg* levels and spatial distribution patterns in subgingival biofilms: Studies on human dental plaque have revealed a highly structured, non-random arrangement of bacterial species forming complex, multi-species biofilms with distinct architecture⁴⁶. Assembly of these highly organized structures is thought to be driven by the metabolic activities of interacting species, but mechanistic studies that test which activities influence biofilm architecture of oral communities are lacking. In supragingival plaque, highly specific inter-species arrangements correlating with localized acidic microenvironments that drive dental caries have been demonstrated^{6,47}. Non-mutans streptococci and *Selenomonas* species have been seen to arrange around microcolonies of *Streptococcus mutans* enhancing acidogenicity and demineralization of the underlying enamel^{6,47}. These studies demonstrate that pH distribution within biofilms is not uniform but exists in discrete micro-patches. These findings are further supported by fluorescence-based pH ratiometric imaging studies, which have also shown heterogenous pH distribution within supragingival biofilms⁴⁸. In contrast, much less is known about how pH and microbial metabolism influence the composition of subgingival plaque and drive the dysbiotic shifts associated with periodontal inflammation. The environment of inflamed deep periodontal pockets has been shown to be alkaline^{28,49}, potentially favoring the growth of asaccharolytic bacteria like *Pg*. However, acid-producing early colonizers including *Streptococcus* and *Actinomyces* species also demonstrate high prevalence in subgingival plaque, and their collective acidogenic activities can lower the local pH, potentially limiting initial *Pg* colonization. Although this acid production may restrict *Pg* establishment at the initial stage, spatial heterogeneity within the biofilm architecture is likely to create pH gradients through the localized distribution of acid-producing and acid-neutralizing species, that may enable *Pg* to establish in specific alkaline micro-niches. Understanding this intricate metabolic balance is critical because once *Pg* successfully outgrows acid-producing species and proliferates, it can modify the environmental pH to favor its own growth, eventually eliminating its dependence on partnering species. This dynamic interplay between spatial organization, metabolic activities, and pH gradients may represent a fundamental mechanism underlying *Pg*-induced periodontal disease that requires further investigation. Our previous work (preliminary data) demonstrated that health-associated commensal *Actinomyces oris* (Ao) inhibits *Pg* growth through lactate

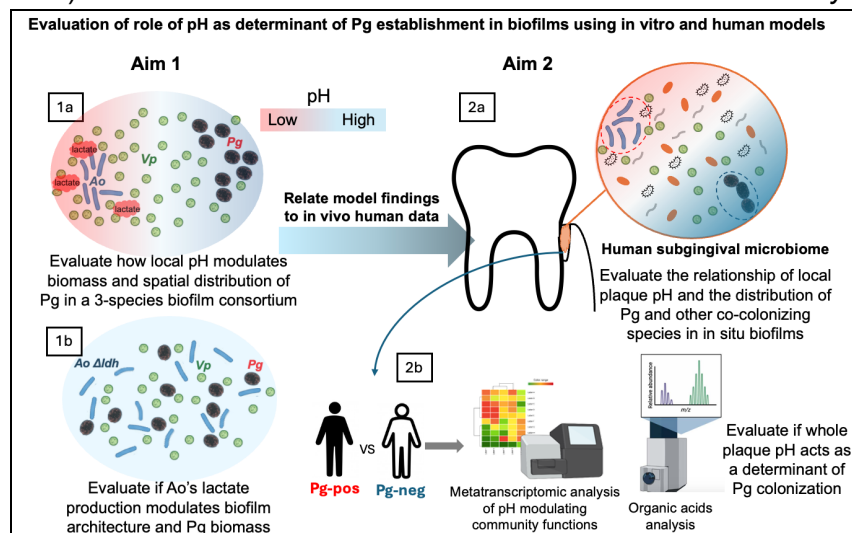


Fig. 2. Schematic of study rationale and aims. This proposal builds on our preliminary data identifying a 3-species interaction network among subgingival plaque colonizers, where species-specific metabolic activities modulate environmental pH promoting or suppressing *Pg* proliferation in batch culture. **Aim 1:** Assess whether pH gradients established by Ao (acid producer) and Vp (acid consumer) determine *Pg* spatial organization and biomass in biofilms grown in flow cells. **Aim 2:** Evaluate the role of these interactions and pH gradients in shaping *Pg* dynamics within the subgingival microbiome, while also identifying novel species and community functions with the potential to influence *Pg* through pH modulation.

production, reducing the medium pH to levels incompatible with *Pg* growth. This inhibitory effect is abrogated by another commensal, *Veillonella parvula* (Vp), which metabolizes organic acids derived from Ao, neutralizing pH and restoring environmental conditions conducive to *Pg* growth. These findings revealed a previously uncharacterized three-species interaction network among subgingival plaque colonizers where collaborative metabolic activities modulate the environmental pH and determine pathogen proliferation. Critical knowledge gaps persist, however, regarding these interactions within the more clinically relevant biofilm growth mode, where pH gradients may establish distinct microenvironments that determine spatial species organization. Therefore, the aim of this study is to investigate whether the establishment of *Pg* in dental biofilms is influenced by the metabolic activities of partnering microbiome species which modify the local pH.

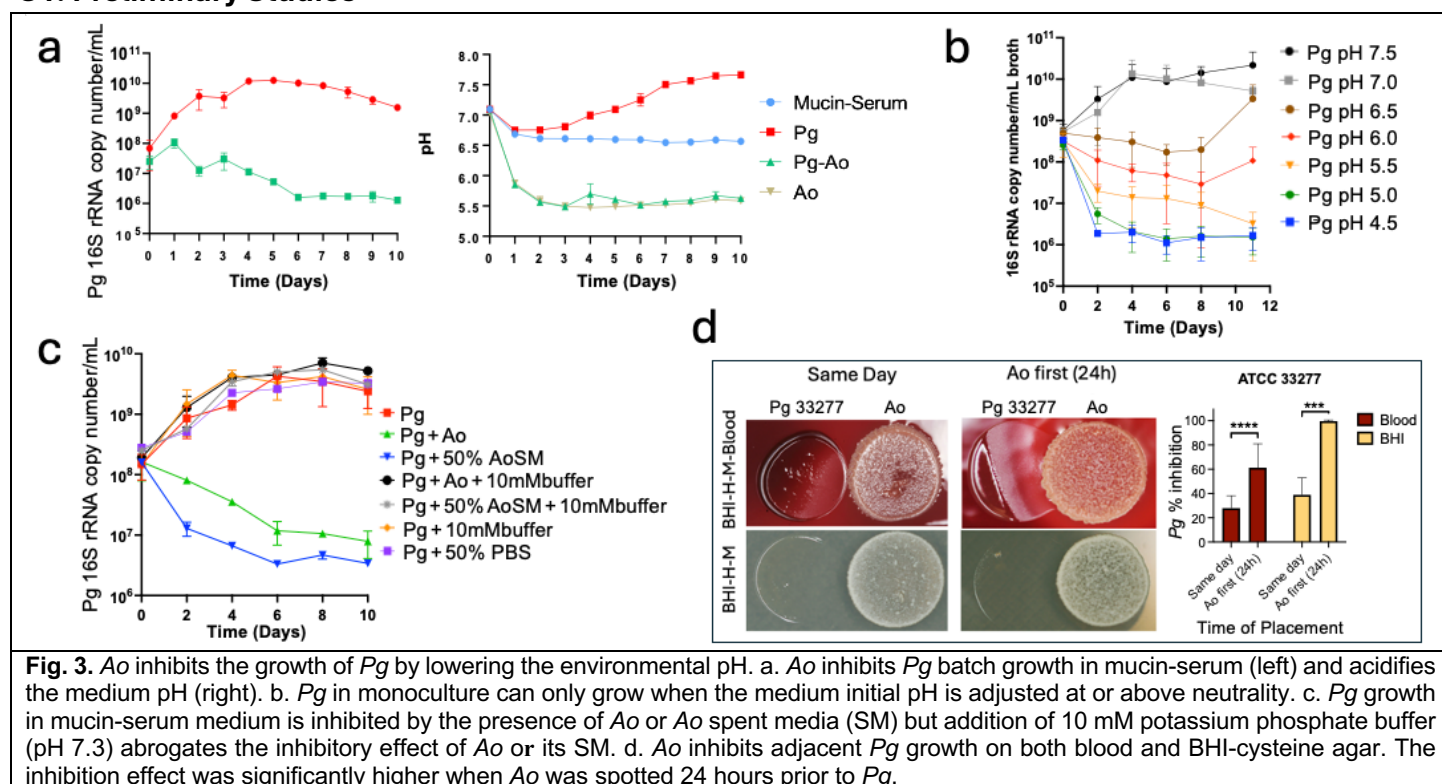
A schematic summary of the rationale and aims of this proposal is shown in Fig. 2.

The proposed studies were design based on my Ph.D work and will enable me to solidify my research training and, after graduation from my dual degree, will facilitate transition into a faculty position. The proposed research will kickstart my independent research program in translational periodontal microbiology.

B. INNOVATION

This work is novel in that (1) it represents the first systematic investigation of pH gradients within subgingival biofilms and their correlation with microbial colonization patterns, addressing a critical gap in understanding how local chemical microenvironments shape community assembly in periodontal disease; (2) using a carrier system that preserves intact biofilm architecture, we will map pH distributions and spatial organization of up to 100 taxa, providing unprecedented resolution of structure–function relationships in natural communities; (3) the study will identify previously uncharacterized bacterial species or metabolic activities with synergistic or antagonistic effects on *Pg* colonization, revealing new therapeutic targets or probiotic candidates and (4) by incorporating isogenic mutants deficient in acid production, we will mechanistically test whether pH-modifying metabolites drive species spatial arrangements within biofilms, a relationship never experimentally validated. Together, this approach will provide direct evidence for pH-mediated biofilm organization and define links among metabolism, spatial structure, and pathogen colonization, advancing our understanding of periodontal disease etiology and informing targeted interventions.

C1. Preliminary Studies



1. Ao inhibits *Pg* by modifying the environmental pH. We discovered that the Gram-positive health-associated commensal Ao inhibits *Pg* growth in mucin-serum batch culture while concurrently causing the medium pH to drop below pH 6.0 (Fig. 3a). This pH reduction is significant because previous studies have established that *Pg* exhibits optimal growth at pH levels above neutrality²⁷. To confirm this pH sensitivity, we tested *Pg*'s growth capacity in culture media with adjusted initial pH values and verified that *Pg* can only grow above pH 7 (Fig. 3b). Both the presence of Ao and its spent medium (SM, cell-free supernatant) inhibited *Pg* growth, however, this inhibition was completely abrogated by adding 10 mM potassium phosphate buffer (pH 7.3), confirming that the inhibitory effect was driven by environmental acidification (Fig. 3c). Furthermore, spot assays revealed that *Pg* fails to grow when adjacent to Ao. This effect was more pronounced when Ao was spotted 24 hours prior to *Pg*, allowing sufficient time for Ao to modulate the environment (Fig. 3d). The shown experiments were conducted with *Pg* strain ATCC 33277, but similar results have been obtained with *Pg* W83.

2. *Vp* restores *Pg* batch growth in presence of Ao by consuming organic acids and neutralizing the environment. Conversely, we found that the addition of the Gram-negative commensal *Vp* neutralized the acidic environment created by Ao, allowing *Pg* to grow even in Ao's presence (Fig. 4a, b). To elucidate the mechanism by which Ao

lowers the pH and inhibits *Pg*, while *Vp* abrogates this antagonistic effect, we quantified organic acids in the culture media of *Ao* and *Vp* monocultures and *Ao-Vp* coculture via liquid chromatography–mass spectrometry (LC-MS). *Ao* released organic acids including lactate and succinate into the medium, which were consumed by *Vp* (Fig. 4c). We confirmed lactate consumption by *Vp* using a colorimetric lactate assay (Fig. 4d).

3. Lactate dehydrogenase in *Ao* is responsible for lactate production and *Pg* inhibition. Previous studies have demonstrated that under anaerobic conditions, *Actinomyces* species catabolize carbohydrates as their primary energy source and produce organic acids⁵⁰. During this process, pyruvate derived from the Embden-Meyerhof-Parnas glycolytic pathway is converted to lactate via lactate dehydrogenase (LDH). In *Ao*, LDH exhibits high affinity for pyruvate and preferentially converts pyruvate to lactate while regenerating NAD⁺ from NADH⁵¹. Therefore, we constructed an *Ao ldh* deletion mutant (Δldh) and found that it exhibited decreased capacity to acidify the medium displaying only a slight growth deficiency compared to the wild-type (WT) (Fig. 5a). Lactate quantification confirmed the Δldh mutant lost its lactate-producing capacity (Fig. 5b). Furthermore, when *Pg* was co-cultured with SM from *Ao* WT or Δldh grown under aerobic or anaerobic conditions, only SM from anaerobic *Ao* WT cultures suppressed *Pg*, while SM from the *Ao* Δldh supported its growth, albeit with a longer lag phase. Consistently, the pH of SM from WT anaerobic cultures had an acidic pH, whereas the Δldh SM was only slightly below neutrality (Fig. 5c). We have determined that the slightly acidic pH in the *Ao* Δldh SM is due to pyruvate accumulation. However, *Pg* can overcome this slightly acidic conditions. We also constructed an *Ao* succinate dehydrogenase mutant, as our LC-MS analysis demonstrated that *Ao* produces succinate. However, this mutant acidified the medium and inhibited *Pg* similarly to *Ao* WT. These results show that LDH-mediated lactate production is the primary mechanism underlying *Ao*'s antagonistic effect on *Pg*.

4. Relationship of *Ao* and *Vp* to *Pg* biomass in the human subgingival microbiome.

Our results suggest that pH-mediated interactions among *Ao*, *Vp*, and *Pg* are important determinants of *Pg* growth and colonization. Preliminary data from our cross-sectional cohort (Fig. 1) shows that *Ao* and *Vp* are both highly prevalent in subgingival plaque and their relative abundances vary across subjects (Fig. 6a). We then selected samples with a *Pg* relative abundance <1%, to evaluate the relationship of *Pg* proportions with *Ao*'s and how *Vp* modifies this relationship. *Ao* levels exhibited a negative correlation with *Pg* at low *Vp* levels, however, this relationship disappeared when *Vp* levels were high (Fig. 6b). These relationships were more evident when the abundance of the genus *Actinomyces* was considered (Fig. 6c), showing that other *Actinomyces* species may have the same effect as *Ao*. Consistently, *Pg* relative abundance was significantly reduced in samples with high *Ao* and low *Vp*, compared to samples with high *Vp* levels, regardless of *Ao* abundance (Fig. 6d). These findings suggest that *Ao* suppresses *Pg* colonization, but this inhibitory effect is counteracted by *Vp*, whose metabolic activity creates conditions favorable for *Pg* growth.

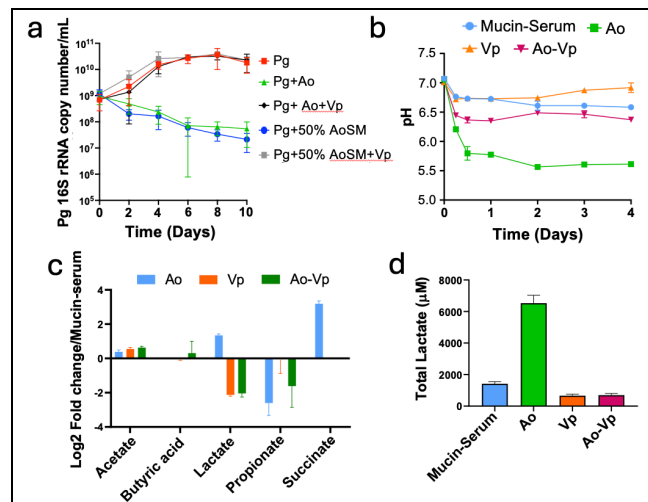


Fig. 4. *Vp* consumes *Ao*-produced organic acids, neutralizing pH and reversing *Pg* inhibition. **a.** Co-culture experiments demonstrate that *Vp* abrogates the inhibitory effect of *Ao* or *Ao* SM on *Pg* growth. **b.** pH measurements show that *Ao* rapidly acidifies the medium, whereas *Vp* counteracts this effect by raising pH to levels *Pg* can tolerate. **c.** Targeted LC-MS metabolomic profiling of SM shows *Ao* produces several organic acids, including lactate and succinate, both of which are consumed by *Vp*. Data are fold change over uninoculated media from 3 replicate experiments. **d.** A lactate colorimetric assay confirmed *Ao* produces lactate, and *Vp* completely consumes it.

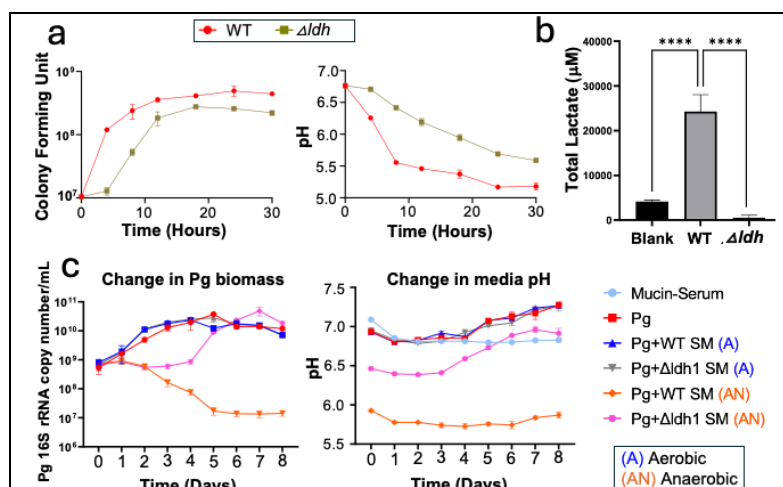


Fig. 5. Deletion of *ldh* reduces *Ao*'s ability to lower medium pH and inhibit *Pg*. **a.** Growth and pH modulation by Δldh mutant, show that *ldh* contributes to lowering the medium pH. **b.** The Δldh mutant is deficient in lactate production compared to WT. **c.** SM from anaerobic *Ao* WT cultures suppresses *Pg* growth, while SM from *Ao* Δldh allowed *Pg* growth. Corresponding culture pH shown in the right.

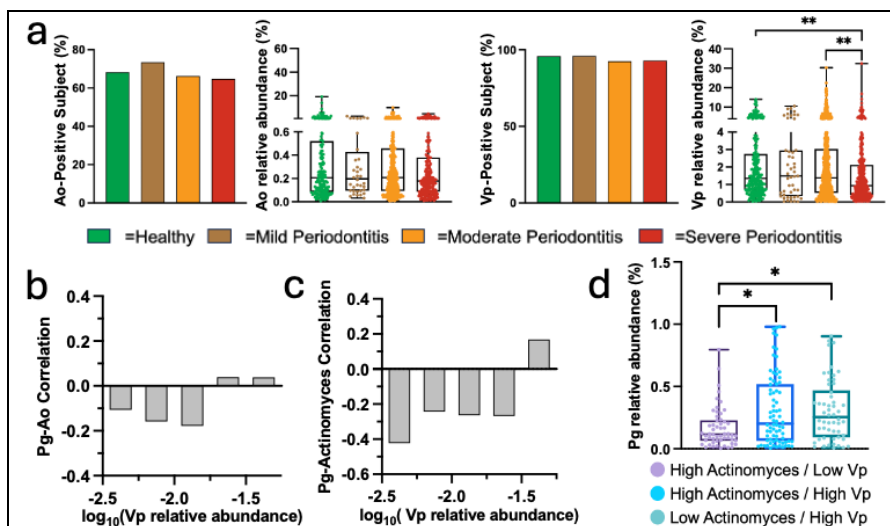


Fig. 6. Prevalence and relative abundance of *Ao* and *Vp* in subgingival microbiome samples from 1,255 subjects characterized by 16S rRNA gene sequencing, and correlation of *Pg* with *Ao* and *Vp*. (a) Prevalence and relative abundance of *Ao* and *Vp* across different disease severities. (b, c) Correlation between *Pg* and *Ao* or *Actinomyces* shifts from negative to positive as *Vp* levels increase. (d) *Pg* relative abundance is significantly lower when *Ao* levels are high and *Vp* levels are low. *Ao* and *Vp* abundances were dichotomized based on their respective median values. Samples with abundances greater than the median were classified as high, whereas those below the median were classified as low.

have provided fundamental insights into microscale pH dynamics within in situ supragingival biofilms^{48,53}. In this aim we will use a combination of ratiometry and fluorescence in situ hybridization (FISH) to visualize spatial arrangements in the *Ao-Vp-Pg* consortium in relation to pH. Subsequently, we will mechanistically evaluate the role of *Ao*'s lactate in shaping biofilm spatial architecture and determining *Pg* biomass. While species create pH niches through their spatial arrangements, these arrangements may, in turn, be initially driven by the metabolic dependencies among community members. By abrogating *Ao*'s ability to produce lactate (*Δldh*) we will mechanistically evaluate whether this organic acid drives biofilm architecture and influences *Pg* colonization patterns and biomass. **We hypothesize that *Ao*'s lactate production creates pH gradients within a three-species model biofilm and that these microenvironments correlate with species-specific localization patterns and *Pg* biomass. In addition, we hypothesize that in the absence of *Ao*'s lactate, spatial organization of species in biofilms is disrupted and *Pg* is able to grow in a spatially unrestricted manner.**

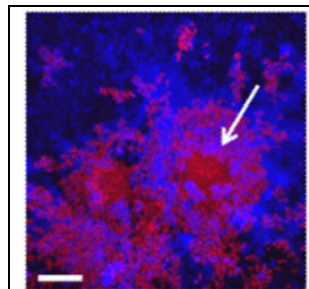


Fig. 7. Biofilms of *Ao* and *Vp* growing under flow in saliva. Red: *Ao*, Blue: *Vp*, visualized via fluorescence in situ hybridization⁵².

Aim 1a: Investigate the correlation between local pH gradients and spatial species distributions in dual or three species biofilms of *Ao*, *Vp* and *Pg* grown under continuous flow.

Research Plan: We will first evaluate spatial architecture and pH gradients in two or three-species biofilms grown under continuous flow. Biofilms will be grown anaerobically at 37° in flow cells with a cover slip as the attachment substratum, with hog gastric mucin (HGM) medium supplemented with 10% (v/v) heat-inactivated human serum (Sigma) at a flow rate of 100 $\mu\text{L min}^{-1}$, as previously described⁵². This medium was used throughout all our preliminary experiments and has been shown to support biofilms of oral microbial consortia^{15,43}. The following combinations of species will be tested: 1) *Ao-Pg* 2) *Vp-Pg* and 3) *Ao-Vp-Pg*. Mono-species biofilms will be also included for comparison purposes. The same strains used in our preliminary study will be employed: *Ao* T14v, *Vp* PK1910 and *Pg* 33277. Each species will be inoculated at 10^7 cells/mL and replicate biofilms will be cultured anaerobically for 4 days to allow sufficient time for growth of *Pg*. After biofilms mature, fluorophore-labelled nanobeads will be added, allowed a brief time to settle into the biofilms, and the excess will be rinsed off. Biofilms will be subjected to pH ratiometric analysis and taxonomic identification by FISH, with the fluorescent nanobeads serving as spatial locators to enable image superimposition. Extracellular pH will be measured using the ratiometric dye C-SNARF-4 by confocal microscopy, with emission intensities at 580 nm and 640 nm. Their ratio will be converted to pH values using a calibration curve generated from C-SNARF-4 in 2-(N-Morpholino) ethanesulfonic acid (MES) buffer titrated to pH 5.0–8.0^{48,54}. Biofilms will be imaged by confocal laser scanning

microscope (CLSM), with z-stacks acquired from at least 4 fields of view per sample and reconstructed into 3D images using IMARIS. pH will be quantified in at least 20 regions of interest per field. Biofilms will be then fixed in 4% paraformaldehyde at 4°C and stained by FISH with species-specific oligonucleotide probes JF201⁵², VEI488⁵⁵ and Bg-8⁵⁶ to detect *Ao*, *Vp* and *Pg*, respectively. For staining, fixed biofilms will be permeabilized with lysozyme, dehydrated in ethanol, incubated for 90 min at 46 °C with oligonucleotide probes in hybridization buffer (25% formamide), and then washed before visualization by CLSM. The biovolume (μm^3) of each species will be calculated from IMARIS surface reconstructions (Surpass mode), and differences among conditions will be tested for significance using ANOVA. Lastly, microbial proximity will be evaluated using the pair correlation function, $g(r)$ in daime software⁵⁷. A $g(r) > 1$ indicates clustering, < 1 indicates avoidance, and ≈ 1 indicates random distribution. At least three independently grown biofilms per group will be analyzed. Significance will be determined by comparing observed patterns to randomized null models generated via Monte Carlo simulations, using 95% confidence envelopes. For visualization of the pH distribution within the 3D biofilm architecture, the finalized stack of pH values will be imported into IMARIS. The fluorescence intensity ratios, corresponding to the full pH range, will be reconstructed and these values converted into a grayscale map. As a control, unlabeled biofilms will be imaged to confirm that autofluorescence from bacterial cells or glucans do not interfere with pH quantification at the wavelengths used⁷.

Expected Outcomes, Potential Problems Alternative Approaches: In aim 1a, we anticipate that flow of the medium will remove *Ao*-produced organic acids. However, localized acidic microenvironments will persist around actively acid-producing *Ao* cells, resulting in distinct spatial organization where *Vp* localizes around *Ao* in a clustered pattern, consuming organic acids produced and neutralizing the biofilm pH. In contrast, *Pg* would likely avoid colonizing in close proximity to *Ao*, displaying an avoidance pattern and instead localizing at the biofilm periphery near *Vp*. Subsequently, *Pg* would increase the pH of its surrounding microenvironment, creating a pH gradient within the biofilm. In the case of *Ao-Pg* biofilms we expect low *Pg* biomass, while in the case of *Pg-Vp* biofilms we expect higher *Pg* biomass and *Pg* association with *Vp* since these two species are known to interact through exchange of soluble factors with *Vp* enhancing *Pg* biomass¹⁵. This interaction, however, is independent of pH. C-SNARF has been shown to penetrate well through extracellular matrix components of biofilms and therefore we do not anticipate problems determining the pH. These experiments will be conducted with *Pg* ATCC 33277 which readily forms biofilms and is the most commonly detected *Pg* genotype in dental plaque⁵⁸. As an alternative approach, we will evaluate *Pg* colonization using clinical strains of *Pg*, *Ao* and *Vp* isolated from a single individual to confirm these relationships with fresh isolates coexisting within a host. These isolates are already available in the Diaz laboratory. Regarding timing of biofilm evaluation, we will consider evaluation of earlier time points as well (1, 2 or 3 days) as spatial patterns may vary during the biofilm assembly process.

Aim 1b: Mechanistically evaluate the role of acid production by *Ao* in governing biofilm spatial organization and *Pg* growth.

Research Plan: In this aim we will utilize our already constructed in-frame deletion mutant of *Ao* (Δldh), which has no lactate producing capacity, to determine if lactate drives spatial arrangements and affects *Pg* biomass in the three-species community. This mutant was constructed in collaboration with Dr. Hung Ton-That (mentoring committee). The following species combinations will be evaluated: 1) *Ao* WT-*Vp*-*Pg* and 2) *Ao* Δldh -*Vp*-*Pg*. We will also compare 3) *Ao* WT-*Pg* and 4) *Ao* Δldh -*Pg*. Biofilms of each group will be cultivated in triplicate using the same methodologies described in Aim 1a. After 4 days of incubation, biofilms will be subjected to pH ratiometric analysis and taxonomic identification with FISH. Average pH values and total species biovolumes will be compared among conditions. In addition, we will evaluate pH gradient distributions and species spatial proximity in relation to pH as described in Aim 1a.

Expected Outcomes, Potential Problems Alternative Approaches: We anticipate biofilms formed with the *Ao* Δldh mutant would be less acidic due to absence of lactate. *Pg* will have a greater capacity to grow, in an unrestricted spatial pattern, in turn further modulating the pH towards alkalinity. The lack of lactate is also expected to disrupt the spatial organization observed with *Ao* WT, with *Vp* showing a more random distribution rather than surrounding *Ao*, and *Pg* growing in closer proximity to *Ao* without an acidic niche. These experiments will tell us if the presence of lactate is a driving force for spatial arrangements. Alternatively, if *Ao* Δldh biofilms are more alkaline but show similar architecture to *Ao* WT, this would suggest that specific adhesin–receptor interactions between *Ao* and *Vp* govern spatial organization independently of pH as previous studies showed that *Ao* and *Vp* coaggregate⁵². In case spatial arrangements are not disrupted in biofilms, we will construct *Ao* and *Vp* mutants for surface structures potentially mediating cell to cell adhesion to evaluate if disruption of *Ao-Vp* spatial arrangements affects *Pg* biomass. Candidate genes include *VtaA* and *VtaE* adhesins from *Vp*⁵⁹. We will also consider constructing *Vp* mutants that do not have the capacity to utilize lactate to evaluate whether

disrupting this interaction with *Ao* has an effect on spatial organization and *Pg* biomass. Dr. Hung Ton-That will provide guidance for mutant construction.

C3. Aim 2: Evaluate the relationship of local plaque pH and pH-related bacterial metabolic activities with *Pg* colonization of human subgingival biofilms.

Aim 2a: Assess whether local plaque pH correlates with the spatial distribution of *Pg* in subgingival plaque.

Rationale: Oral microbes are organized in complex biofilm communities that contain many bacterial species with various genetic potentials⁶⁰. Periodontitis is associated with dysbiotic microbiome shifts, but it remains unclear which inter-species interactions influence pathobiont colonization and outgrowth. In our preliminary data we show that *Pg* colonization varies, and even in *Pg*-positive subjects the levels of *Pg* greatly differ among subjects (Fig. 1). It is likely that interactions with other community members determine whether *Pg* can colonize and if it can flourish in subgingival plaque. We also show that in subjects with low *Pg* levels (less than 1%), the relative abundance of *Pg* in individual subgingival plaque positively correlates with the relative abundance of *Vp*, and negatively with the relative abundance of *Ao*, suggesting that the interactions identified through our work may also be relevant in vivo (Fig. 6). However, whether *Actinomyces* spp., *Veillonella* spp. and *Pg* spatially organize according to their pH-mediated interactions in natural subgingival plaque is unknown. In this aim we will evaluate this question, also exploring other species for their spatial interactions with *Pg* during in situ subgingival plaque development. Obtaining undisturbed subgingival plaque presents significant challenges due to limited accessibility. Thus, current understanding of inter-species interactions in subgingival biofilms is largely derived from in vitro model systems, leaving fundamental questions unanswered about complex multi-species interactions in clinically relevant settings. Wecke et al.⁶¹ introduced a carrier system to enable analysis of in situ intact subgingival biofilms (Fig. 8). This innovative approach can help overcome the accessibility limitations and provides a pathway to study natural biofilm communities. Using this system, placed in subjects recruited through a prospective clinical study, we will examine how pH gradients within biofilms correlate with the specific positioning of *Pg*, and which species show physical interactions with *Pg*. We hypothesize that heterogeneous pH microenvironments within natural subgingival plaque correlate with *Pg* localization, and *Pg* colocalizes with specific species that support an alkaline pH while avoiding taxa known to be acid producers.

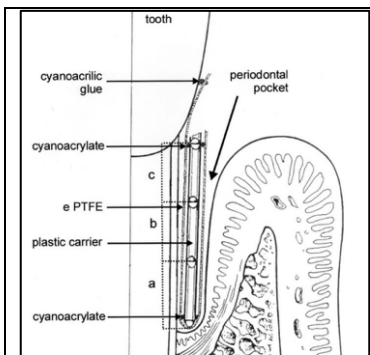


Fig. 8. Schematic diagram of carrier system. An ePTFE membrane is inserted inside the gingival crevice and biofilms are allowed to develop for 7 days before retrieval⁶¹.

Research Plan: In this aim, we will evaluate the pH gradient and spatial distribution of *Pg* in in situ subgingival biofilms allowed to develop in a carrier system (Fig. 8) in subjects with mild to moderate periodontitis.

Clinical Study Procedures: Adults with untreated mild to moderate periodontitis with at least 2 sites with probing depth ≥ 5 mm and concomitant clinical attachment loss will be recruited. Subgingival plaque collected from the deepest sites will be screened for *Pg* using qPCR, as previously described¹⁵. *Pg*-positive subjects, determined to have at least 10^3 copies of *Pg* 16S rRNA gene per sample will be enrolled. *Pg* relative microbiome abundance and that of other species will be quantified via Illumina amplicon sequencing of the V1-V2 region of the 16S rRNA gene as previously published²⁹. Amplicon reads will be processed in mothur⁶² for quality trimming, chimera removal, and taxonomic assignment using the latest version of Human Oral Microbiome Database for species-level classification. This analysis will determine *Pg* relative abundance in enrolled subjects. We aim to analyze 10 participants with *Pg* relative abundance $< 1\%$, as we expect low abundance *Pg* to depend on specific interactions with pH-modifying species, like

Veillonella spp., for its survival in plaque (Fig. 6). In contrast, high abundance *Pg* is likely to already inhabit an alkaline environment contributed by its own metabolism. For enrolled *Pg*-positive subjects ($< 1\%$ abundance), the in situ biofilm carrier system will be placed as described previously⁶¹. Briefly, expanded polytetrafluoroethylene (ePTFE) membranes attached to plastic carriers will be inserted into the gingival crevice to the base of the deepest pockets and secured supragingivally with cyanoacrylate adhesive. After 7 days of in situ development, carriers will be retrieved, and fluorescent beads will be added as in Aim 1 to provide spatial coordinates for aligning pH and CLASI-FISH images.

pH ratiometric analysis and taxonomic identification with CLASI-FISH: pH ratiometric analysis will be performed as described in Aim 1. Membranes will then be fixed in 4% paraformaldehyde, rinsed with PBS, and stored in 50% ethanol at 4 °C until hybridization. Species-specific 16S rRNA probes targeting up to 100 of the most abundant oral taxa in a sample, will be designed using ARB-SILVA databases and combinatorially labeled with

12 spectrally distinct fluorophores following the CLASI-FISH framework⁶³. Each taxon will receive a unique binary fluorophore code for multiplex detection. Hybridization will be carried out at 46°C, followed by washing for 15–20 min. Samples will be mounted in antifade medium and imaged on a Zeiss LSM 980 confocal microscope with Airyscan 2 and a 32-channel spectral array, using six excitation wavelengths (639, 594, 561, 514, 488, 445 nm). Z-stacks will be collected at 0.25 µm intervals. Single-labeled references and unlabeled controls will generate endmember spectra and background profiles. Spectral data will be unmixed using a multi-view machine learning algorithm⁶⁴, and 3D per-species spatial maps will be reconstructed in IMARIS. Quantitative analyses will assess species abundance, co-localization, and interspecies spatial relationships. pH distribution within 3D biofilms will be mapped as described in Aim 1.

Expected Outcomes, Potential Problems Alternative Approaches: In this aim, we expect that natural subgingival plaque grown on the membrane will exhibit pH gradients, with patches of alkaline niches correlating with *Pg* presence. These alkaline niches are anticipated to form at a distance from acidic microenvironments that correlate with acid-producing species such as *Ao*. A potential challenge is subject compliance, as biofilm integrity may be compromised if the cyanoacrylate adhesive detaches. To mitigate this, participants will receive detailed post-insertion instructions, and stents may be provided to protect the carrier during eating and oral hygiene. Imaging will be performed under the guidance of Dr. Alex Valm, whose CLASI-FISH method allows simultaneous imaging of up to 100 taxa. However, depending on the relative abundance of species in each sample, based on 16S rRNA gene sequencing, we will determine the optimal number of taxa to target, which is likely to be less than 100 taxa.

Aim 2b: Evaluate inter-individual differences in pH-modulating metabolic activities of subgingival plaque communities and their relationship to *Pg* colonization status.

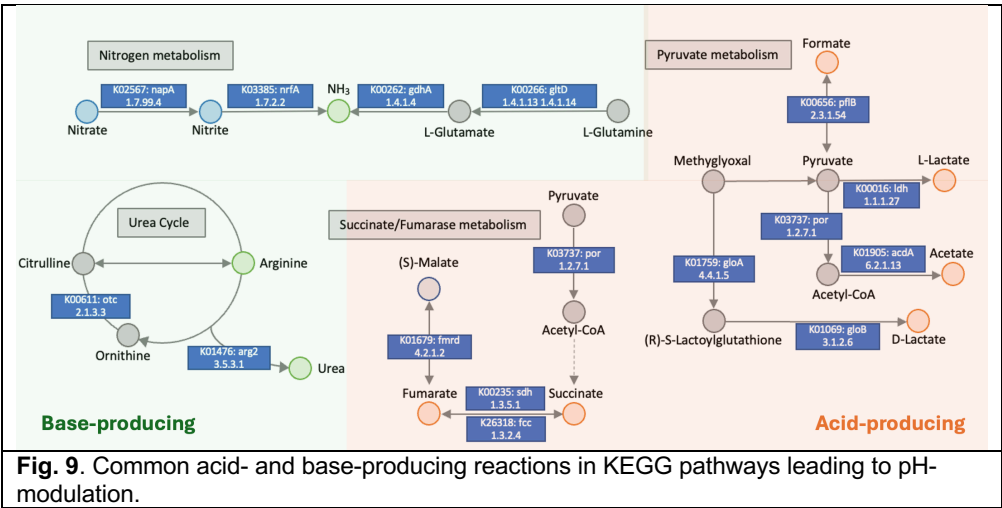
Rationale: Utilization of multi-omics approaches can address questions of both microbial community function and underlying mechanisms and moreover identify functional genes and microbial metabolic pathways that are expressed in subgingival plaque further contributing to our understanding of microbiota in health versus periodontitis. Several studies have characterized the functional capacity of the oral microbiome in health and disease utilizing metatranscriptomic analysis, where they have found distinct expression patterns of genes associated with pathogenesis during periodontal disease⁶⁵. The rationale for studying community function in this proposal is to understand which expressed genes are likely to influence the environmental pH and how that pH affects *Pg* colonization and abundance. While direct measurement of pH in the subgingival environment is technically challenging due to probe sensitivity and the heterogeneous nature of biofilm microenvironments, we can infer the overall pH-modifying capacity of the microbiome by examining acid and base-producing metabolic functions through metatranscriptomics and quantifying relevant metabolites in gingival crevicular fluid (GCF). To the best of our knowledge, there have been no human studies that investigate acid and base-producing community functions to indirectly predict the ecosystem dynamics and its impact on *Pg* growth in vivo. Thus, we seek to utilize metatranscriptomics to compare the pH-modifying metabolic potential of subgingival plaque in *Pg*-positive and negative subjects, cross-sectionally. This analysis will reveal specific metabolic functions, and the species associated with these functions, that might facilitate favorable environments for *Pg* growth or antagonize it. Subsequently, we will employ metabolomic analysis of matched gingival crevicular fluid (GCF) samples to quantify the actual metabolites that may contribute to the overall environmental pH in the gingival crevice of individuals with varying levels of *Pg* colonization. We hypothesize acid/base-producing gene functions in subgingival plaque will correlate with levels of organic acids and ammonia in GCF and with *Pg* colonization levels.

Research Plan:

Clinical cohort description: In this aim we will compare the metatranscriptomic profiles of 40 *Pg*-positive and same number of *Pg*-negative subjects matched by periodontitis severity to find differentially represented functional activities. These subjects have been already enrolled under IRB STUDY00005287 at the University at Buffalo and samples are available. Enrolled subjects had untreated moderate periodontitis with at least 2 sites with probing depth ≥ 5mm and concomitant clinical attachment loss. Subjects were screened for *Pg* in subgingival plaque via qPCR as described in Aim 2a. Subgingival plaque from the deepest site in each quadrant was subsequently obtained via curettes and stored in RNAlater at -80°C. GCF was collected via paper strips.

Power and sample size justification: An a priori power analysis was conducted to determine the required sample size for detecting a significant difference in the relative abundance of pH-related KEGG pathways (Fig. 9) between two independent groups. The calculation aimed to detect a moderate effect size (Cohen's $d = 0.63$). Based on these parameters, a sample of 40 subjects per group is required to achieve approximately 80% power with a significance level (α) of 0.05.

Metatranscriptomics of subgingival plaque: Plaque RNA extraction will be performed after bead-beating via the Qiagen RNeasy Mini Kit using protocols optimized in the Diaz lab. Libraries will be prepared using the Illumina Stranded Total RNA Prep with Ribo-Zero Plus and sequenced with a NovaSeq instrument. For data processing, we will first perform quality control using Kneaddada (trimming, remove host reads). Then, utilizing HUMAnN we will perform functional annotations via searching sequences against the UniRef90 gene database. The obtained gene expression for gene families will be then summarized using KEGG pathways. We will determine gene



families or KEGG reactions/pathways differentially expressed in *Pg*-positive and negative subjects via DeSeq2. We will focus on evaluation of differences in community functions that are capable of pH modulation (common functions in Fig. 9). We will also assign taxonomic origin to RNASeq reads by utilizing Kraken2 and our custom reference database of oral genomes, to determine the species associated with acid- or base-producing functions in each microbiome.

Targeted LC-MS Metabolomics for organic acids and ammonia assay: GCF will be analyzed using targeted LC-MS metabolomics to assess common organic acids. This method has been already developed in collaboration with the Wood lab (mentoring committee) and was used to measure organic acids in spent media (preliminary data, Fig. 4c). For this analysis reference-grade standards of sodium acetate, succinic acid, valeric acid, butyric acid, propionic acid, fumaric acid, sodium L-lactate, 1-¹³C-sodium acetate, 3-¹³C-lactic acid, 1-¹³C-butyric acid, and 2,3-¹³C₂-succinic acid will be prepared at 1 mg/mL in water. A ThermoScientific Accucore™-150-Amide-HILIC (2.6 μm, 2.1 × 150 mm) analytical column will be used. Calibration standards (0.78–50 μg/mL) will be prepared in acetonitrile and spiked with ¹³C₂-succinic acid (19 μg/mL) as an internal standard. GCF samples will be eluted from strips, diluted in acetonitrile, and analyzed in triplicate, with blanks between runs to monitor carryover. Calibration and quantification will be performed using TraceFinder v4.1, ensuring linear response without weighting. Ammonia levels will be measured using a colorimetric assay kit (Abcam #ab83360).

Expected Outcomes, Potential Problems Alternative Approaches: In sub-aim 2b, we expect to see associations between acid/base-producing gene functions with *Pg* colonization and levels. There might be increased acid-producing and depleted base-producing transcripts in *Pg*-negative samples, whereas the contrary is expected in *Pg*-positive subjects. It is also possible that these acid- and base-producing transcriptional activities correlate with *Pg* relative abundance in *Pg*-positive subjects. The metatranscriptomic analysis will also reveal which species are associated with acid/base functions that correlate with *Pg* levels. We expect to find *Actinomyces* and other acid-producing species such as *Streptococcus* to be enriched in *Pg*-negative subjects, with genes related to lactic production also enriched. Candidate novel interactions revealed by this analysis will be tested in future work using model systems such as those presented in preliminary data and Aim 1. We expect no difficulties measuring organic acids in GCF, since our preliminary data (Fig. 4c) establishes the feasibility of identifying and quantifying trace amounts of organic acids. From GCF samples, we expect to see higher concentrations of organic acids with strong pKa such as lactate in *Pg*-negative samples.

D. FUTURE DIRECTION. Successful completion of this project will pave the way for future research on periodontitis, focusing on two key areas: (1) Test therapeutic interventions to manipulate biofilm pH or target specific pH-modifying species to prevent *Pg* outgrowth and restore health-compatible microbial communities. (2) Expand the three-species model to include additional pathogenic or protective species identified through metatranscriptomic analysis in Aim 2b.

TIMELINE						
Aim	Experiment	Yr 1	Yr 2	Yr 3	Yr 4	Yr 5
1a	Imaging of extracellular pH and FISH imaging of 3-species model biofilm	X	X			
1b	Defining role of Ao's lactate production in governing biofilm arrangement		X			
2a	Imaging of extracellular pH and CLASI-FISH imaging of natural subgingival biofilms	X	X	X	X	
2b	Metatranscriptomic & LC-MS analysis of pH modulating community functions and metabolites		X	X	X	X

Training in the Responsible Conduct of Research

1. Format. As a foundation, I have completed the Collaborative Institutional Training Initiative (CITI) RCR program, which provides baseline instruction in biomedical research ethics. However, consistent with NIH guidance, this online program will be supplemented with substantial in-person and interactive components. Specifically, I will complete the Responsible Conduct of Research Workshop Series offered by the University at Buffalo's (UB) Clinical and Translational Science Institute (CTSI) Workforce Development Core. This series includes at least six interactive 90-minute sessions per year and uses a case-based format with structured discussion, video examples, and group exercises. In parallel, I will engage in weekly mentor-led discussions with Dr. Patricia Diaz and present case studies in our laboratory meetings to integrate formal instruction with day-to-day research practices.

2. Subject Matter. The CTSI program offers comprehensive instruction across all NIH-recommended RCR domains. Core topics include research misconduct, conflicts of interest and commitment, mentor–mentee responsibilities, responsible data acquisition, management, sharing, and reproducibility, authorship and publication ethics and the integrity of the peer review process. The curriculum also covers protections for human subjects and IRB requirements, the ethical use of animals in research under IACUC oversight, and the broader professional and societal responsibilities of researchers. Instruction is highly interactive and emphasizes the development of critical thinking and ethical decision-making skills through case studies, real-world scenarios, and group discussion. To formally recognize completion, the CTSI additionally offers a Responsible Conduct of Research micro-credential, which provides both a certificate and a digital badge documenting mastery of the training content.

3. Faculty Participation. The CTSI workshop series is led by rotating UB faculty and compliance experts from diverse academic and clinical disciplines, including the Office of Research Compliance. This broad participation ensures that trainees are exposed to multiple perspectives on ethical research practice. My primary mentor, Dr. Diaz, will oversee my progress, assign supplemental readings, and integrate RCR discussions into our weekly one-on-one meetings. In addition, I will contribute to the training environment by presenting RCR-related topics during our regular laboratory meetings, which will encourage dialogue across the research team.

4. Duration of Instruction. The CTSI RCR program alone exceeds eight contact hours, satisfying NIH's requirement for substantive instruction. When combined with ongoing mentor-led discussions, regular lab-based case presentations, and preparatory assignments associated with the workshops, my training will total more than ten hours during the project period. This ensures both breadth and depth of exposure to the ethical dimensions of research.

5. Frequency of Instruction. I will complete the CTSI RCR workshop series during the first year of my award. Weekly mentor meetings will provide sustained opportunities for discussion throughout the project period, and I will revisit RCR principles annually by leading case-based discussions within our lab. In accordance with NIH policy, if my project extends beyond four years, I will repeat or update my formal RCR training to ensure ongoing compliance and engagement with current ethical standards.



Patricia I. Diaz, DDS, MSc, PhD
Sunstar Robert J. Genco Endowed Chair
Professor of Oral Biology

09/26/2025

Re: Mentor Statement of Support: Patricia I. Diaz, DDS, MSc, PhD

It is my great pleasure to write in support for Dr. Dam Soh's application to the K08 Mentored Clinical Scientist Research Career Development Award and to serve as her primary mentor. Dr. Soh is an extremely talented clinician scientist currently starting the 5th year of her dual residency in Periodontology and PhD in Oral Biology program. Dr. Soh has worked under my supervision as research advisor leading towards her PhD thesis for the last 4 years. As detailed below, Dr. Soh has almost completed all the requirements of her clinical residency and has also successfully become a Ph.D candidate after passing her qualifying requirements. Dr. Soh will focus the last years of her dual training in completing her research requirements and seeking mentorship and training to transition to a faculty position with her own independent research program. Dr. Soh is an incredibly talented, hard-working, thoughtful, creative, and effective clinician scientist working at the interface between periodontology and microbiology, who is an ideal fit for this award.

Research Qualification and Previous Mentoring Experience

My background uniquely qualifies me to serve as Dr. Soh's primary mentor for this award. I am Professor in the Department of Oral Biology of the University at Buffalo and Director of the UB Microbiome Center. Similar to Dr. Soh's career goals, I am a clinician-scientist with training in Periodontology and Microbiology. My research program focuses on understanding the ecology of the oral microbiome and its impact in oral and systemic disease. Since establishing my laboratory, I have led several clinical studies to evaluate perturbations in oral microbiome communities that occur with the development of oral disease, especially focusing on periodontitis and oral comorbidities of cancer treatment. My laboratory has optimized sequencing and bioinformatic approaches to study the oral microbiome. We also utilize in vitro and animal models to study inter-species interactions in oral microbiome communities and their role in dysbiosis. I have co-authored more than 60 peer-reviewed research articles (h-index: 45). Some trainees in my research group focus mostly on bench research using model systems, while others work closely with our clinical research team, and others focus on bioinformatics. Dr. Soh is being trained in all aspects, combining in vitro model systems, clinical research and bioinformatics. Therefore, my laboratory provides Dr. Soh with a well-rounded environment where she can continue exploring unresolved key questions pertaining the microbiome and periodontitis.

I am highly committed to the mentorship of my trainees. Currently, I serve as primary mentor to 2 PhD students and 2 postdoctoral fellows and I co-mentor with Dr. Troy Wood a PhD student. I also serve as co-mentor to a D43-supported PhD student at the University of West Indies in Jamaica. One of my previous trainees, an MD-PhD, obtained a competitive NIH F30 Fellowship award (F30GM146451). One current trainee was recently awarded a K99 Pathway to Independence Award (K99DE034829). Five of my former trainees are currently full-time faculty in medical and dental schools in the US, Chile and Turkey. I closely work with my trainees meeting regularly and providing them with opportunities to develop presentation and writing skills. I am currently MPI and co-director of a D43 FIC-NIDCR training grant to develop research capacity in Jamaica and I am co-director of the K12 specialty-PhD training in Oral Biology at the University at Buffalo. I have been PI or co-PI of 3 R01s (two current), three R21, one R56 and multiple foundation and industry grants. I also have a very collaborative research program and currently serve as co-investigator in one R01, one U01 and one R21. These resources will allow me to cover the costs of Dr. Soh's research and training not directly covered by the K08 mechanism.

Department of Oral Biology
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 Phone: (716) 829-2844 Fax: (716) 829-3942
<http://dental.buffalo.edu/departments/oral-biology.html>

Training Plan

I will support Dr. Soh in her training plan to gain all the necessary skills to graduate from her Ph.D and transition to a faculty position. During the past years, Dr. Soh acquired expertise in clinical research and in in vitro microbial community models. Under the guidance of Dr. Troy Wood (mentoring committee) she developed expertise in LC-metabolomics. She has also collaborated with Dr. Hung Ton-That (mentoring committee) to develop *Actinomyces* mutants, and under my mentorship is currently receiving training in microbiome bioinformatics. Dr. Soh will continue to have me as her primary mentor and will continue to interact with Drs. Wood and Ton-That as part of her mentoring committee. In addition, Dr. Soh has sought mentorship from Dr. Alex Valm who will help her gain expertise in CLASI-FISH and other methods to evaluate in situ biofilm architecture. Thus, Dr. Soh has assembled a strong mentoring committee to guide her career development and research goals.

In addition, Dr. Soh developed a solid plan to gain further expertise in microscopy and grant/scientific writing skills by attending courses and workshops. Dr. Soh will have access to facilities in my laboratory, will attend our weekly laboratory meetings and will be given the opportunity to contribute to the mentoring of undergraduate and junior graduate students. Dr. Soh will also have access to already collected plaque and gingival crevicular fluid samples from Pg-positive and Pg-negative subjects (Aim 2b), and I will support her own clinical study to evaluate in situ biofilm architecture (Aim 2a). She will also continue to attend and present at the monthly research seminars of the University at Buffalo-University of Rochester Microbiome working group, and will present her work at local conferences (Finger Lakes Microbiome Symposium, Microbial Pathogenesis Meeting at the University at Buffalo) in addition to other national and international conferences. Upon graduating from her residency/Ph.D. (anticipated 2028), Dr. Soh will pursue a tenure-track position. I will mentor her during this transition and throughout the rest of the K08 to support the establishment of her lab and independent funding.

Research Plan

Dr. Soh's goal is to conduct research in the periodontal microbiome to develop an understanding of species that modulate the colonization of pathogens to identify, in the long-term, strategies to disrupt dysbiotic shifts. She will seek an academic faculty position to establish her independent research program. Dr. Soh's research plan for this K08 was developed as a continuation of the studies she conducted under my mentorship during the past years of her Ph.D training. These studies have led to a high-quality first author publication (currently in preparation for submission). Dr. Soh has developed a plan to take these studies in an independent direction now exploring the relationship of biofilm architecture and inter-species interactions through metabolic exchange and environment modulation. She will conduct this exploration of biofilm architecture using her three species model consortium in combination with flow cells and confocal microscopy ([Aim 1](#)), for which all equipment and expertise are available in my laboratory. If any new mutants are required for these studies, Dr. Ton-That will advise Dr. Soh in molecular methods to create new strains.

In addition, Dr. Soh plans to examine the interactions of *P. gingivalis* with *Actinomyces*, *Veillonella* and other genera in subgingival plaque in situ ([Aim 2a](#)). I will provide any required support for her clinical study to collect these samples, but I would like to emphasize that this study was designed by Dr. Soh and is her own human subjects research protocol. To analyze biofilm architecture in her clinical samples, Dr. Soh has sought the co-mentorship of Dr. Alex Valm who is an expert in advanced imaging of oral biofilms and has a laboratory at the University of Albany, which is at driving distance from the University at Buffalo. Albany is also the hometown of Dr. Soh. During the initial training in CLASI-FISH microscopy methodologies Dr. Soh plans to travel to Albany as frequently as needed to interact in person with Dr. Valm and his laboratory. Later, after she collects biofilms from her participants, she will travel to Albany to access Dr. Valm's spectral imaging microscope and collect images. Dr. Valm has committed to provide Dr. Soh with access to his laboratory and equipment, in addition to providing mentorship for the analysis of her samples.

For [Aim 2b](#), Dr. Soh will have access to plaque and gingival crevicular samples already collected in my laboratory, with Dr. Soh's assistance. She will oversee sequencing of plaque samples and will conduct LC-metabolomics to evaluate organic acids under the co-mentorship of Dr. Troy Wood. Dr. Soh has already worked with the Wood laboratory developing an accurate LC-MS-based methodology to quantify common organic acids produced by microbes in extremely small volume samples (like GCF). Therefore, this methodology and mentorship relationship are already in place. For her bioinformatics training, Dr. Soh has already taken courses

to learn basic coding skills (R and MatLab) and will continue to receive my mentorship to implement bioinformatics pipelines for analysis of oral metagenomes and metatranscriptomes.

Plan for coordination with the mentoring team

Drs. Diaz, Valm, Wood and Ton-That will meet formally every 2 months with Dr. Soh to discuss her progress. Each mentoring team member will also meet independently with Dr. Soh as required since we are all advising on different aspects of her project. Once Dr. Soh transitions to an independent faculty position, the mentoring team will continue to advise her, online if required, until the end of her K08 career development award.

Transition Plan

I am fully supportive of Dr. Soh taking the proposed research into her own laboratory and having complete ownership of the work. In addition, I will continue to serve as a resource for Dr. Soh in reviewing her future grants and papers. I will advise Dr. Soh during her job application process and interview to help her prepare in the best possible way to secure a tenure-track position.

Assessment of the candidate's qualifications and potential for a research career

Dr. Soh has my strongest support for a K08 Award. She combines microbiology and periodontology expertise with strong training in both the clinical and basic science aspects. She is extremely hard-working, productive, and rigorous in her research. Although the first four years of her dual degree training were mostly occupied by her clinical degree, Dr. Soh has shown to be very productive not only completing all her clinical requirements but also successfully completing her qualifying examinations to become a Ph.D candidate and producing high quality work in my laboratory which is now being prepared for submission with Dr. Soh as first author. During her time in my lab, she has worked in subject recruitment and clinical data and sample collection, she learned to work with oral community in vitro models, gained expertise in molecular biology and other assays, and learned basic bioinformatic skills to analyze 16S rRNA gene data and metagenomics datasets. She also worked with the Wood Lab to learn LC-MS methodologies. Dr. Soh has presented her work at numerous scientific meetings and is the recipient of many awards. Most recently, she was selected to give an oral presentation at the AADOCR 9th Annual Mini Symposium for Young Investigators organized by the IADR Oral Microbiology and Immunology research group. She received 2nd place in the post-doctoral category. Dr. Soh was also selected to give an oral presentation at the Finger Lakes Microbiome Symposium (FILMS) in Rochester, NY, with her presentation winning 1st place for PhD category. These are just a few of the many accolades she has received for her research work and attest to her ability to communicate her science to others. Therefore, Dr. Soh brings to the field of Dentistry a combination of clinical and research skills and excellent communication ability which I am sure will lead to her becoming a prominent investigator and academician.

In summary, Dr. Soh has the dedication and skill to succeed as a clinician scientist and independent investigator. I enthusiastically support her K08 application.

Sincerely,



Patricia I. Diaz, DDS, MS, PhD
Sunstar Robert J. Genco Endowed Chair in Oral Biology
SUNY Empire Innovation Professor
Director of UB Microbiome Center
Department of Oral Biology
School of Dental Medicine
University at Buffalo
The State University of New York (SUNY)
Buffalo, NY 14214-8024

October 4, 2025

Co-Mentoring Statement: Alex Valm, PhD

I am Associate Professor of Biological Sciences at the State University of New York at Albany. I have broad training in Microbiology, Biophysics and Quantitative Biological Imaging. My laboratory develops multispectral fluorescence imaging technologies, including methods for preparing and labeling intact biofilms, and novel algorithms for analyzing highly multi-plex labeled samples and applies these methodologies to study systems-level structure function relationships in oral biofilm communities. I currently mentor 4 PhD students, a postbac researcher, three undergraduates, and three postdocs in my lab, all supported on their own training fellowships (NIH F31) or my NIH grants. Particularly relevant to the Co-mentor plan here, I have previously hosted a visiting postdoctoral researcher from the Pasteur Institute, Dr. Rebecca Stevick that resulted in one manuscript, currently pre-print on BiorXiv and two manuscripts close to submission.

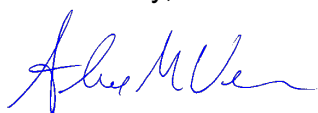
Specifically, my lab will host Dam as a visiting scholar at SUNY Albany. With this status, Dam will have full access to my laboratory and all shared equipment in the Life Sciences Research Institute, including our Zeiss LSM 980 multispectral confocal microscope. I will provide hands on training in FISH probe design and testing and Combinatorial Labeling and Spectral Imaging Fluorescence in situ Hybridization (CLAS-FISH). I will provide Dam with both technical training as well as intellectual guidance in imaging experimental design, quantitative image analysis, and interpretation of multispectral image data. Beyond technical training, I will advise integration of imaging results with other datasets to address key research questions, supporting Dam's development as an independent researcher in microbial spatial ecology. As the Coordinator of AI and Imaging in Biology at SUNY Albany, I will especially train Dam in modern A.I. approaches for analyzing large image datasets, including the ones she will generate as part of this proposal.

To grow her professional network, Dam will be invited to join and present in the Center for Translational Imaging, an interdisciplinary group of imaging scientists in the New York Capital District made up of researchers from SUNY Albany, Albany Medical College, Rensselaer Polytechnic Institute and the New York Dept of Health Wadsworth Center. As Dam gains expertise in quantitative imaging, she will be further invited to assist Dr. Valm in teaching microbiome imaging workshops and to mentor a visiting summer undergraduate researcher in the Valm lab as appropriate.

As part of the mentoring team, I will meet with Dam and the entire team bi-monthly to discuss her projects and review progress toward the proposal aims. The co-mentoring plan at SUNY Albany will be closely coordinated with the primary mentor, Dr. Diaz, to ensure a cohesive, non-redundant, and integrated training experience. As needed, I will hold one-on-one sessions with Dam over Zoom to provide tailored guidance, constructive feedback and problem-solving support as she designs her imaging experiments. In addition, co-mentors will advise on strategies to build a professional network, establish collaborations, and navigate career pathways in academia and research, ensuring Dam acquires the skills, experience, and confidence necessary to become a successful independent investigator.

At the conclusion of her training in my laboratory, Dam will be free to take with her reagents and software tools including FISH probe sequences for oral microbiome members, labeled E. coli cells as fluorescence standards for implementing CLAS-FISH in her own independent lab, and machine learning MATLAB and other code developed in the Valm lab by postdocs and students that have been published.

Yours truly,



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 10833 LE CONTE AVENUE, BOX 951668
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October 7, 2025

Re: Mentoring Statement for Dr. Dam Soh

Dear Colleagues,

I am pleased to serve as a co-mentor for Dr. Dam Soh's NIH K08 Mentored Clinical Scientist Development Award. I am a Professor and Chair in the Division of Oral and Systemic Health Sciences at the UCLA School of Dentistry. My laboratory focuses on elucidating the molecular mechanisms underlying the pathogenicity of both Gram-negative and Gram-positive bacteria, with a particular emphasis on oral pathogens. Our research encompasses cell surface biogenesis, pilus assembly, protein secretion, and bacterial pathogenesis. We employ a multidisciplinary approach that integrates classical and modern methodologies, including bacterial genetics, biochemical techniques, electron microscopy, cryo-electron tomography, X-ray crystallography, biophysics, mass spectrometry, cell-based assays, and rodent models of infection, hence providing a rich training environment to the lab trainees. Our work has been continuously supported by the NIH for over two decades.

Having successfully mentored numerous graduate students, postdoctoral fellows, and early-stage investigators who have gone on to establish independent academic and research careers, I am fully committed to supporting Dr. Soh's development as an independent scientist. This will be achieved through rigorous research training and structured professional guidance. As a co-mentor, I will provide Dr. Soh with training and mentorship in bacterial genetics and molecular techniques directly relevant to her research on oral microbial communities. Specifically, I will guide her in designing and generating targeted gene knockouts, constructing mutant strains, and applying advanced molecular cloning and genome-editing methods. I will also assist her in interpreting experimental results and troubleshooting technical challenges to ensure scientific rigor and reproducibility. Through this mentorship, Dr. Soh will gain a strong mechanistic understanding of microbial genetics, complementing her broader training in microbial ecology and biofilm biology.

The co-mentoring team—comprising Drs. Diaz, Valm, Wood, and myself—will meet with Dr. Soh bi-monthly to review research progress, discuss publications, and evaluate career development milestones. When appropriate, I will also meet with Dr. Soh individually to provide targeted feedback and problem-solving support. Our mentoring plan will be closely coordinated with the primary mentor to ensure a cohesive and integrated training experience encompassing experimental design, data analysis, manuscript preparation, and grant writing.

Beyond technical training, I will advise Dr. Soh on strategies for building collaborations, expanding her professional network, and navigating academic career advancement. I will encourage her participation in national and international scientific meetings, assist in identifying appropriate publication venues, and provide feedback on research presentations and proposals. Through this structured and collaborative mentoring framework, I am confident that Dr. Soh will acquire the expertise, independence, and professional foundation necessary to transition successfully to an independent investigator role.

Sincerely,

Hung Ton-That

HUNG TON-THAT, PHD
 PROFESSOR & CHAIR OF ORAL & SYSTEMIC HEALTH SCIENCES
 DR. NO-HEE PARK ENDOWED CHAIR
 DIRECTOR, T90/R90 DS-OHR TRAINING PROGRAM
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October 6, 2025

RE: Co-mentoring Statement Troy Wood

I am a Professor of Chemistry at the University at Buffalo and the Laboratory Director of the University at Buffalo's Center for Integrated Biomedical Sciences (CIGBS). My formal training is in mass spectrometry (Ph.D. with Alan Marshall, the Ohio State University, and Postdoc with Fred McLafferty, Cornell University). My laboratories are involved in development of analytical methods for the detection and quantification of proteins, pharmaceuticals, and metabolites in biological specimens. Ms. Soh's research fits well within the mission of CIGBS, and I will advise her on experimental design to ensure robust and reproducible metabolite identification and quantification. I will provide further analytical chemistry technique guidance as needed to support the completion of her experimental aims. Relevant to the mentoring plan, Ms. Soh has worked with my former doctoral student Dr. Erin Tiede (now at Kodak) on developing an LC-MS approach for detection of organic acids from microbial communities, which Dr. Tiede presented at the American Society for Mass Spectrometry annual conference.

As part of your Ms. Soh's team, we will meet with her bimonthly to discuss projects and review research progress. Tailored advising in developing your analytical methods which we will coordinate with Professor Diaz. The CIGBS labs and my South Campus office are two floors below Professor Diaz's laboratories in the Biomedical Research Building, which facilitates our meetings. In addition to experimental design, I will mentor Ms. Soh on the finer points of LC-MS data analysis, grant writing, scientific communication, and manuscript preparation. In addition, as Ms. Soh's research will have interest in the mass spectrometry community, as opportunities arise I will invite her to accompany my group to present at mass spectrometry-based meetings to help broaden her scientific network.

The resources provided by our lab highly complement Ms. Soh's proposed project to help develop scientific independence. Furthermore, I am eager to help her build her laboratory skill set and experience, as researchers who work and communicate across disciplines are truly essential in 21st century scientific research.

Sincerely,

A handwritten signature in black ink that reads 'Troy D. Wood'. The signature is written in a cursive, flowing style.

Troy D. Wood, Ph.D.
 Professor, Department of Chemistry
 Laboratory Director, Center for Integrated Global Biomedical Sciences (CIGBS)

Department of Chemistry
 College of Arts and Sciences

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 twood@buffalo.edu

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Description of Institutional Environment

The University at Buffalo (UB) School of Dental Medicine (SDM) is internationally recognized for its innovative research and major contributions to dental science. Ranked among the top 10 dental institutions in NIH research funding, UB provides a robust scientific environment with extensive resources, state-of-the-art facilities, and exceptional intellectual capital. As a research-intensive university with long-standing strengths in oral biology, UB fosters an interdisciplinary setting that will enhance the proposed project and support Dr. Dam Soh's career development.

The Department of Oral Biology at UB, established as the first of its kind in a U.S. dental school and the pioneer in offering a Ph.D. in Oral Biology, continues to set national standards in oral health research. The department consistently ranks in the top 25% of U.S. dental institutions for NIDCR funding and has a strong record of NIH training grants and faculty research awards. This robust funding base sustains a dynamic training program that cultivates the next generation of leaders in dental research. The Department of Oral biology currently employs 20 faculty, all of which maintain funded research programs. Areas of faculty expertise include Immunology, Craniofacial Biology, Bone and Mineralization, Stem Cell Biology, Saliva and Salivary glands, Microbial Ecology and Pathogenesis and Cancer Biology. The faculty includes nationally distinguished scholars who serve as editors of leading journals and officers of major dental societies, as well as rising investigators who bring new perspectives and innovation. This breadth of expertise provides an outstanding mentorship network for Dr. Soh, ensuring exposure to cutting-edge science, guidance in research independence, and support in establishing a competitive trajectory in microbiome and periodontal research. The Department of Oral Biology is home to highly successful predoctoral (PhD in Oral Biology) and postdoctoral training programs. It is also associated with the PhD Program in Biomedical Sciences (PPBS) run by the School of Medicine and Biomedical Sciences. The Department provides a nurturing environment for all Ph.D and Masters students and post-doctoral scholars working under the mentorship of faculty. The Department holds a weekly journal club seminar where students have the opportunity to present and discuss a current paper with peers and faculty. External and internal researchers at the cutting edge of science are invited to present at the Department of Oral Biology Research Seminars Series, with students and faculty are provided with the opportunity to interact with the invited speaker through a lunch with trainees and independent meetings. All together, the Department of Oral Biology provides an excellent environment for Dr. Soh's career development with diverse opportunities and experiences.

Dr. Diaz's laboratory and the UB Microbiome Center (UBMC), where Dr. Soh conducts her research, serve as a hub for investigations into the oral microbiome in both human and laboratory animal models. The UBMC focuses on the role of microbial communities in chronic diseases, including periodontal disease. The UB's Genomics and Bioinformatics Core provides advanced sequencing technologies, including 16S rRNA gene and metatranscriptomic platforms essential to this proposal. The Optical Imaging and Analysis Facility at the School of Dental Medicine offers state-of-the-art imaging systems, including confocal microscopy, which will be critical for spatial analyses of biofilm architecture. The Chemistry Instrument Center at the Department of Chemistry provides cutting-edge analytic chemistry platforms and training. Dr. Soh has already gained experience using this facility and will further expand her metabolomics expertise there to support the proposed studies. In addition, UB maintains a comprehensive electronic library system with extensive online access to scientific literature and databases, ensuring continuous access to critical resources.

UB promotes a highly collaborative and interactive research culture. Faculty across departments share resources and expertise, creating a supportive and interdisciplinary environment. Dr. Soh's prior training in UB's periodontics program established strong connections with clinical faculty, offering continued opportunities to align research with clinical needs. She will also participate in monthly seminars of the University at Buffalo—University of Rochester Microbiome Working Group, broadening her professional network and exposure to innovative science. In addition, UB provides workshops, seminar series, and grant development programs provided by Office of Research Advancement (ORA) that support the career advancement of early-stage investigators.

In sum, the University at Buffalo offers an exceptional environment for Dr. Soh's research and career development. Access to advanced infrastructure, a collaborative and interdisciplinary culture, and a distinguished mentorship network will collectively ensure her success in completing the proposed studies and establishing a strong, independent research career.



October 02, 2025

Dear Members of the Review Panel:

I am writing to express my strong support for Dr. **Dam Soh's** application for the K08 NIH Mentored Clinical Scientist Research Career Development Award. Dr. Soh is a PhD candidate in the Department of Oral Biology and a Periodontology Resident, currently supported by the NIH-funded K12 program at the School of Dental Medicine at the University at Buffalo. Dr. Soh is conducting her research under the primary mentorship of Dr. Patricia I. Diaz. Her work focuses on inter-species interactions and ecological determinants that influence the fitness of the periodontal pathogen *Porphyromonas gingivalis*. As a long-term goal, Dr. Soh aims to establish an independent research program that advances translational research in oral microbiome and periodontology. She is pursuing the K08 award to gain additional training and experience necessary to achieve this objective and successfully transition to independence under the comprehensive mentorship of Dr. Diaz as well as the mentoring committee Dr. Soh has assembled.

The Department of Oral Biology fully supports Dr. Soh's career development and is committed to providing the environment, resources, and mentorship necessary for her success, independent of this award. At the time of the award, Dr. Soh would have completed all the requirements for her clinical training. Therefore, as a full-time PhD candidate, Dr. Soh will have protected time to devote 100% effort to research and career development activities.

Dr. Soh will have dedicated office and laboratory space, with access to the necessary equipment, instrumentation, and core facilities required to conduct her proposed research. She will have opportunities to collaborate with researchers throughout the Department and across the University, leveraging multidisciplinary expertise. These collaborations will provide access to diverse perspectives, technical support, and resources that will enhance the scope and impact of her work. She will be encouraged to participate in weekly departmental seminars and journal clubs with opportunities to engage with invited speakers, fostering professional networking and collaborative partnerships.

The Department is committed to providing a collaborative, resource-rich environment enabling Dr. Soh to develop the skills and knowledge necessary to become an independent investigator. Her position in the Department is not contingent upon receipt of the K08 award, and her highly innovative research will be supported regardless of the outcome of this proposal. However, the K08 award will allow Dr. Soh the opportunity to accelerate her career development by providing more opportunities for scientific training by engaging with her mentoring committee, providing support to attend courses and scientific conferences and enabling her to start her independent line of research thereby increasing her potential to obtain a competitive faculty position and establish as an independent investigator by the end of the K08 award period.

Dr. Soh is a promising scientist who is expected to make impactful contributions to oral microbiome research. In summary, the Department of Oral Biology is fully committed to providing Dr. Dam Soh with the time, resources, mentorship, and support necessary to ensure the successful development of her research career.

Sincerely,

A handwritten signature in blue ink that reads 'Stefan Ruhl'.

Stefan Ruhl, D.D.S., Ph.D.

Professor and Chair of Oral Biology

Professor (HS) of Medical Microbiology and Immunology

Department of Oral Biology

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PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001

Expiration Date: 12/31/2027

Use of Human Specimens and/or Data

Does any of the proposed research in the application involve human specimens and/or data *

☒ Yes

☐ No

Provide an explanation for any use of human specimens and/or data not considered to be human subjects research.

Are Human Subjects Involved

☒ Yes

☐ No

Is the Project Exempt from Federal regulations?

☐ Yes

☒ No

Exemption Number

☐ 1

☐ 2

☐ 3

☐ 4

☐ 5

☐ 6

☐ 7

☐ 8

Other Requested Information

Human Subject Studies

Study#	Study Title	Clinical Trial?
<u>1</u>	Imaging of natural human subgingival biofilm	No
<u>2</u>	Community-wide metabolic profiling of the subgingival microbiome in relation to P. gingivalis colonization	No

Section 1 - Basic Information (Study 1)

1.1. Study Title *

Imaging of natural human subgingival biofilm

1.2. Is this study exempt from Federal Regulations *

☐ Yes ☒ No

1.3. Exemption Number

☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8

1.4. Clinical Trial Questionnaire *

1.4.a. Does the study involve human participants?

☒ Yes ☐ No

1.4.b. Are the participants prospectively assigned to an intervention?

☐ Yes ☒ No

1.4.c. Is the study designed to evaluate the effect of the intervention on the participants?

☐ Yes ☒ No

1.4.d. Is the effect that will be evaluated a health-related biomedical or behavioral outcome?

☒ Yes ☐ No

1.5. Provide the ClinicalTrials.gov Identifier (e.g. NCT87654321) for this trial, if applicable

Section 2 - Study Population Characteristics (Study 1)

2.1. Conditions or Focus of Study

- Periodontal disease

2.2. Eligibility Criteria

- Ability to understand and sign the consent form
- Adult females and males more than 25 years old
- At least 15 teeth present with at least 8 posterior teeth including pre-molars and molars
- At least 2 sites PD ≥ 5 mm
- Positive for the bacteria *P. gingivalis* present at less than 1% abundance after screening

2.3. Age Limits	Min Age: 25 Years	Max Age: N/A (No limit)
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2.3.a. Inclusion of Individuals Across the Lifespan 1260-Inc Ind acx life span_study 1_FINAL.pdf

2.4. Inclusion of Women and Minorities 1261-Incl_women_min_childr_study1.pdf

2.5. Recruitment and Retention Plan 1262-Recruitment and Retention Study1.pdf

2.6. Recruitment Status	Not yet recruiting
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2.7. Study Timeline

2.8. Enrollment of First Participant	09/01/2026	Anticipated
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Inclusion of Individuals Across the Life Span for Study 1 (Aim 2a): “Imaging of natural human subgingival biofilm”

The study is designed to enroll individuals aged 25 and older, a demographic in which chronic periodontitis is most prevalent and clinically significant. This age range is scientifically justified by the natural history of the disease and its primary pathogen, *Porphyromonas gingivalis*. By the age of 25, both the oral microbiome and the host immune response have typically reached a stable, mature state. This targeted recruitment ensures that the data will reflect the pathophysiology of established chronic periodontitis in a fully developed adult context, thereby enhancing the clarity of the findings. The research questions are not relevant to pediatric populations, and children (individuals under 18) are excluded to avoid the specific ethical considerations associated with their inclusion in research. The investigative team possesses extensive experience in adult periodontal disease research and clinical care, with previous clinical study involvement with adult populations, including expertise in informed consent procedures, patient recruitment, and managing the specific health considerations of middle-aged and older adults. The clinical facilities at University at Buffalo are fully equipped to accommodate adult participants aged 25 and older, with accessible examination rooms, appropriate equipment, and full ADA accessibility compliance. The inclusion of adults across the lifespan (25 years and older) will enable meaningful analysis of chronic periodontitis and *P. gingivalis* colonization patterns, contributing to a comprehensive understanding of disease pathophysiology in oral environments.

Inclusion of Women and Minorities for Study 1 (Aim 2a): “Imaging of natural human subgingival biofilm”.

In order to conduct Study 1 (Aim 2a): “Imaging of natural human subgingival biofilm”, we will recruit women and men, age 25 years and older, as well as minorities to this study. See the table below for estimated distribution and numbers of participants.

- *Planned distribution of subjects by sex/gender, race, and ethnicity.*

Below is a table of our estimate of the numbers of females and males by gender, race and ethnicity we expect to recruit. The estimates are based on the distribution of gender, race and ethnicity in Western New York State and in previous studies.

	Females	Males	Total
Hispanic	1	1	2
Not Hispanic or Latino	4	4	8
American Indian/Alaska Native	0	0	0
Asian	1	0	1
Native Hawaiian/Other Pacific Islander	0	0	0
Black/African American	1	1	2
White/Caucasian	3	3	6
More than one race	1	0	0
Total	6	4	10

- *Rationale for selection of sex/gender, race, and ethnic group members in terms of the scientific objectives and proposed study design.*

The estimated numbers are based on the distribution of gender, race and ethnicity in Western New York State and on the numbers and distributions in previous studies. Participation will be open to the general population in the Western New York area using flyers, posters, advertisements and/or word-of-mouth and from already-completed studies – all directed at the entire population in this geographic area. Both men and women are colonized by *Pg*. The distribution is in line with the scientific aims of Aim 2a and the larger grant proposal.

- *Proposed outreach programs for recruiting sex/gender, race, and ethnic group members.*

We will recruit subjects from the Western New York Community using a multi-faceted approach. To recruit a cohort representative of the Western New York population, we will utilize community-based outreach via eye-catching flyers and posters that pique interest in community centers and local clinics, digital media, and our established database of prior research participants who have consented to be re-contacted for future studies. In addition, we have contacts at churches, senior centers, libraries, social groups, and similar venues that we can leverage for this study. We have successfully used these sources in the past as opportunities for education in dental and general health (providing guidelines for oral health, taking blood pressures at health fairs, etc.) as well as recruitment for previous studies. We have often been the leading and most successful center in recruiting for multi-center studies because of these methods and the contacts we have developed over the years. These broad methods will ensure that the populations recruited are diverse and reflect the demographics of the area.

Inclusion and Excluded Groups: No individual will be excluded from the study based on sex/gender, race and/or ethnicity.

Recruitment and Retention Plan for Study 1 (Aim 2a): “Imaging of natural human subgingival biofilm”.

For this proposed study, participants will be recruited from the community using flyers, posters, advertisements, and/or word-of-mouth, as well as from participants in already-completed studies. The clinical research center maintains lists of participants from previous studies who have consented to be contacted for new studies. Existing patient charts will be screened to determine if a comprehensive oral examination has been performed and periodontitis has been diagnosed.

Responders to posters, flyers, and advertisements will be screened over the phone using a Telephone Script and Screening Checklist. If eligible, they will be scheduled for a screening visit. Former participants will be called and asked about their interest in this proposed study using a similarly scripted format and the Screening Checklist. If needed, we may leave voicemail messages about this study and ask them to call us back if interested.

We will mail consents to participants prior to their appointment to allow them to review the consents ahead of the visit so they can formulate questions. A trained staff member will review the study consent form with the participant prior to completion of any study activities. Any questions raised by the participant about risks, benefits, confidentiality, voluntary participation, or any other aspect of the study will be answered until the potential participant is comfortable. All eligible men and women will be required to sign the consent before any study activities occur.

Our methods of recruitment will be respectful of potential participants' preferences and rights so they can make a comfortable, informed decision about participating in our studies. In all contacts, respondents will be provided information about the study, screened for interest and eligibility, and those who remain interested will be scheduled for an appointment. Prior to the scheduled appointment, they will be mailed a packet of information including a brief summary of the study, instructions regarding the appointment (fasting requirements), a consent form for initial review, questionnaires to complete, and a parking pass. To maximize retention, we will employ a participant-centered plan that includes flexible scheduling, systematic appointment reminders via the participant's preferred method, building positive rapport, and providing fair compensation for their time and travel. This comprehensive approach is designed to ensure the timely enrollment of our target sample, minimize attrition, and uphold the scientific integrity of the proposed research.

2.9. Inclusion Enrollment Reports

IER ID#	Enrollment Location Type	Enrollment Location
<u>Study 1, IER 1</u>	Domestic	The State University of New York at Buffalo (Buffalo, New York)

Inclusion Enrollment Report 1

1. Inclusion Enrollment Report Title* : Imaging of natural human subgingival biofilm
2. Using an Existing Dataset or Resource* : ☐ Yes ☒ No
3. Enrollment Location Type* : ☒ Domestic ☐ Foreign
4. Enrollment Country(ies): USA: UNITED STATES
5. Enrollment Location(s): The State University of New York at Buffalo (Buffalo, New York)
6. Comments:

Planned

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	0	0	0	0	0
Asian	1	0	0	0	1
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	1	1	0	0	2
White	2	2	1	1	6
More than One Race	1	0	0	0	1
Total	5	3	1	1	10

Cumulative (Actual)

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/ Alaska Native	0	0	0	0	0	0	0	0	0	0
Asian	0	0	0	0	0	0	0	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	0	0	0	0	0	0	0
Black or African American	0	0	0	0	0	0	0	0	0	0
White	0	0	0	0	0	0	0	0	0	0
More than One Race	0	0	0	0	0	0	0	0	0	0
Unknown or Not Reported	0	0	0	0	0	0	0	0	0	0
Total	0	0	0	0	0	0	0	0	0	0

Section 3 - Protection and Monitoring Plans (Study 1)

3.1. Protection of Human Subjects

1263-Protection of Human subject_Study1.pdf

3.2. Is this a multi-site study that will use the same protocol to conduct non-exempt human subjects research at more than one domestic site?

☐ Yes ☒ No ☐ N/A

Single IRB plan attachment

3.3. Data and Safety Monitoring Plan

1264-Data and Safety Monitoring Plan_Study 1.pdf

3.4. Will a Data and Safety Monitoring Board be appointed for this study?

☐ Yes ☒ No

3.5. Overall structure of the study team

Protection of Human Subjects for Study 1 (Aim 2a): “Imaging of natural human subgingival biofilm”

1. Risks to Human Subjects

a. Human Subjects Involvement, Characteristics, and Design

Proposed involvement of human subjects: The goal of this study is to evaluate the pH gradient and spatial distribution of *Porphyromonas gingivalis* (Pg) at its initial establishment stage in *in situ* subgingival biofilms allowed to develop in a carrier system (Aim 2a). To accomplish this goal, we will develop and collect intact human subgingival biofilms by placing an ePTFE membrane attached to a carrier system into the periodontal pockets of study participants. Using advanced imaging systems capable of taxonomic identification of up to 100 species within a biofilm, we will characterize the spatial architecture of subgingival species and evaluate whether the localization pattern of Pg positively or negatively correlates with pH microenvironments or specific co-localized bacterial species.

Characteristics of the subject population:

To meet the goals of this aim, we will recruit 10 adult males and females with detectable Pg that is less than 1% relative abundance in subgingival plaque as assessed via qPCR and 16S rRNA gene sequencing. Other eligibility criteria are:

- Ability to understand and sign the consent form
- Adult females and males more than 25 years old
- At least 15 teeth present with at least 8 posterior teeth including pre-molars and molars
- At least 2 sites with PDs ≥ 5 mm
- Ability to understand the instructions and comply with study procedures

Exclusion Criteria:

- Individuals with unstable medical conditions or conditions associated with immunosuppression
- Chronic use of corticosteroids, cyclosporine or other immunosuppressive medication
- Pregnancy or lactation
- Oral lesions (lichen planus, candidiasis, leukoplakia, pemphigus, aphthous or herpetic lesions)
- Orthodontic therapy
- Acute necrotizing gingivitis or periodontitis
- Pericoronitis, dental abscesses or any other urgent dental condition that requires immediate care

Subject Enrollment and Retention: This study is expected to enroll subjects representing the demographic makeup in Western New York State.

	Females	Males	Total
Hispanic	1	1	2
Not Hispanic or Latino	4	4	8
American Indian / Alaska Native	0	0	0
Asian	1	0	1
Native Hawaiian / Other Pacific islander	0	0	0
Black/African American	1	1	2
White / Caucasian	3	3	6
More than one race	1	0	1
Total	6	4	10

Recruitment and Retention Plan: We will recruit subjects from the Western New York Community using a multi-faceted approach. To recruit a cohort representative of the Western New York population, we will utilize community-based outreach via flyers and posters in community centers, local clinic and digital media, and our established database of prior research participants who have consented to be re-contacted for future studies. Following an initial screening, all potential subjects will undergo a

comprehensive informed consent process conducted by principal investigator. To maximize retention, we will employ a participant-centered plan that includes flexible scheduling, systematic appointment reminders via the participant's preferred method, building positive rapport, and providing fair compensation for their time and travel. This comprehensive approach is designed to ensure the timely enrollment of our target sample, minimize attrition, and uphold the scientific integrity of the proposed research.

Responders to posters, flyers and advertisements will be screened over the phone using a standardized telephone script and eligibility checklist. Individuals who appear eligible and remain interested will be scheduled for an in-person screening visit. Before their appointment, we will mail them an information packet containing a confirmation letter, directions to our clinic, medical history forms to complete, and a copy of the consent form for their review. To ensure meticulous oversight, we will use a Screening and Enrollment Log to monitor all recruitment efforts and document the status of every prospective participant.

Study design: This prospective, observational study is designed to examine the relationship between local pH gradients and the spatial distribution of the pathogen *Pg* within the biogeography of naturally developed subgingival biofilms. The study will employ an in situ carrier system, where expanded polytetrafluoroethylene (e-PTFE) membranes are placed inside human periodontal pockets to allow for undisturbed biofilm development over a 7-day period. After this maturation phase, the intact biofilms will be collected and subjected to a sequential imaging workflow. First, pH ratiometric imaging using confocal microscopy will generate a three-dimensional map of local pH microenvironments. Subsequently, Combinatorial Labeling and Spectral Imaging-Fluorescence *in situ* Hybridization (CLASI-FISH) will be used to taxonomically identify and map the spatial organization of up to 100 of the most abundant taxa within the same biofilm. By superimposing the pH map with the species distribution data, we will directly evaluate how pH gradients correlate with the establishment and localization of *Pg*, providing a deeper understanding of this pathogen's colonization dynamics.

b. Study Procedures, Materials, and Potential Risks

Planned Research Procedures:

1. **Screening Visit - Subgingival Plaque Collection:** After receiving a detailed explanation of all study procedures, eligible participants will provide written informed consent. They will then undergo a routine periodontal examination that includes measuring probing depths to assess their clinical status. For microbial analysis, subgingival plaque samples will be collected from the deepest site in each quadrant using sterile curettes. These samples are essential for the initial screening, which involves detecting the *Pg* level via qPCR and analyzing the overall microbiome composition through Illumina amplicon sequencing

Screening visit activities

- A. Obtain informed consent for study and review eligibility criteria
- B. Collect data on:
 - a. Demographic information: age, gender, contact information
 - b. Medical history data: disease history, medications, surgeries
- C. Oral health examination with clinical periodontal parameters measured
 - a. Clinical attachment levels (CAL): The distance from the cemento-enamel junction to the base of the pocket in mm measured. Six sites around the tooth are examined: mesial-buccal, buccal, distal-buccal, distal-lingual, lingual and mesial-lingual
 - b. Pocket depth (PD): The distance from the gingival margin to the base of the pocket is measured in mm measured at six sites per tooth: mesial-buccal, buccal, distal-buccal, distal-lingual, lingual and mesial-lingual
 - c. Bleeding on Probing (BOP):
 1. 0 = No bleeding on probing
 2. 1 = Presence of bleeding on probing

3. Each of the six sites around the tooth (mesial-buccal, buccal, distal-buccal, distal-lingual, lingual and mesial-lingual) is scored after probing depth measurements are taken
- d. Plaque score (O'Leary): It is a dichotomous measure determined with the use of disclosing solution at 6 sites on all teeth:
 1. 0 = no plaque
 2. 1 = presence of plaque
- D. If eligible on level of periodontal disease, collect subgingival plaque sample for PCR determination of the presence or absence of *Pg*

2. **Carrier System Placement:** For subjects meeting enrollment criteria, e-PTFE membranes attached to plastic carriers will be carefully inserted into the periodontal pocket at the deepest site, reaching the base of the pocket. Each carrier will be secured supragingivally to the tooth surface using cyanoacrylate adhesive (dental-grade tissue adhesive). Subjects will maintain the carrier system in place for 7 days while continuing normal oral hygiene practices (avoiding direct brushing of the carrier site).
3. **Carrier Removal Visit:** After 7 days, carriers will be carefully removed from the periodontal pockets. A brief oral examination will be conducted to assess the carrier site.
4. Depending on preliminary findings, we may ask subjects to carry two e-PTFE membranes or at subsequent time-intervals to examine biofilm formation at different time points (eg. 3 or 4 days).

Research Materials to be Obtained:

- Subgingival plaque samples from periodontal pockets
- In situ developed biofilm on e-PTFE carrier membranes
- Clinical periodontal measurements
- Demographic and medical history information

Identifiable Information: Private identifiable information will be collected, including name, date of birth, contact information, medical and dental history. All data will be coded with a unique study identification number, and a master linking key will be maintained separately in a secure, password-protected database accessible only to authorized study personnel.

Potential Risks:

Physical Risks (Minimal): The potential risks to participants are minimal and primarily consist of temporary, localized discomfort associated with the study procedures. Subjects may experience minor transient discomfort or bleeding during subgingival plaque sampling, similar to what might occur during a routine dental cleaning, as well as a mild gingival irritation at the carrier insertion site. There is a rare possibility of an allergic reaction or temporary tissue irritation from the cyanoacrylate adhesive used to secure the device. During the 7-day period when the carrier is in place, there is a potential for increased plaque accumulation, which could cause temporary gingival inflammation, and a possibility that the carrier could become prematurely dislodged, necessitating its removal.

Privacy and Confidentiality Risks (Minimal): The primary risks involve a potential breach of confidentiality of personal health information and study data, along with an extremely low risk that an individual could be identified through their unique microbiome data.

Risk Level: This study presents minimal risk to subjects. The procedures are similar to those encountered during routine dental examinations and periodontal treatment. The carrier system has been used successfully in previous published research without significant adverse events.

2. Adequacy of Protection Against Risks

a. Informed Consent and Assent

Informed Consent Process: Informed consent will be obtained from all prospective subjects before any study-related procedures are performed. The consent process will be conducted in a private setting by trained study personnel (principal investigator) who are knowledgeable about the study procedures and risks.

Consent Circumstances and Information: Potential subjects will be identified through the periodontal clinic at University at Buffalo or through approved recruitment materials. Subjects will be provided with adequate time to review the consent document and ask questions. The informed consent document will include:

- Purpose and background of the research
- Description of all study procedures and timeline
- Potential risks and discomforts
- Potential benefits (or lack thereof) to subjects
- Confidentiality protections
- Voluntary nature of participation and right to withdraw
- Compensation
- Contact information for questions or concerns

Capacity to Consent: All subjects will be adults (≥ 25 years) capable of providing informed consent. If study personnel have concerns about a potential subject's capacity to provide informed consent, the individual will not be enrolled.

Documentation of Consent: Written informed consent will be obtained using an IRB-approved consent form. Both the subject and the person obtaining consent will sign and date the document, and the subject will receive a copy for their records.

Waiver of Consent: No waiver of informed consent will be requested.

b. Protections Against Risk

Strategies to Minimize Physical Risks: To ensure participant safety, all study procedures will be performed by the Principal Investigator with ample experience in periodontal procedures and the handling of ePTFE membranes. All interventions will use sterile or disposable instruments in strict accordance with standard infection control protocols. Prior to enrollment, subjects will be carefully screened for any medical conditions or allergies. During the 7-day carrier exposure period, participants will receive clear instructions for maintaining oral hygiene while avoiding the carrier site and will be provided with contact information to immediately report any concerns, such as significant pain, swelling, or carrier dislodgement. Emergency dental care will be available if needed throughout the study, and upon removal, the carrier insertion site will be thoroughly examined for any adverse effects to confirm the participant's well-being.

Privacy and Confidentiality Protections: To protect participant confidentiality, all study data will be coded with unique identification numbers, and biological specimens will be labeled only with these non-identifiable codes. The master key linking these study codes to personal information will be stored separately in a secure, encrypted, and password-protected database. Access to identifiable information will be strictly limited to authorized study personnel who have completed human subjects research and HIPAA training and have signed confidentiality agreements. All research materials will be securely stored, with paper records kept in locked file cabinets within secure offices and electronic data housed on password-protected, encrypted institutional servers with regular backups. Any data shared for future research purposes will be fully de-identified to ensure participant privacy.

Management of Incidental Findings: During the periodontal examination and plaque collection procedures, incidental findings may be discovered (e.g., dental caries, oral lesions, severe periodontal disease). The informed consent document will explain that subjects will be informed of any clinically significant findings and referred for appropriate dental care. If urgent conditions are discovered, subjects will be referred immediately for treatment.

c. Vulnerable Populations and Special Considerations

Vulnerable Populations: This study does not specifically target vulnerable populations.

Children, Prisoners, and Neonates: This study will not enroll children (under 18 years), prisoners, pregnant women specifically, human fetuses, or neonates. These populations are not relevant to the research objectives.

3. Potential Benefits of the Proposed Research to Research Participants and Others

Benefits to Research Participants: Individual subjects enrolled in this study will receive a comprehensive periodontal examination, including probing depth measurements and assessment of periodontal disease status. They will also receive information about their oral health status. Subjects will not receive periodontal treatment as part of this study, though they will be referred for standard care as needed. Subjects should not expect their periodontal condition to improve as a result of study participation.

Benefits to Others: This research has the potential to significantly advance scientific knowledge about the microbial ecology of periodontal disease by clarifying how pH gradients in natural subgingival biofilms correlate with the spatial distribution of pathogenic bacteria like *Pg* and identifying specific inter-species interactions that influence pathogen colonization. This foundational knowledge is crucial for developing novel, targeted therapeutic strategies aimed at manipulating biofilm microenvironments to prevent or treat periodontitis. Given that periodontitis affects a significant proportion of adults worldwide and is associated with consequences ranging from tooth loss to complications with systemic health conditions like cardiovascular disease, the insights gained from this work could ultimately lead to more effective interventions and improved health outcomes for future patients.

Risk-Benefit Ratio: The risks to subjects are minimal and temporary, involving procedures similar to routine dental care. The potential knowledge gained about periodontal disease mechanisms and inter-species interactions in natural biofilms is substantial and could inform future therapeutic development. Therefore, the risks are reasonable in relation to the anticipated benefits to society, even though individual subjects will not receive direct therapeutic benefit.

4. Importance of the Knowledge to be Gained

Scientific Importance: Periodontitis is a prevalent chronic inflammatory disease linked to tooth loss and systemic conditions like cardiovascular disease, yet the mechanisms by which key pathogens like *Pg* colonize the complex polymicrobial biofilms that drive the disease remain poorly understood. Current knowledge is largely derived from simplified laboratory models, creating a significant gap in understanding how these pathogens exploit specific microenvironmental niches within natural communities. The proposed study will overcome this challenge by employing an innovative carrier system to study intact, undisturbed subgingival biofilms, enabling the first-ever detailed spatial analysis of pH gradients and bacterial distributions in this clinically relevant setting. This novel approach is expected to provide direct evidence of whether pH heterogeneity correlates with *Pg* localization, identify specific bacterial species that spatially co-localize with or are excluded from the pathogen, and validate in vitro findings in natural biofilms, thereby generating foundational data for the development of new microenvironment-targeted therapeutic strategies.

Data and Safety Monitoring Plan

Although this project does not constitute a clinical trial and involves no therapeutic intervention, a Data and Safety Monitoring Plan is included to ensure appropriate oversight, participant protection, and data integrity. This single-site, minimal-risk observational study involves the temporary placement of a biocompatible carrier system within periodontal pockets to allow subgingival biofilm development for imaging. Oversight of all study activities will be provided by the Principal Investigator (PI) and the University at Buffalo Institutional Review Board (IRB), in accordance with NIH guidelines for human subjects research.

The PI will be responsible for the day-to-day monitoring of all research procedures, participant safety, and data accuracy. All study activities, including subject screening, informed consent, plaque collection, carrier placement and removal, and data management, will be conducted by trained and IRB-approved study personnel under the PI's direct supervision. The IRB will review and approve the protocol, consent documents, and all modifications, and will perform continuing review of the study at least annually. Given the limited sample size, single-site design, and minimal risk level, the establishment of an independent Data and Safety Monitoring Board is not required.

Monitoring will focus on participant eligibility, informed consent documentation, procedural adherence, and any adverse events. Adverse events will be assessed at each visit and documented in the study record. Minor and expected events, such as mild gingival irritation or temporary bleeding during sampling, will be recorded and reviewed by the PI. Any serious or unanticipated adverse event, including hospitalization or significant injury, will be reported to the IRB within 24 hours and to the NIH awarding institute according to their reporting requirements. Participants will be instructed to contact study staff immediately if they experience discomfort, swelling, or dislodgement of the carrier system. Emergency dental care will be available at the University at Buffalo Dental Clinic as needed.

Data integrity and confidentiality will be safeguarded through the use of coded study identifiers, secure electronic databases, and password-protected institutional servers compliant with HIPAA standards. The master linking file will be stored separately from research data in a restricted-access encrypted folder. All paper records will be maintained in locked offices accessible only to authorized study personnel. The PI will ensure that all investigators and staff complete required human subjects protection and HIPAA training prior to engaging in study activities.

Given the minimal-risk nature of this observational study, no interim analyses or stopping rules are required. However, the PI will review study progress monthly, and any unexpected pattern of adverse events will prompt immediate review and potential suspension of study activities pending IRB evaluation. Annual progress reports to NIH will include a summary of safety monitoring findings, any adverse events, and corrective actions implemented.

Section 4 - Protocol Synopsis (Study 1)

4.1. Study Design

4.1.a. Detailed Description

4.1.b. Primary Purpose

4.1.c. Interventions

Type	Name	Description
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4.1.d. Study Phase

Is this an NIH-defined Phase III Clinical Trial? ☐ Yes ☐ No

4.1.e. Intervention Model

4.1.f. Masking ☐ Yes ☐ No

☐ Participant ☐ Care Provider ☐ Investigator ☐ Outcomes Assessor

4.1.g. Allocation

4.2. Outcome Measures

Type	Name	Time Frame	Brief Description
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4.3. Statistical Design and Power

4.4. Subject Participation Duration

4.5. Will the study use an FDA-regulated intervention? ☐ Yes ☐ No

4.5.a. If yes, describe the availability of Investigational Product (IP) and Investigational New Drug (IND)/ Investigational Device Exemption (IDE) status

4.6. Is this an applicable clinical trial under FDAAA? ☐ Yes ☐ No

4.7. Dissemination Plan

Section 1 - Basic Information (Study 2)

1.1. Study Title *

Community-wide metabolic profiling of the subgingival microbiome in relation to P. gingivalis colonization

1.2. Is this study exempt from Federal Regulations *

☐ Yes ☒ No

1.3. Exemption Number

☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8

1.4. Clinical Trial Questionnaire *

1.4.a. Does the study involve human participants?

☒ Yes ☐ No

1.4.b. Are the participants prospectively assigned to an intervention?

☐ Yes ☒ No

1.4.c. Is the study designed to evaluate the effect of the intervention on the participants?

☐ Yes ☒ No

1.4.d. Is the effect that will be evaluated a health-related biomedical or behavioral outcome?

☒ Yes ☐ No

1.5. Provide the ClinicalTrials.gov Identifier (e.g. NCT87654321) for this trial, if applicable

Section 2 - Study Population Characteristics (Study 2)

2.1. Conditions or Focus of Study

- Periodontal disease

2.2. Eligibility Criteria

- Ability to understand and sign the consent form
- Adult females and males more than 25 years old
- At least 15 teeth present with at least 8 posterior teeth including pre-molars and molars
- More than 2 sites $\geq 5\text{mm}$
- Ability to understand the instructions and comply with study procedures

2.3. Age Limits	Min Age: 25 Years	Max Age: N/A (No limit)
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2.3.a. Inclusion of Individuals Across the Lifespan 1265-Incl_ind_accr_lifespan_study2.pdf

2.4. Inclusion of Women and Minorities 1266-Incl_women_min_childr_study2.pdf

2.5. Recruitment and Retention Plan 1267-Recruitment and Retention Study2.pdf

2.6. Recruitment Status Completed

2.7. Study Timeline

2.8. Enrollment of First Participant	06/21/2021	Actual
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Inclusion of Individuals across the life span for Study 2 (Aim 2b): “pH-modulating metabolic activities of subgingival plaque communities and their relationship to *Pg* colonization status”.

This study recruited adult males and females aged 25 years and older, a demographic in which chronic periodontitis is most prevalent and clinically significant. This age range was selected based on the natural history of the disease and its primary pathogen, *Porphyromonas gingivalis*. By age 25, the oral microbiome and host immune system are typically stable and mature, allowing for accurate assessment of established chronic periodontitis in a fully developed adult context. The study’s research questions are not applicable to pediatric populations, and children were excluded to avoid additional ethical considerations.

The investigative team has extensive experience conducting clinical research on adult periodontal disease, including recruitment, informed consent, and management of age-specific health considerations. The clinical facilities at the University at Buffalo are fully equipped for adult participants aged 25 and older, with accessible examination rooms, appropriate equipment, and full ADA compliance. The inclusion of adults across the lifespan (25 years and older) enables meaningful analysis of chronic periodontitis and *P. gingivalis* establishment, advancing understanding of disease pathophysiology in adult oral environments.

Inclusion of Women and Minorities for Study 2 (Aim 2b): “pH-modulating metabolic activities of subgingival plaque communities and their relationship to *Pg* colonization status”.

In order to conduct Study 2 (Aim 2b): “pH-modulating metabolic activities of subgingival plaque communities and their relationship to *Pg* colonization status”, we recruited women and men, age 25 years and older, as well as minorities to this study. Significant numbers of women were recruited, comprising 52.5% of the participants included in the proposed study. Minorities were also recruited and enrolled in lesser numbers, whose distribution is similar to the racial and ethnic composition of Western New York Community. See the table below for the distribution and numbers of participants.

	Females	Males	Total
Hispanic	4	2	6
Not Hispanic or Latino	38	36	74
American Indian/Alaska Native	0	0	0
Asian	2	2	4
Native Hawaiian/Other Pacific Islander	0	0	0
Black/African American	6	6	12
White/Caucasian	30	28	58
More than one race	4	2	6
Total	42	38	80

The distribution of participants by sex aligns with the scientific aims of this study and the larger grant proposal. This population study was designed to recruit individuals with moderate periodontitis with or without *Porphyromonas gingivalis* colonization. Since periodontitis occurs in men and women at comparable rates, balanced enrollment of both sexes was scientifically appropriate and ensured the generalizability of study findings to the broader population affected by periodontal disease. No vulnerable populations were included in this study.

Inclusion and Excluded Groups: No individual will be excluded from the study based on sex/gender, race and/or ethnicity.

Recruitment and Retention Plan for Study 2 (Aim 2b): “pH-modulating metabolic activities of subgingival plaque communities and their relationship to *Pg* colonization status”.

For this proposed study, participants have already been recruited from the community using flyers, posters, advertisements and/or word-of-mouth and from participants from already-completed studies.

At the time of submission, 40 *Pg*-positive and 40 *Pg*-negative participants have already been enrolled.

2.9. Inclusion Enrollment Reports

IER ID#	Enrollment Location Type	Enrollment Location
<u>Study 2, IER 1</u>	Domestic	The State University of New York at Buffalo (Buffalo, New York)

Inclusion Enrollment Report 1

1. Inclusion Enrollment Report Title* : Community-wide metabolic profiling of the subgingival microbiome in relation to P. gingivalis colonization
2. Using an Existing Dataset or Resource* : ☒ Yes ☐ No
3. Enrollment Location Type* : ☒ Domestic ☐ Foreign
4. Enrollment Country(ies): USA: UNITED STATES
5. Enrollment Location(s): The State University of New York at Buffalo (Buffalo, New York)
6. Comments:

Planned

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	0	0	0	0	0
Asian	0	0	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	0	0	0	0	0
White	0	0	0	0	0
More than One Race	0	0	0	0	0
Total	0	0	0	0	0

Cumulative (Actual)

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/ Alaska Native	0	0	0	0	0	0	0	0	0	0
Asian	2	2	0	0	0	0	0	0	0	4
Native Hawaiian or Other Pacific Islander	0	0	0	0	0	0	0	0	0	0
Black or African American	6	6	0	0	0	0	0	0	0	12
White	26	26	0	4	2	0	0	0	0	58
More than One Race	4	2	0	0	0	0	0	0	0	6
Unknown or Not Reported	0	0	0	0	0	0	0	0	0	0
Total	38	36	0	4	2	0	0	0	0	80

Section 3 - Protection and Monitoring Plans (Study 2)

3.1. Protection of Human Subjects

1268-Protection of Human subject_Study2.pdf

3.2. Is this a multi-site study that will use the same protocol to conduct non-exempt human subjects research at more than one domestic site?

☐ Yes ☒ No ☐ N/A

Single IRB plan attachment

3.3. Data and Safety Monitoring Plan

1269-Data and Safety Monitoring Plan_Study 2.pdf

3.4. Will a Data and Safety Monitoring Board be appointed for this study?

☐ Yes ☒ No

3.5. Overall structure of the study team

Protection of Human Subjects for Study 2 (Aim 2b): “Community-wide metabolic profiling of the subgingival microbiome in relation to *P. gingivalis* colonization”.

1. Risks to Human Subjects

a. Human Subjects Involvement, Characteristics, and Study Design

Proposed involvement of human subjects:

The goal of this study is to identify community-wide, pH-modulating species or metabolic activities within the subgingival microbiome that are associated with the colonization status of pathogen *Porphyromonas gingivalis* (*Pg*). To accomplish this goal, we have already collected samples of subgingival plaque and gingival crevicular fluid (GCF) from 40 *Pg*-positive and 40 *Pg*-negative subjects with moderate periodontitis. We will perform metatranscriptomic analysis on the subgingival plaque samples to compare the expressed pH-modifying gene functions between the *Pg*-positive and *Pg*-negative groups. Concurrently, we will conduct targeted liquid chromatography-mass spectrometry (LC-MS) metabolomics on matched GCF samples to quantify key metabolites. By integrating these functional and metabolic datasets, we will determine if specific species or community-wide activities in the subgingival environment correlate with *Pg* colonization status, thereby providing critical *in vivo* evidence for the metabolic factors that shape the microbial ecosystem in periodontitis.

We have already obtained IRB approval for the study (ID: STUDY00005287) and completed subject enrollment.

Characteristics of the subject population:

To meet the goals of this aim, we will analyze 80 already recruited adult males and females with detectable *Pg* in subgingival plaque as assessed via PCR. Other eligibility criteria are:

- Ability to understand and sign the consent form
- Adult females and males more than 25 years old
- At least 15 teeth present with at least 8 posterior teeth including pre-molars and molars
- More than 2 sites $\geq 5\text{mm}$
- Ability to understand the instructions and comply with study procedures

Exclusion Criteria:

- Individuals with unstable medical conditions or conditions associated with immunosuppression
- Periodontal therapy within the past one year
- Systemic antibiotic therapy within 6 months prior to the study initiation
- Chronic use of non-steroidal anti-inflammatory medication
- Chronic use of corticosteroids, cyclosporine or other immunosuppressive medication
- Pregnancy or lactation
- Oral lesions (lichen planus, candidiasis, leukoplakia, pemphigus, aphthous or herpetic lesions)
- Orthodontic therapy
- Acute necrotizing gingivitis or periodontitis
- Pericoronitis, dental abscesses or any other urgent dental condition that requires immediate care

Subject Enrollment and Retention:

Subject enrollment for this study has been completed. The study satisfies demographic diversity requirements, as shown in the table below. The enrollment is reflective of the demographic diversity of Western New York State.

	Females	Males	Total
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Hispanic	4	2	6
Not Hispanic or Latino	38	36	74
American Indian/Alaska Native	0	0	0
Asian	2	2	4
Native Hawaiian/Other Pacific Islander	0	0	0
Black/African American	6	6	12
White/Caucasian	30	28	58
More than one race	4	2	6
Total	42	38	80

Recruitment and Retention Plan: Participation was open to the general population in the Western New York area through multiple channels, including flyers, posters, advertisements, word-of-mouth, and referrals from previously completed studies. Our database of participants from prior studies who have expressed interest in future research is substantial and comprises reliable, conscientious individuals who are committed to contributing to scientific advancement. We utilized eye-catching posters and flyers designed to generate interest, which yielded strong response rates. Additionally, we have cultivated positive relationships with advertising representatives at local newspapers, radio stations, and television stations. Colorful, engaging advertisements in community newspapers and *Buffalo Healthy Living*, accompanied by detailed articles about the studies, have proven highly effective recruitment strategies in previous research. Radio interviews and associated advertisements have also been valuable recruitment tools. The interviewer we work with is well-known and respected in Western New York and demonstrates enthusiasm for health-related initiatives. She conducts engaging, encouraging interviews with investigators and staff that effectively stimulate public interest in our research.

Responders to posters, flyers and advertisements are screened over the phone (using a Telephone Script and Screening Checklist) and if eligible, scheduled for a screening visit. Former participants are called and asked about their interest in this proposed study using a similarly scripted format and a Screening Checklist.

Screening involves two phases: a telephone screening and a clinical screening visit. The telephone contact can be initiated either by the potential participant in response to an advertisement or by a staff member/screener. A script with a screening checklist is used for telephone calls. The script provides information about every aspect of the study including number of visits, activities at each visit, amount of time each visit takes, and the screener encourages questions to promote greater understanding of the commitment the caller may make. If the caller remains interested, a screening visit is scheduled. Prior to the visit, a packet of information is sent to the caller and includes a letter confirming the date and time of the screening appointment, directions and a map to the clinic, the consent form that they are encouraged to read but not sign, and Medical and Dental Histories to complete before the visit. A Screening and Enrollment Log is used to monitor participant recruitment and document the enrollment status of prospective study participants.

Retention: Recruitment, data collection and biological sample collection were completed. Hence, plans for recruitment and retention have been effective and no additional recruitment is planned.

Study design: This study will employ a cross-sectional design to investigate the relationship between the subgingival microbiome's metabolic functions and *Porphyromonas gingivalis* (Pg) colonization status. To accomplish this goal, we collected subgingival plaque and gingival crevicular fluid (GCF) samples from Pg-positive and Pg-negative individuals and will conduct a multi-omics analysis. Metatranscriptomic sequencing of plaque will identify community-wide, pH-modulating gene expression patterns, while targeted LC-MS metabolomics and colorimetric assays will quantify corresponding organic acids and ammonia in matched GCF samples. By integrating these functional and metabolic datasets, we aim to

determine if specific species or community-wide activities create an environment that is either favorable or antagonistic to *Pg* colonization, providing critical *in vivo* insight into the ecosystem dynamics that influence this pathogen

b. Sources of Materials – Study Visit Activities

- Screening visit
 - Obtain informed consent for study and review eligibility criteria
 - Collect data on:
 - Demographic information: age, gender, contact information
 - Medical history data: disease history, medications, surgeries
 - Oral examination and periodontal measurements to determine level of periodontal disease
 - If eligible on level of periodontal disease, collect subgingival plaque sample for PCR determination of the presence or absence of *Pg*
 - Schedule sample collection appointment
- Sample collection visit:
 - Update data on:
 - Demographic information
 - Medical history, disease history, medications, surgeries
 - Height and weight, blood pressure and pulse
 - Oral health examination with clinical periodontal parameters measured
 - Clinical attachment levels (CAL): The distance from the cemento-enamel junction to the base of the pocket in mm measured. Six sites around the tooth are examined: mesial-buccal, buccal, distal-buccal, distal-lingual, lingual and mesial-lingual
 - Pocket depth (PD): The distance from the gingival margin to the base of the pocket is measured in mm measured at six sites per tooth: mesial-buccal, buccal, distal-buccal, distal-lingual, lingual and mesial-lingual
 - Bleeding on Probing (BOP):
 - 0 = No bleeding on probing
 - 1 = Presence of bleeding on probing
 - Each of the six sites around the tooth (mesial-buccal, buccal, distal-buccal, distal-lingual, lingual and mesial-lingual) is scored after probing depth measurements are taken
 - Plaque score (O'Leary): It is a dichotomous measure determined with the use of disclosing solution at 6 sites on all teeth:
 - 0 = no plaque
 - 1 = presence of plaque
 - Collect biological samples:
 - Subgingival plaque via curettes from the site in each side (Right and Left) that shows the deepest PD
 - Gingival crevicular fluid

Biological samples to be collected: Subgingival plaque collected via curettes from the deepest pockets, and gingival crevicular fluid collected using paper points.

c. Potential risks

Periodontal measurements and collecting plaque samples and GCF may be uncomfortable and there is a small risk of gingival bleeding; however, the examiners are experienced and skilled in proper collection technique. None of the study procedures (e.g., oral health examination, periodontal measurements, collection of biological specimens) are considered experimental; they are considered standard of care.

There is a potential risk to confidentiality due to protected health information being collected and stored.

2. Adequacy of Protection Against Risks

a. Recruitment and Informed Consent

Recruitment: Participants were recruited from previous studies (those who had indicated interest in future research), as well as through responses to posters and flyers (placed around the University and local community), advertisements, and word-of-mouth. Our recruitment methods were consistently respectful of potential participants' preferences and rights, enabling them to make comfortable, informed decisions about study participation. In all contacts, respondents were provided with information about the study, screened for interest and eligibility, and those who remained interested were scheduled for a screening visit. Prior to their scheduled appointment, participants were mailed an information packet that included a brief summary of the study, appointment instructions (including fasting requirements), a consent form for initial review, questionnaires to complete, and a parking pass. This approach provided participants with additional time to review materials and consider what study participation entailed, thereby facilitating more informed decision-making.

Consent: Informed consent was obtained from each participant before any other study activities took place, in accordance with the policies of the University at Buffalo Institutional Review Board. Prior to the screening visit, extensive discussion of the risks and possible benefits of the study was provided to potential participants. At the screening visit, the study was again explained in detail, and participants were encouraged to ask questions, providing ample opportunity to understand all activities, procedures, and responsibilities before signing the consent document. After consents were signed and dated, they were copied and the copy was given to participants; the original was retained with participants' clinical records.

Screening Visit: Following the consenting process, a review of the medical and dental histories was completed. An oral health examination was conducted to assess the level of periodontal disease and to collect subgingival plaque for PCR testing for the presence of *P. gingivalis*. Participants were notified of their eligibility after PCR testing was completed and their *P. gingivalis* status was determined.

Other Study Visits: If eligible, participants were scheduled for a Baseline visit. Medical and dental histories were reviewed, and any changes since the screening visit were noted. A complete oral health examination with periodontal measurements was completed, providing documented assessment of the level of periodontal disease and subgingival plaque and gingival crevicular fluid (GCF) were collected, and Plaque Score was obtained.

b. Protections Against Risk

For the clinical examinations and collection of biological samples, only trained, calibrated, and skilled examiners were used. To maintain the confidentiality of study records, paper records are kept in locked cabinets and/or locked rooms. Computerized datasets are stored on study computers accessible only to authorized study personnel and are password protected. Participant identity is coded and is not associated with any published results. Code numbers and identities are kept in secured files maintained by the Principal Investigator. Participant identity can only be revealed through an on-site review by an IRB or NIDCR representative of clinical records to assess accuracy and consistency with study/research records. During such reviews, the representative might see a name with other information, but they were not allowed to remove any clinical record containing a name from the secured area unless mandated by a judicial court order. HIPAA policies and practices were followed in the handling of participants' dental records and study documents. Identifying data are kept in files separate from study data files for as long as there is interest in re-contacting participants, such as for a follow-up study.

3. Potential Benefits of the Proposed Research to Human Subjects and Others

Individual participants will benefit from clinical evaluation and diagnosis of periodontal infections. Furthermore, by identifying specific community-wide metabolic functions that create environments either conducive or antagonistic to *Pg*, this study will provide a fundamental understanding of the ecological mechanisms driving periodontal disease. This knowledge is critical for overcoming current therapeutic limitations and may enable the development of targeted strategies aimed at preventing *Pg* colonization and promoting a microbiome compatible with oral health. Ultimately, this work has the potential to reduce the burden of periodontitis, a prevalent chronic disease associated with significant systemic health consequences.

4. Importance of the Knowledge to be Gained

The activities in this study will provide additional information for making conclusions related to the general hypotheses proposed and tested in this overall project, leading to greater information related to the colonization of *Pg*. *Pg* is an important human pathogen, associated with periodontitis and also a risk factor in several systemic conditions, including cardiovascular disease, rheumatoid arthritis, and Alzheimer's disease. The purpose of this study is to add to elucidate the environmental factors that influence *Pg* colonization in the oral cavity so that the new knowledge can be used in the development of prevention and treatment tactics for periodontal disease and against the systemic spread of *Pg*.

Data and Safety Monitoring Plan

This study is a single-site, minimal-risk observational project involving the collection of subgingival plaque and gingival crevicular fluid (GCF) samples from adult participants for metatranscriptomic and metabolomic analyses. Participant enrollment has been completed, and no investigational drugs, devices, or interventions are involved. The procedures pose minimal risk, limited primarily to mild and transient discomfort associated with plaque and GCF sampling.

The Principal Investigator (PI) will provide continuous oversight to ensure compliance with the approved IRB protocol and institutional policies for human subjects research. Given the minimal-risk, non-interventional design, this level of monitoring is appropriate and consistent with NIH guidelines. The PI will oversee data collection, sample handling, and record-keeping to ensure participant confidentiality, data accuracy, and protocol adherence. Any adverse events or protocol deviations will be promptly reported to the PI, who will determine whether they require IRB notification in accordance with institutional procedures. Because enrollment is complete and no further participant contact or intervention is planned, no interim safety analyses are required.

All data will be de-identified prior to analysis and managed using coded participant identifiers. A master linking file that connects participant identifiers to study codes is securely stored on an encrypted, access-restricted institutional server. Analytical data, including sequencing and metabolomic datasets, will be stored on institutional servers with encryption, access control, and routine data backup. Only study personnel approved by the IRB have access to identifiable data, and all data handling follows institutional and NIH data security standards.

Although adverse events are not anticipated, any unexpected events such as gum irritation or minor bleeding during sample collection will be documented and reported to the IRB per institutional policy. Serious or unanticipated problems involving risk to participants will be reported promptly to the IRB and, if required, to the NIH program officer.

Quality assurance will be maintained through periodic internal review of consent documentation, sample labeling, and data entry accuracy conducted by the PI or designated study staff. The IRB will continue to provide ongoing oversight through continuing review as required. Overall, the proposed data and safety monitoring plan is appropriate for the minimal-risk nature of this completed, observational study.

Section 4 - Protocol Synopsis (Study 2)

4.1. Study Design

4.1.a. Detailed Description

4.1.b. Primary Purpose

4.1.c. Interventions

Type	Name	Description
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4.1.d. Study Phase

Is this an NIH-defined Phase III Clinical Trial? ☐ Yes ☐ No

4.1.e. Intervention Model

4.1.f. Masking ☐ Yes ☐ No

☐ Participant ☐ Care Provider ☐ Investigator ☐ Outcomes Assessor

4.1.g. Allocation

4.2. Outcome Measures

Type	Name	Time Frame	Brief Description
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4.3. Statistical Design and Power

4.4. Subject Participation Duration

4.5. Will the study use an FDA-regulated intervention? ☐ Yes ☐ No

4.5.a. If yes, describe the availability of Investigational Product (IP) and Investigational New Drug (IND)/ Investigational Device Exemption (IDE) status

4.6. Is this an applicable clinical trial under FDAAA? ☐ Yes ☐ No

4.7. Dissemination Plan

Delayed Onset Studies

Delayed Onset Study#	Study Title	Anticipated Clinical Trial?	Justification
The form does not have any delayed onset studies			

Resource Sharing Plan

Data and biomaterials generated from this research project will be disseminated following the Public Health Service (PHS) Policy on Distribution of Unique Research Resources Produced with PHS Funding and all applicable Federal Regulations.

1. Sharing Biomaterials

Unique resources developed through this research will be made readily available to the scientific community after the publication of the work. These resources may include, but are not limited to, bacterial mutants created to deplete acid production. Mutant strains will be made available to other investigators upon request.

2. Dissemination of Data

In addition to the annual report to the funding agency, progress on the research project will be reported at least once per year at scientific meetings. Manuscripts reporting scientifically significant findings will be prepared promptly for publication in peer-reviewed journals. Sequencing data generated through the research will be deposited in the GenBank and Sequence Read Archive (SRA) of NCBI. Data will be made available to the scientific community following the publication of the work or by end of the performance period of the award.

NIH Generated message:

The Other Plan(s) attachment included with the application is not evaluated during the peer review process but will be evaluated prior to a funding decision. Although part of the official submission, the attachment is maintained as a separate document in eRA Commons viewable by authorized users and is not part of this assembled application.

Authentication of Key Biological and/or Chemical Resources

Biologic and chemical reagents involved in this project are purchased from well-established, reputable suppliers and lot numbers are being recorded. Moreover, all key resources for this project will be authenticated as outlined below to enhance the reproducibility of our results.

Experiments with microorganisms will be conducted with strains of valid identity as established by 16S rRNA gene sequencing or specific sequencing of deletion mutants. The purity of routinely-grown cultures will be verified via colony morphology and Gram-staining prior to each experiment.

Regarding all reagents, to ensure that there are no batch effects, we will be testing new batches of reagents via side-by-side comparison with the previous, before continuing with new experiments.