

Electronic Cover Sheet		
PI: Elias, Jeremy	Title: Three-Dimensional Hydrogel Models for Analyzing Cariogenic Bacterial and Antimicrobial Responses	
Received: 11/11/2025	Opportunity: PA-24-191	Council: 05/2026
Competition ID: FORMS-I	FOA Title: Mentored Quantitative Research Development Award (Parent K25 Independent Clinical Trial Not Allowed)	
1K25DE035216-01A1	Dual:	Accession Number: 5219466
IPF: 2705601	Organization: ADA FORSYTH INSTITUTE, INC.	
Former Number: 1K25DE035216-01	Department: Mineralized Tissue Bioengineering	
IRG/SRG: ZRG1 MSOS-F (22)S	AIDS: N	Expedited: N
<u>Subtotal Direct Costs</u> <u>(excludes consortium F&A)</u> Year 1: 151,700 Year 2: 151,700 Year 3: 151,700 Year 4: 151,700 Year 5: 151,700	Animals: N Humans: N Clinical Trial: N Current HS Code: 10 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>
Jeremy Elias	ADA Forsyth Institute, Inc.	PD/PI
Xuesong He	ADA Forsyth Institute, Inc.	Other Professional-Co-Mentor
Jirun Sun	ADA Forsyth Institute, Inc.	Other Professional-Mentor
Felicitas Bidlack	ADA Forsyth Institute, Inc.	Other Professional-Scientific Advisory Committee

Reference Letters

Thomas Angelini	University of Florida	11/11/2025
Josephine Allen	University of Florida	11/11/2025
mark martindale	Univ. Floirda	11/11/2025

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE		State Application Identifier
1. TYPE OF SUBMISSION*		4.a. Federal Identifier DE035216
<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*: MZ9DFVC2J1B7		
Legal Name*: ADA Forsyth Institute, Inc. Department: Office of Sponsored Programs Division: Street1*: 245 First Street Street2: City*: Cambridge County: Middlesex State*: MA: Massachusetts Province: Country*: USA: UNITED STATES ZIP / Postal Code*: 02142-1200		
Person to be contacted on matters involving this application Prefix: First Name*: Anastacia Middle Name: L. Last Name*: Feldman Suffix: Position/Title: Street1*: 245 First Street Street2: City*: Cambridge County: Middlesex State*: MA: Massachusetts Province: Country*: USA: UNITED STATES ZIP / Postal Code*: 02142-1200 Phone Number*: (617)892-8425 Fax Number: (617)892-8434 Email: osp@forsyth.org		
6. EMPLOYER IDENTIFICATION NUMBER (EIN) or (TIN)*		1042104230A2
7. TYPE OF APPLICANT*		M: Nonprofit with 501C3 IRS Status (Other than Institution of Higher Education)
Other (Specify): 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 type="radio"/> New <input checked="" type="radio"/> Resubmission <input type="radio"/> Renewal <input type="radio"/> Continuation <input type="radio"/> Revision		<input type="radio"/> A. Increase Award <input type="radio"/> B. Decrease Award <input type="radio"/> C. Increase Duration <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* Three-Dimensional Hydrogel Models for Analyzing Cariogenic Bacterial and Antimicrobial Responses		
12. PROPOSED PROJECT		13. CONGRESSIONAL DISTRICTS OF APPLICANT
Start Date* Ending Date* 07/01/2026 06/30/2031		MA-007

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

Prefix: First Name*: Jeremy Middle Name: Last Name*: Elias Suffix:

Position/Title: Postdoctoral Fellow

Organization Name*: ADA Forsyth Institute, Inc.

Department: Mineralized Tissue Bioengineering

Division:

Street1*: 245 First Street

Street2:

City*: Cambridge

County: Middlesex

State*: MA: Massachusetts

Province:

Country*: USA: UNITED STATES

ZIP / Postal Code*: 02142-1200

Phone Number*: 909-342-4582 Fax Number: Email*: jelias@forsyth.org

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested* \$819,180.00

b. Total Non-Federal Funds* \$0.00

c. Total Federal & Non-Federal Funds* \$819,180.00

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*: Shawn Middle Name: H. Last Name*: Eung Suffix:

Position/Title*: Sr. Director, Office of Sponsored Programs

Organization Name*: ADA Forsyth Institute, Inc.

Department: Office of Sponsored Programs

Division:

Street1*: 245 First Street

Street2:

City*: Cambridge

County: Middlesex

State*: MA: Massachusetts

Province:

Country*: USA: UNITED STATES

ZIP / Postal Code*: 02142-1200

Phone Number*: (617) 892-8334 Fax Number: (617) 892-8434 Email*: osp@forsyth.org

Signature of Authorized Representative*
Anastacia L Feldman

Date Signed*
11/11/2025

20. PRE-APPLICATION File Name:**21. COVER LETTER ATTACHMENT** File Name:1240-Cover Letter_Elias_K25_11.11.25.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: ADA Forsyth Institute, Inc.
UEI: MZ9DFVC2J1B7
Street1*: 245 First Street
Street2:
City*: Cambridge
County: Middlesex
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 02142-1200
Project/Performance Site Congressional District*: MA-007

Additional Location(s)

File Name:

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* <input type="radio"/> Yes <input checked="" type="radio"/> No	
1.a. If YES to Human Subjects Is the Project Exempt from Federal regulations? <input type="radio"/> Yes <input type="radio"/> No If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6 _ 7 _ 8 If NO, is the IRB review Pending? <input type="radio"/> Yes <input type="radio"/> No IRB Approval Date: Human Subject Assurance Number	
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 1254-Project Summary_11.10.25.pdf
8. Project Narrative*	1255-Project Narrative_11.03.25.pdf
9. Bibliography & References Cited	1256-Bibliography_11.03.25.pdf
10. Facilities & Other Resources	1257-Facilities and Resources_11.03.25.pdf
11. Equipment	1258-Equipment_11.03.25.pdf

Project Summary: Biofilm-mediated diseases, including dental caries, impose a significant global health and economic burden, underscoring the need for precision treatments that account for the dynamic nature of complex three-dimensional (3D) biofilms. In addition, pathogenic bacteria thrive in structured environments shaped by porosity, physical confinement, and chemical gradients. Bridging the gap between planktonic cultures or traditional surface-grown biofilms and realistic 3D biofilms is crucial to advancing our understanding and developing targeted treatments. This project seeks to create an innovative 3D microbial culture platform offering *precise spatiotemporal control and real-time monitoring* to reveal how bacteria behave and respond to antimicrobials within biofilms. Insights from this platform will guide the design of precision therapies for biofilm-associated infections. To accomplish this, we integrate expertise in caries microbiology, advanced materials engineering, and single-cell imaging. The project is structured around three specific aims: **Aim 1:** Define the extent of spatial precision regarding the control and analysis of viability, proliferation, and chemical/physical gradients in a bacteria-laden 3D model using *Streptococcus mutans*, the main cariogenic bacterium causing dental caries. We will adapt and optimize bacteria-laden hydrogels, which in preliminary studies have supported *S. mutans* proliferation, aggregation, and metabolism, to construct printed 3D microenvironments. Within these constructs, we will map viability and proliferation while quantifying the profiles of controlled nutrient and pH gradients. **Aim 2:** Evaluate the spatial specificity and efficacy of pH-responsive antimicrobial agents in targeting *S. mutans* within single-species 3D environments. By integrating pH-responsive antimicrobial agents into the bacteria-laden hydrogel systems, we will test how these agents target cariogenic *S. mutans* in response to their metabolic activity (acid production via carbohydrate fermentation). We will quantify antimicrobial efficacy with single-cell resolution and assess how spatial localization and chemical gradients influence treatment outcomes. **Aim 3:** Assess how interspecies interactions of *S. mutans* with other bacteria may affect biofilm architecture, acidogenesis, and antimicrobial susceptibility in 3D hydrogels. Leveraging our ability to spatially position bacterial aggregates, we will create co-culture models with *S. mutans* and partner species to examine how interspecies co-aggregation, competition, or cooperation shape biofilm formation, acid production, and antimicrobial behavior in 3D environments. During this K25 Mentored Quantitative Research Development Award, I will receive both theoretical and hands-on training in microbiology, advanced imaging techniques, and bioengineering under the guidance of experts at ADA Forsyth. The skills and knowledge I acquire will prepare me to establish an independent research program centered on microbial interactions, using advanced material models and fine-scale microstructural control to explore deeper questions and promote a healthy and balanced oral microbiome.

Project Narrative: Biofilm-mediated diseases such as dental caries impose a substantial global burden with no effective prevention strategy, as bacteria within biofilm communities exhibit dramatically different physiology, often showing greater tolerance to antimicrobial treatment. The proposed 3D culture platform will fill a critical gap by enabling high-resolution exploration of bacterial behavior, chemical variation, and therapeutic responses in realistic biofilm architectures, paving the way for smarter, localized and precise antimicrobial strategies.

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ADA FORSYTH INSTITUTE, Inc.

FACILITIES AND OTHER RESOURCES

ADA Forsyth Institute, Inc. is an independent, nonprofit research foundation whose mission is to improve human health through innovative research and education in the biomedical sciences. Forsyth is located at 100 Chestnut St. in Somerville, Massachusetts. The 73,317 sq. ft. facility near key locations in Cambridge and Boston brings ADA Forsyth Institute into close proximity to its counterparts in the biotech industry, fostering collaborations and creating opportunities to expand its research. Investigators at Forsyth have access to state-of-the-art equipment and resources located in the Boston area, including Harvard, Tufts and Boston Universities and affiliates. The institute maintains strong ties with the Longwood Medical Area and collaborates with neighboring institutions such as the Massachusetts Institute of Technology, the Broad Institute, and MGH. The Forsyth facility features state-of-the-art labs and equipment, a research clinic, multiple conference spaces and administrative offices.

SCIENTIFIC ENVIRONMENT

ADA Forsyth Institute has a long history of successfully conducting studies related to oral and systemic health and in providing research training to dental students and postdoctoral fellows. The institute hosts local, national and international speakers in weekly seminars, holds an in-house speaker series, several weekly group meetings and journal clubs, a weekly brainstorming session for new ideas and many other opportunities for scientific interactions, giving ample opportunity for interaction and collaborative dialogues with experts across many fields. Investigators at Forsyth provide invaluable constructive feedback and informal intellectual input collaboratively. Forsyth's Scientific Symposium has become an annual event over the last six years. With global attendance, this event brings together scientists with diverse research backgrounds and different career stages to share their research and ideas. The meeting encourages collaborations and provides a great opportunity for trainees to interact with experts in the field and promote their research.

RESEARCH FACILITIES

Forsyth's facilities are distributed over four floors: one floor for clinical research, meeting facilities (conference and seminar rooms), financial, development and upper administration offices, a second floor for research administration, bullpen space for postdoctoral fellows, library, data services, and staff lunchroom, and two wet lab research floors that include conferencing and casual meeting space.

Investigators have their own offices (120 sq. ft.) which are located on the laboratory level. There are separate shared bullpen areas for postdoctoral fellows. All office spaces have desks, chairs and filing cabinets and are hardwired for high-speed access to the internet and to the internal Forsyth intranet. These facilities ensure that investigators and trainees have space to plan experiments, analyze data and prepare manuscripts for publication.

Investigators each have their own PC computers, typically Intel i5/i7 machines with computers linked to shared black & white and color laser jet printers. Computers are connected to Forsyth's internal intranet and the internet via 1 Gbps connections to Forsyth's Office of Computing & Network Technology (OCNT). The combination of these linked computers contributes to efficient data handling and optimal communication between members of the research team.

CORE FACILITIES

Advanced Microscopy Core: The Forsyth Advanced Microscopy Core is equipped with a suite of light microscopes. There is a Zeiss Axio Observer inverted microscope with epi-fluorescence illumination and Colibri LED light source, seven DIC/FL objectives, an Orca-Flash4 CMOS camera, AxioCam 712 color camera, and a ZEN Pro T/Z workstation. This set up is optimized for live cell imaging including incubation equipment with heating insert, temperature and CO₂ module, and cultivation system that provides imaging options for a great range of samples. The core has in addition, a Zeiss LSM 780 confocal microscope with a GaAsP 34 channel detector and 7 laser lines, ACR beam combiner set, filter sets for DAPI, CY3, and GFP, and five objective lenses. The GaAsP detector and ZEN software package allow for spectral imaging of many fluorophores simultaneously.

A Zeiss LSM 880 confocal microscope is housed in a temperature-controlled chamber and is capable of performing CLARITY imaging, as well as super-resolution imaging using a 32-channel GaAsP Airyscan detector. The core has a Zeiss LSM 980 confocal microscope equipped for simultaneous spectral imaging of many fluorophores as well as NIR detection. This microscope can be used for correlative imaging workflows utilizing the Zen Connect software module.

The workstations involved in image capture, analysis and storage have a gigabit pipe to a 7.5TB storage area network. An image analysis workstation, equipped with a Dell Precision T7610, 64 GB RAM, 16-core Dual Intel Xenon Processor E5-2687W v2, 4 GN NVIDIA Quadro K5000 and dual 27-inch monitors, is also available. Core staff provide support services including training and consultation on data analysis and protocol development.

MicroCT Core: The Forsyth Micro Computed Tomography Core is an NIH-funded imaging facility equipped with a Scanco μ CT 40, ex vivo μ CT scanner, and with a high throughput scanning option (auto sample exchanger). The scanner is designed for 3D X-ray imaging of small samples in high resolution, and provides images and quantitative analyses of internal structures of ex vivo samples without any destructive procedures. The non-destructive nature of this technology allows investigators to carry out complementary analyses (e.g., histology) of the same samples. For image processing, a Scanco GPU Reconstruction Hardware (hp Z4 Windows workstation P5000 GPU) with Software (RDK022719YH-GPU) can integrate with Scanco MicroCT 40 system to accelerate the reconstruction of data. The workstation includes the Amira software package for mCT data processing and visualization. Core staff provide support services including training and consultation on data analysis and protocol development.

Oral Microbiome Core: The Forsyth Oral Microbiome Core (FOMC) offers Next Generation Sequencing (NGS) and comprehensive data analyses and interpretation for 16S rRNA gene amplicon sequences and other big data sequence applications. Analyses of previously obtained sequences are also offered. Many investigators are familiar with the Human Oral Microbe Identification using Next Generation Sequencing (HOMINGS) as well as its predecessor the Human Oral Microbe Identification Microarray (HOMIM) for rapid 16S rDNA analyses of oral clinical samples. The Core continues in that tradition and now provide 16S rRNA and fungal ITS NGS amplicon sequencing services with state-of-the-art bioinformatic methodology, which will provide the best possible taxa identification.

Dr. Bruce Paster, an internationally known oral microbiome researcher, is the director of FOMC and Dr. George Chen, director of Forsyth Bioinformatics Core, is a well-known bioinformatic specialist in oral microbiome research. The primary focus of the FOMC involves the microbial analysis of samples derived from the human oral cavity, however, analyses of samples from all human, animal, and environmental sites are also available.

Animal Facility: The Forsyth Animal Facility provides animal housing, care and associated services for investigators wishing to perform research using live animals at Forsyth. The onsite Forsyth Animal Facility comprises five rooms for housing animals, three procedure rooms and facilities for cage washing and storage. Adjacent to the standard animal facility is a germ-free facility including twelve independent CBC isolators (with required accessories) and a Tlive animal imaging facility. Currently, the facility is able to house mice, rats, guinea pigs and hamsters; additional species may be accommodated with IACUC approval. Animals can be housed under BL2 conditions upon request. The Forsyth Animal Facility maintains a staff of animal care technicians who provide 7 day/week care of the animals. Veterinary care is provided by the consulting veterinarian and backup veterinary care is available 24 hours/day through the MIT Division of Comparative Medicine. The Forsyth IACUC oversees and approves all aspects of animal care in compliance with requirements of the Office of Laboratory Animal Welfare (OLAW) at NIH, the US Department of Agriculture, and the City of Cambridge. Animals are maintained in appropriate caging with free access to food and water; and caging is changed at regular intervals. In addition, all animals are provided with devices that confer environmental enrichment, as appropriate for specific species. Animal care standards described in the 8th edition of the "Guide for the Care and Use of Laboratory Animals" are generally adhered to. The Forsyth Animal Facility is currently fully accredited by AAALAC (most recent accreditation 11/17/23) and has maintained that status continuously for more than 40 years.

The live animal imaging center is located in a dedicated space attached to the Forsyth Animal Facility, including a Lumina IVIS XRMS *in vivo* imaging system and a Ex-Works Cellvizio Dual Band System probe-based laser endomicroscopy system. The IVIS allows for simple, user-guided spectral unmixing for the detection and separation of multiple reporters to monitor simultaneous biologic events in the same animal by either fluorescent or bioluminescent reporters or dyes. The system is equipped with 21 filter sets to image reporters that emit from green to near-infrared and high resolution, sharp cut-off emission filters to achieve the highest performance, sensitivity and spectral unmixing. The system is used with PerkinElmer's Living Image® software to automate all the controls and settings, yielding images that can be overlaid to see optical reporters together with the anatomical surface or X-ray features. The Cellvizio system allows real-time images of tissues in live animals at cellular resolution, *in vivo*. The dual-color capability (488 nm and 660 nm excitations) allows capture of functional and morphological images of molecular mechanisms in real time.

Bioinformatics Core: The Forsyth Bioinformatics Core was established alongside the Human Oral Microbiome Database (HOMD) since 2006. Led by Dr. Tsute (George) Chen, a human oral microbiome data analysis expert, the core has been developing bioinformatics tools to analyze the NGS data for research community in the oral/dental and nasal study fields. The core houses an array of high-performance multi-CPU computers with high memory that can analyze NGS data. The key feature of our pipelines is the use of the HOMD curated 16S rRNA genes and whole genome reference sequences. Thus, the results are tailored to, but not limited to, oral and nasal microbiological and clinical research fields. Please follow this link to the publications involving these pipelines. We also provide consultation and analytical services in oral microbiomics, genomics, taxonomy, phylogenetics and all next generation sequence (NGS) data analysis. The services are provided to both internal and external researchers including the Harvard CATALYST community, for funded projects and funding applications requiring preliminary bioinformatics analysis or method writing.

Epidemiology and Biostatistics Core: The Forsyth Epidemiology and Biostatistics Core collaborates with clinical and laboratory investigators by providing support for experimental design, statistical methods, and analysis and management of data for epidemiologic studies, clinical trials and laboratory investigations. Collaborations have included Center grants, Program Projects and numerous individual investigator grants. These studies have largely focused on oral health research, particularly dental caries and periodontitis, with an emphasis on the microbial and immunologic aspects as well as diagnosis and treatment of these conditions and use of high throughput technologies in their evaluation. Services of the ADA Epidemiology and Biostatistics staff are also available to Forsyth scientists.

Flow Cytometry Core: The Core staff provide consulting and support services as well as protocol development and data analysis expertise. The Forsyth Flow Cytometry Core is equipped with two flow cytometers. The BD FACS Aria II cell sorter is a high-speed fixed alignment benchtop cell sorter capable of multicolor analysis of up to 13 fluorescent markers and two scatter parameters at a time. The cytometer can be operated at varied pressures, acquire up to 70,000 events per second up to 4 populations per sample can be sorted simultaneously into varied collection devices for further research. The Invitrogen Attune NxT Flow Cytometer is equipped with a high throughput auto-sampler and features acoustic-assisted hydrodynamic focusing, allowing for rapid acquisition of up to 35,000 events/second and analysis of up to 14 fluorescent markers.

Multiplex Core: The Forsyth Multiplex Core provides high-throughput analysis of a variety of sample types for simultaneous quantitative analysis of up to 100 or 500 different proteins, peptides, or DNA molecules in a single microplate well. The two platforms available for use at the Core include the Luminex® 200™ and FLEXMAP 3D™ both of which operate on a 96-well plate platform, though the FLEXMAP 3D platform has the capacity to operate on a 386-well plate.

Luminex results are obtained and computed using Bio-Plex Manager, v6.0 (Bio-Rad Laboratories, Hercules, CA) and then exported as an excel file. Data include raw and computed mean fluorescence intensity, observed concentrations, standard curves, %CV, standard deviations, internal controls, and plate-to-plate variations, among others. Both Luminex systems are calibrated and validated weekly to assure accurate results, and both systems are fully covered under service contracts with annual preventive maintenance visits.

The Core Team will be responsible for working closely with the principal investigators, designing the assays, communicating with the companies, and presentation of the data. The Core will be responsible for sample preparation and performance of the assays as well as day-to-day operations and maintenance of the systems.

OTHER RESOURCES

The Office of Computing & Network Technology (OCNT): The Office of Computing & Network Technology (OCNT) is staffed by two technical professionals, including a Director of Information Technology and an FTE Cloud Administrator.

All of Forsyth's front end (Help desk and ticketing), as well as all backend (server patching and maintenance) is handled by a company called Aqueduct Technologies. Aqueduct monitors the environment 24 hours a day, 365 days a year. The network 10gb switched Ethernet LAN with a fiber backbone providing connectivity for more than 225 desktop computers and workstations, including 165 Intel i5/i7 machines, 20 Apple, and 10 UNIX workstations.

All Forsyth servers have been virtualized, and sit on a Cisco Hyperflex, hyper converged computing environment. This environment has a total of 210ghz of computing power. 2TB of memory, and 80TB of useable storage. All of which are upgradable with additional compute nodes.

For backup and retention, Forsyth uses the Cisco Hyperflex, alongside Cohesity Cloud storage device. Backups are taken locally in three separate intervals daily. Depending on the level of importance, servers are backed up on a one, four and 8-hour interval daily. These local backups are then sent via Cohesity to the Microsoft Azure cloud platform every 8 hours.

For security and intrusion monitoring, Forsyth Utilizes two Cisco ASA firewalls, coupled with Cisco Umbrella web filtering and monitoring. All Email traffic is monitored and protected with Mimecast email protection services. Forsyth's environment is also protected and monitored 24/7 by Alert Logic's servers and Components.

The Microsoft Windows Server network operating system utilizes the TCP/IP protocol offering: file and print services; messaging (Microsoft O365); database applications (Microsoft SQL); and business applications including desktop access to grant accounts and generation of purchase orders. Network access to SigmaPlot, Reference Manager and Lasergene is available to all investigators. Several networked departmental printers. A networked, high-speed (65 ppm) printer/copier (Canon IR ADV C7565) for print-once/copy-many technology utilized in printing of grant proposals. The Canon IR ADV C7565 also provides network scanning for bringing legacy documents and other paper documents to the desktop. Full-time access to the Internet is provided via a dedicated, 1Gbps fiber line to our Internet Service Provider, Crown Castle Fiber. Back-up access to the Internet is provided via a fixed wireless 10Mbps line from Netblazr.

The Office of Technology Development: The Office of Technology Development provides the link between Forsyth's research and commercialization efforts. The office evaluates new technologies at Forsyth and facilitates their efficient translation into products and services that improve public health globally. Forsyth's technologies extend well beyond the oral health arena, and include oral-systemic disease connections, vaccines, global infectious diseases, bone, and the prevention of inflammation.

Library access: ADA Forsyth maintains a collection of journals and books related to oral health and biology in its own **Percy Howe Memorial Library** and also provides access to electronic resources and journals through the superb ADA library services that are staffed by several librarians, and comprise nearly 10,000 dental, biomedical, and scientific journals and eBooks. Article delivery service through interlibrary loan is available on request. Many faculty and labs also have access to a university library through their appointments at the Harvard School of Dental Medicine, with access to the Countway Medical Library, or adjunct appointments at various dental schools.

HARVARD UNIVERSITY

Harvard Center for Nanoscale Systems (CNS): The Harvard CNS is an open/shared core facility with two campuses in Cambridge and Allston, Massachusetts that are available and easily accessible to Forsyth Researchers and those at other institutions. CNS offers state-of-the-art facilities and tools including scanning electron microscopy, transmission electron microscopy, and focused ion beam imaging tools as well as micro- and nanomechanical testing and sample preparation tools. CNS also provides training and assistance to users for the imaging, fabrication, and analysis of nanoscale and microscale structures.

EQUIPMENT

ADA FORSYTH INSTITUTE:

Sun Lab:

- Cellink BIOX 3D Bioprinter
- Omnicure S1500 Spot UV Curing System

Shared Equipment:

- 4C cold room
- 37C warm room
- Agilent 2100 Bioanalyzer
- Alpha Innotech FluorChem Q DNA and protein electrophoresis gel documentation system
- BioTek Synergy HT plate reader
- BioRad ChemiDoc MP imager
- Eppendorf BioPhotometer 6131
- Eppendorf 5430R refrigerated tabletop centrifuge
- Eppendorf Vacufuge Plus
- Molecular Devices SpectraMax i3x
- ThermoFisher QuantStudio 3 Real-Time PCR Systems
- Roche Lightcycler 96
- Beckman Coulter Optima L-100 XP ultracentrifuge
- ThermoFisher Lynx4000 superspeed centrifuge (this is shared)
- Nexcelom Cellometer Auto X4 cell viability counter
- Equipment for histological staining
- Heat plates

Department of Mineralized Tissue Biology

The Department of Mineralized Tissue Biology is equipped with:

- Equipment for histological staining
- -20C freezers
- Eppendorf/New Brunswick U410 Premium -80°C freezer
- Several lab computers
- Sartorius precision balance
- Mettler UMT2 microbalance
- Eppendorf 5417R Refrigerated Centrifuge
- Perkin Elmer FTIR-microscope (Multiscope)
- Buehler Hard Tissue Slow Saw
- 2 Zeiss Stemi Dissecting microscopes
- Zeiss Primo Star light microscope
- Constant temperature incubator
- Boekel TTT incubator
- Leco M-400-H1 Hardness Testing Machine
- Labconco Purifier Logic Class II Type A2 Biosafety Cabinet
- BioRad T100 Thermal Cycler
- BioRad Mini Protean Tetra System
- BioRad Turbo Transfer System
- Canon EOS 7D Digital Camera with 65mm Macro lens
- Benchtop equipment including hot plate, vortex, orbital and rotisserie shakers, digital dry bath, pH meter with data storage capacity
- Buehler Hard Tissue Microtome
- Minimet (Buehler) grinder-polisher
- Milli-Q Ultrapure Water System
- Real-time PCR

- iD3 plate reader
- Microtome

Other shared Equipment at Forsyth Institute

Clean bench, CO₂ incubator, -80°C freezers, 4°C refrigerators, Microwaves, Water bath, Bio UV cabinets, Labconco freeze dry system. **The Live Animal imaging center** located in the Animal Facility includes an Illumina IVIS XRMS *in vivo* imaging system and a CellVizio probe-based laser endomicroscopy system.

CORE FACILITIES

Histology Laboratory

Histology and Sectioning equipment:

- RMC Powertome ultramicrotome
- 2 manual Spencer 820 Rotary microtomes
- 1 Microm HM 315 manual rotary microtome
- 1 Automated Microm HM 355S microtome for both paraffin and methyl and glycol methacrylate embedded specimens
- Knife holders allowing for use of both low- and high-profile disposable blades, stainless steel knives and tungsten carbide knives
- Stainless steel knives
- Tungsten carbide knives
- Thermo Scientific Cryomicrotome FSE with high profile and stainless-steel blade holders
- Boekel InSlide Out *in situ* hybridization oven
- Premiere lighted tissue bath xh-1003
- Retriever 2100 for thermally processing slides
- Slide warmer
- Logos X-Clarity Tissue Clearing System
- Two incubators
- Two fume hoods, one of them set up as workstation for histological staining
- Equipment for histological staining
- Workstation with equipment for paraffin embedding
- Heat plates

Advanced Microscopy Core

- Zeiss LSM 980 confocal microscope equipped for simultaneous spectral imaging of many fluorophores as well as NIR detection. This microscope can be used for correlative imaging workflows utilizing the Zen Connect software module.
- Zeiss Evo LS10 scanning electron microscope with Peltier cooling stage and Oxford INCA 250 micro-analysis system for EDS as well as Shuttle & Find software and sample holders for correlative light and electron microscopy.
- Zeiss Axio Observer inverted microscope with epi-fluorescence illumination and Colibri LED light source including 365 nm, 470 nm, 540-580 nm, and 625 nm modules, as well as 425 nm, 490nm, and 600 nm beam combiners, seven DIC/FL objectives including 40x oil DIC, 40x water immersion, and 63x as well as 100x oil immersion objectives. The instrument is equipped with an Orca-Flash4 CMOS camera, an Axiocam 712 color camera, and the Zen Blue Pro T/Z software. There is a Temp Module S1 with stagetop heating insert, objective heater, and CO₂ control for live cell imaging.
- Zeiss LSM 780 confocal microscope with GaAsP 34 channel detector and 7 laser lines (405, 458, 488, 514, 561, 594 and 633 nm), ACR beam combiner set, filter sets for DAPI, CY3, and GFP, and five objective lenses, including 40x water immersion, 40x and 63x oil immersion objective lenses. The GaAsP detector and ZEN software package allow for spectral imaging of many fluorophores simultaneously.

- An image processing workstation, equipped with a Dell Precision T7610, 64 GB RAM, 16-core Dual Intel Xenon Processor E5-2687W v2, 4 GN NVIDIA Quadro K5000 and dual 27 inch monitors.

Flow Cytometry Core

- BD FACS Aria II cell sorter
- Attune NxT Flow Cytometer

MicroCT Imaging Core:

- Scanco μ CT 40, ex vivo μ CT scanner, and with a high throughput scanning option (auto sample exchanger).

Multiplex Core:

- Luminex $\text{\textcircled{R}}$ 200TM
- Flexmap 3DTM
- KingFisherFlex Automated Extraction Instrument
- BioPlex Pro Wash Station for plate washing

Salivary Diagnostic Center

- Thermo Scientific Orbitrap Fusion mass spectrometer coupled with an easy-nanoLC 1000 system
- Thermo Scientific Q Exactive Orbitrap LC-MS/MS system coupled with an easy-nLC 1000 system
- AB Sciex 4800 Plus MALDI TOF/TOF system complemented by an AB Sciex Tempo LC MALDI Spotting system
- Protein Technologies Symphony automated 12 Channel solid phase peptide synthesizer
- BIAcore 3000 surface plasmon resonance instrument.
- AB Sciex TripleTOF 6600 coupled with a Shimadzu Nexera UHPLC system
- AB Sciex QTrap 6500 coupled with a Shimadzu Prominence HPLC system
- Dionex 3000 LC-system
- For data collection, processing and analysis, a storage array and personal computers are available as well as a Linux-based server along with a wide range of data analysis software packages. These facilities are supported by Forsyth's Bioinformatics and Biostatistics Cores, which assist Center investigators in the analysis and interpretation of complex data sets. Commercialization efforts are spearheaded by Forsyth's Office of Technology Development.

Bioinformatics Core

The hardware infrastructure consists of two load-balancing computer clusters

- PowerWulf Compute Engine that contains 176 total processor cores, 368GB System RAM and 19.8TB Accessible RAID Storage with a DDR InfiniBand Network
- 32-core TORQUE-based cluster with a total of 128GB system RAM and 2 TB hard drive storage.

Additional Instruments

- Horiba XploRA Plus Confocal Raman Microscope
- Rigaku SmartLab SE X-ray Diffraction System
- JEOL JCM-6000 Benchtop Scanning Electron Microscope with EDX

Equipment available at Harvard University's Center for Nanoscale Systems (CNS)

- several FE-SEMs (Jeol 7900F SEM, Zeiss Ultra55, and Zeiss UltraPlus)
- several TEMs (Jeol 2010 TEM/STEM, Jeol 2100 TEM, FEI Tecnai Artica CryoTEM, and Jeol ARM 200F STEM)
- several FIB-SEM (FEI Helios 660, and Zeiss Crossbeam FIB)
- HR 20 Discovery Hybrid Rheometer
- Critical Point Dryer Tousimis 931 GL

- Bruker Hysitron Nanoindenter
- Asylum Research Cypher Atomic Force microscope
- Cross section polisher (Jeol IB-09010CP)
- Carbon, gold and Pt/Pd coater (EMS 150T ES Plus)
- Automatic dicing saw (Disco DAD-321)
- Horiba Xplora Raman microscope

OTHER RESOURCES

The Office of Computing & Network Technology (OCNT)

- 10/100/1000 switched (CISCO) Ethernet LAN as well as a Cisco Meraki WLAN mesh network with a fiber backbone providing connectivity for more than 230 desktop computers, comprised of mostly Windows PC. Many workstations involved in image capture, analysis and storage have a gigabit pipe to a 125.15TB storage area network.
- Microsoft Windows 2012 & 2016 Servers utilizes the TCP/IP protocol offering: file and print services; messaging (Microsoft Office 365); database applications (Microsoft SQL 2012); and a Windows-based accounting system that allows desktop access to grant accounts, and generation of requisition and purchase orders.
- Network printing services include: several high-speed, high volume monochrome printers (HP 9050, HP 4000N(6), HP 3005DN); several networked departmental color laser printers; a color laser printer (Xerox Phaser 8860DN); and 3 networked, high-speed (65 ppm) color printer/copiers (Canon Image Runner Advance c7565) for print-once/copy-many technology.
- Full-time access to the Internet for scientific applications is provided via a dedicated, fiber optic connection from Crown Castle Fiber Networks providing up to 1Gbps bandwidth as well as a 10Mbps backup affixed wireless connection with NetBlazr as a backup connection. Some public facing web servers include Apache 2.2.6. Several of the research departments host their own websites on these servers. Forsyth's main external site is hosted by Pantheon.
- A Level II data center renovation project funded by NIH (G20 RR031082). The 425 square-foot data center, named "The Douglas B. Hanson Data Center" was formally launched in October 2012 and is currently hosting all of the Institute's computational servers, storage servers, and network infrastructure. The space has 10-42U racks for equipment. The equipment is powered by redundant 40kVA APC UPS's with redundant APC in-row cooling units. The in-row cooling units utilize chilled glycol which is cooled by two redundant chillers with a free-cooling option which allows the Institute to cool the space for minimal cost in the colder months. In the event of commercial power failure, all of the equipment can be powered by the 125kW standby natural gas generator which is capable of running all the equipment in the room for an extended period of time.

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator				
Prefix:	First Name*: Jeremy	Middle Name	Last Name*: Elias	Suffix:
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Project Role*: PD/PI		Other Project Role Category:		
Degree Type: PhD		Degree Year: 2017		
Attach Biographical Sketch*:		File Name:	1234-Elias_Biosketch_11.10.25.pdf	
Attach Current & Pending Support:		File Name:		

PROFILE - Senior/Key Person				
Prefix:	First Name*: Jirun	Middle Name	Last Name*: Sun	Suffix:
Position/Title*:	Full Professor			
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Division:				
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Phone Number*: 617-892-8201	Fax Number:			
E-Mail*: jsun@forsyth.org				
Credential, e.g., agency login: JIRUN_SUN				
Project Role*: Other Professional		Other Project Role Category: Mentor		
Degree Type: PhD		Degree Year: 2006		
Attach Biographical Sketch*:	File Name:	1235-Bio-Sun-2025-K25_11.09.25.pdf		
Attach Current & Pending Support:	File Name:	1236-Sun_Other Support_07.02.25_signed.pdf		

PROFILE - Senior/Key Person				
Prefix:	First Name*: Xuesong	Middle Name	Last Name*: He	Suffix:
Position/Title*:	Full Professor			
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County:	Middlesex			
State*:	MA: Massachusetts			
Province:				
Country*:	USA: UNITED STATES			
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Phone Number*: 617-892-8228	Fax Number:			
E-Mail*: xhe@forsyth.org				
Credential, e.g., agency login: xuesonghe2				
Project Role*: Other Professional		Other Project Role Category: Co-Mentor		
Degree Type: PhD		Degree Year: 2006		
Attach Biographical Sketch*:	File Name:	1237-BIO-He-11-5-2025.pdf		
Attach Current & Pending Support:	File Name:	1238-He_Other Support_08.07.25 - signed flat.pdf		

PROFILE - Senior/Key Person				
Prefix:	First Name*: Felicitas	Middle Name	Last Name*: Bidlack	Suffix:
Position/Title*:	Full Professor & Sr Dir of RA			
Organization Name*:	ADA Forsyth Institute, Inc.			
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Division:				
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County:	Middlesex			
State*:	MA: Massachusetts			
Province:				
Country*:	USA: UNITED STATES			
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E-Mail*: fbidlack@forsyth.org				
Credential, e.g., agency login: BIDLACK_FELICITAS				
Project Role*: Other Professional		Other Project Role Category: Scientific Advisory Committee		
Degree Type: PhD		Degree Year: 2003		
Attach Biographical Sketch*:		File Name:	1239-Bio_Bidlack_11 11 2025.pdf	
Attach Current & Pending Support:		File Name:		

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: Elias, Jeremy

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

POSITION TITLE: Postdoctoral Fellow

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)	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
University of Arizona	BS	08/2013	05/2017	Materials Science and Engineering
University of Florida	PHD	08/2017	05/2022	Materials Science and Engineering

A. Personal Statement

My long-term research goal is to advance the development of biomimetic materials and analytical methods to improve oral health outcomes. Specifically, I aim to leverage biomineral structures and biomaterial platforms to quantitatively assess cell-microbe interactions and treatment efficacy in real time at the cellular level. My graduate and postdoctoral training in biomimetic material design, characterization, and manipulation has provided a strong foundation for this work. In preparation for the K25 Quantitative Research Development Award, I am expanding my expertise in advanced materials analysis, including electron microscopy, X-ray diffraction, and mechanical testing, applying these techniques to study and modulate the oral microbiome. My past and ongoing collaborations with experts in biomimetics, microbiology, and tissue engineering have uniquely positioned me to integrate these disciplines in my current and future work.

Under the mentorship of Drs. Jirun Sun, Felicitas Bidlack, and Xuesong He, I have contributed to research on biomineral characterization, targeted caries prevention strategies, and the development of advanced biomaterial systems for real-time microenvironment analysis. My recent work optimizing a 3D printing platform to enhance the versatility of biomaterial inks and bioinks, is contributing to novel applications in hydrogels and bioprinting for tissue engineering, antimicrobial testing, and oral microbiome studies. Moving forward, I plan to design next-generation biomaterials and antibacterial delivery systems to promote the regeneration of mineralized tissues of bone and teeth. I am eager to lead and collaborate on projects that bridge materials science, microbiology, and translational dental research using my skills and expertise.

Ongoing projects I am contributing to include:

NIH/NIDCR R01 DE029479AS

PI: He/Sun

09/01/2021 – 08/31/2026

Title: Preventing dental caries through targeted treatment of acid-producing bacteria

Role: Postdoctoral Fellow

Forsyth Pilot Grant FP85

Title: 3D-bioprinting of heterogeneous and biologically functional materials.

Role: Investigator

Relevant Citations:

1. **Elias, J.**, Dong, P.T., Liu, J., Cen, L., He, X., Sun, J., 2025 (in progress) Single-Cell Level Analysis of Metabolism-Activated Antibacterial Mechanisms in Customizable Hydrogels. *Proc. Natl. Acad. Sci.*
2. **Elias, J.**, Klein, C., He, X., Sun, J., 2025 (in review) Precision Hydrogel Environments for Advanced Microbial Culture and Patterning. *Int. J. Bioprinting*.
3. Liu, J., **Elias, J.**, et al., 2025 (in review) Nano-Gatekeepers Triggered by Metabolism: From Broad-Spectrum to Precision Release. *Nat. Mater.*
4. **Elias, J.**, Matheson, B.-A., Gower, L., 2023. Influence of Crosslinking Methods on Biomimetically Mineralized Collagen Matrices for Bone-like Biomaterials. *Polymers*, 15, 1981.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2023 – Present	Member, International Association for Dental Research
2022 – Present	Postdoctoral Fellow, ADA Forsyth Institute
2017 – 2022	Graduate Research Assistant, University of Florida
2015 – 2017	Secretary, National Society of Black Engineers, University of Arizona Chapter
2014 – Present	Member, National Society of Black Engineers

Honors

2024	2025 AADOCR Bloc Travel Grant Award, AADOCR
2023	2024 AADOCR Bloc Travel Grant Award, AADOCR
2017-2022	Graduate Student Preeminence Award, University of Florida
2013-2017	National Merit Scholarship, National Merit Scholarship Corporation

C. Contributions to Science

1. **Graduate Career:** My graduate research contributions focused on biomineralization and biomaterials, utilizing biomimetic fabrication processes for the creation and analysis of novel bone-like materials. My work involved the synthesis of collagen-based biomaterials, which I functionalized via chemical crosslinking to tailor their properties. I characterized these scaffolds using a suite of materials analysis techniques to quantitatively evaluate how specific fabrication and processing methods influenced their mechanical behavior upon mineralization. This has yielded scaffolds with bone-like mechanical properties, and the findings of this work have been disseminated through multiple publications and conference presentations. My broader expertise in biomineralization includes developing chitin-based composites to mimic crustacean shells and conducting cell culture studies to evaluate the osteogenic properties of biomimetic collagen-based scaffolds.
 - a. Gower, L., **Elias, J.**, 2022. Colloid assembly and transformation (CAT): The relationship of PILP to biomineralization. *Journal of Structural Biology*: X, 6, 100059.
 - b. **Elias, J.**, Angelini, T., Martindale, M.Q., Gower, L., 2022. Assessment of Optimal Conditions for Marine Invertebrate Cell-Mediated Mineralization of Organic Matrices. *Biomimetics*, 7, 86.
 - c. **Elias, J.**, Matheson, B.-A., Gower, L., 2023. Influence of Crosslinking Methods on Biomimetically Mineralized Collagen Matrices for Bone-like Biomaterials. *Polymers*, 15, 1981.
2. **Postdoctoral Research:** In my current research, I am contributing to developments in both biomineralized tissue and dental materials applications. Recently completed work has utilized materials analysis methods of electron microscopy, computed tomography and nanoindentation to reveal trends across the dentin-enamel junction that uncover information about dentin and enamel formation and properties during development.
 - a. **Elias, J.**, Kattinanon, R., Kraemer, S., Depalle, B., Sun, J., Bidlack, F.B., 2024. Collagen Point-Mutation and Altered Enamel Matrix Change Dentin-Enamel Junction Properties. *J Dent. Res.* Vol #103 (Spec Iss A): 2039.
 - b. **Elias, J.**, Kattinanon, R., Kraemer, S., Depalle, B., Sun, J., Bidlack, F.B., 2025 (in review) Collagen mutation or enamel matrix disruptions have sex-specific effects on dentin-enamel junction and beyond. *J. Dent. Res.*

3. My current research regarding dental materials has involved pioneering and adapting a custom 3D printing system to provide pathways for a range of bioprinting applications. This has included the creation of tissue engineering scaffolds for bone repair as well as systems to better manipulate and analyze bacterial behavior and interactions. This work addresses biofabrication challenges of balancing printability with biological function in engineered bioinks. This effort is foundational to my goal of leveraging 3D and 4D bioprinting to create versatile models for studying the oral microbiome, enabling investigations into biofilm behavior and targeted antimicrobial strategies.
 - a. **Elias, J.**, Dong, P.T., Liu, J., Cen, L., He, X., Sun, J., 2025 (in progress) Single-Cell Level Analysis of Metabolism-Activated Antibacterial Mechanisms in Customizable Hydrogels. *Proc. Natl. Acad. Sci.*
 - b. **Elias, J.**, Sun, J., He, X., 2025. S. Mutans Distribution and Proliferation in Customizable 3D Hydrogel Environments. *J Dent. Res.* Vol #104 (Spec Iss A): 0467.
 - c. **Elias, J.**, Klein, C., He, X., Sun, J., 2025 (in review) Precision Hydrogel Environments for Advanced Microbial Culture and Patterning. *Int. J. Bioprinting*.
 - d. Liu, J., **Elias, J.**, et al., 2025 (in review) Nano-Gatekeepers Triggered by Metabolism: From Broad-Spectrum to Precision Release. *Nat. Mater.*

Selection of Public Works:

<https://orcid.org/0000-0002-9782-4377>

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: Sun, Jirun

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

POSITION TITLE: Associate Member

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
Qingdao University, China	B.S.	07/1994	Chemistry
Shandong University, China	M. Sc.	07/1997	Polymer Chemistry
Louisiana State University, USA	Ph.D.	08/2006	Polymer Physics
NIST Polymers Division, USA	Postdoctoral	03/2009	Dental Materials and Tissue Engineering

A. Personal Statement

I am a material scientist focusing on biomaterial development for dental applications and tissue engineering in the last 20+ years. Before joining Forsyth and Harvard School of Dental Medicine, I worked at the National Institute of Standards and Technology (NIST) and American Dental Association (ADA). Over the years, I collaborated with Dr. Rafael Bowen who was one of the godfathers of dental materials including the invention of Bis-GMA-based dental materials, which are the dominant products as dental adhesives and dental composites for over 60 years. As the PI of an NIH cooperative grant (U01 DE023752), I led a multidisciplinary team and developed new dental restoratives. For example, I have two patent families on photo-polymerizable and hydrolytically stable dental resins, e.g., TEG-DVBE (*highlighted by C&En news, Oct 2015, US Patent 9,572,753, granted in 2017*). My team successfully prepared stronger and tougher dental composites comprised of TEG-DVBE, self-healing filler systems, and stimuli-responsive antimicrobial compounds. Supported by this U01 grant, I was awarded 8 US patents and published 28 high-level academic papers. Prior to these, I have developed combinatorial platforms with nano-scale surface patterns. Using these platforms, I have demonstrated complete control of cell contact on biomaterials, in terms of cell orientation, from randomly oriented to aligned in parallel (*Advanced Materials, 2011*). In addition, I am well-accomplished in designing, synthesizing, and characterizing organic compounds and polymers (*ACS Applied Materials & Interfaces, 2018*).

I am extremely excited to provide my expertise helping Jeremy. Over the last 10 years, I have trained more than 30 trainees including undergraduate students, graduate students, and dentists. Many of them become dentists or independent PIs.

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

NIH/NIDCR R01 DE029479A PI: He/Sun 09/01/2021 – 08/31/2026
Preventing dental caries through targeted treatment of acid-producing bacteria
 NIH/NIDCR RM1DE034233 PI: Bidlack/Mirmomen/Sun/Zhang 07/01/2024 – 06/30/2029
Virtual Twin-Powered Rapid Development of Bioactive Multifunctional Dental Restoratives
 NIH/NIDCR R01DE033442A PI: Sun/Xu 07/01/2024 – 06/30/2029
Hydrolysis-resistant resin networks for durable and multifunctional dental restorations

Citations:

- a. Yang, Y., Reipa, V., Liu, G., Meng, Y., Wang, X., Mineart, K., Prabhu, V., Shi, W., Lin, N., He, X., **Sun, J.**, pH-sensitive Compounds for Selective Inhibition of Acid-producing Bacteria *ACS Applied Materials & Interfaces*, 2018, 8566–8573 PMID: 29436821 DOI: 10.1021/acsami.8b01089
- b. Wang, X., Song, S., Chen, L., Stafford, C., **Sun, J.**, Short-time dental resin biostability and kinetics of enzymatic degradation *Acta Biomaterialia* 2018, 326-33 PMID: 29751113 DOI: 10.1016/j.actbio.2018.05.009
- c. Yamauchi, S., Wang, X., Egusa, H., **Sun, J.**, High performance dental adhesives containing an ether-based monomer *Journal of Dental Research* 2019 99(2) 189-195 PMID: 31861961 DOI: 10.1177/0022034519895269
- d. Gonzalez-Bonet A., Kaufman G., Yang Y., Wong C., Jackson A., Huyang G., Bowen R., and **Sun, J.**, Preparation of dental resins resistant to enzymatic and hydrolytic degradation in oral environments *Biomacromolecules*, 2015 3381-3388 PMID: 26358180 PMCID: PMC4788384 DOI: 10.1021/acs.biomac.5b01069. This paper was highlighted in Chemical & Engineering News, Oct 12, 2015

B. Positions and Honors.

Positions and Employment

2024 – present	Professor, ADA Forsyth Institute, Somerville, MA, USA;
2023 – present	Professor, Dean's Faculty, University of Maryland Baltimore, School of Dentistry, Baltimore, MD, USA;
2022 – 2024	Adjunct Faculty, Harvard School of Dental Medicine, Cambridge, MA, USA;
2021 - 2024	Associate Member of Staff, the Forsyth Institute, Cambridge, MA, USA;
2016 - 2021	<i>Senior Project leader</i> at Volpe Research Center (VRC), American Dental Association (ADA), Gaithersburg, MD, USA;
2009 - 2016	<i>Project leader</i> at VRC, ADA, Gaithersburg, MD, USA;
2006 - 2021	<i>Research associate</i> at National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA;
2006 - 2009	<i>Postdoctoral Fellow</i> at NIST, Gaithersburg, MD, USA;
2001 - 2006	<i>Research Assistant</i> at Louisiana State University (LSU), Baton Rouge, LA, USA;
2001 - 2006	<i>Teaching Assistant</i> at LSU, Baton Rouge, LA, USA, (taught organic synthesis lab and macromolecular characterization);
1997 - 2001	<i>Group leader, Project leader and Research Scientist</i> at Dainippon Ink & Chemicals Inc.(DIC), Qingdao, China

Postdoctoral Associates and Students Mentored (total 27):

Dr. Youyuan Man, PhD, The University of Tokyo, Tokyo, Japan, 2025-present
 Dr. Mohammed Zahedul Islam Nizami, GDCSc, BDS., PhD, Okayama University, Okayama, Japan, 2023-present
 Dr. Jianhui Liu, PhD, Hong Kong University of Science and Technology, Hong Kong, 2022-present
 Dr. Jeremy Elias, PhD, University of Florida, FL, USA, 2022-present
 Dr. Waad Alomran, BDD, DMSc candidate, Harvard School of Dental Medicine, Boston, MA, USA, 2022-present
 Dr. Huilin Cao, MS, BDD, Harvard School of Dental Medicine, Boston, MA, USA, 2021-present
 Dr. Takaaki Sato, DDS, PhD, Tokyo Medical and Dental University, Japan, 2018
 Dr. Han Byul Song, University of Colorado at Boulder, 2019-2020
 Dr. Xiaohong Wang, Institute of Chemistry, Chinese Academy of Sciences, China, 2016-2021
 Dr. Yuan Meng, University of Rochester, 2017-2018, now at UCLA
 Dr. Yin Yang, Dartmouth College, 2015 – 2017, now at Oblon, McClelland, Maier & Neustadt, LLP
 Dr. Xiaohui Liu, University of Massachusetts Amherst, 2016 – 2017, now at BASF
 Dr. Sheng Song, Georgetown University, 2015 – 2016, now at Amarex
 Dr. George Huyang, University of Sydney, 2014 – 2016, now at NICNAS, Department of Health, AU
 Dr. Andres Gonzalez Bonet, Purdue University, 2013- 2015, now at FDA
 Dr. Andrews Xu, Dentist, DDS, MS West Virginia University, School of Dentistry, 2014
 Dr. Javoris Hollingsworth, Louisiana State University, 2012, now Assistant Prof. at Univ. of St. Thomas
 Dr. Evan Whitbeck, DDS, MS, Naval Postgraduate Dental School, 2012-2013
 Dr. Kelli Swenson, DDS, Endodontic Resident, Naval Postgraduate Dental School, 2011-2012

Ethan Finley, Appalachian State University, summer 2017
Heetae Jeon, Undergraduate, University of Maryland College Park, summer 2016
Christopher Wong, Undergraduate, University of Maryland College Park, summer 2014
Evalyn Myerly, Undergraduate, Mount St. Mary's Univ., 2013
Alexander Kassman, Undergraduate, University of Maryland College Park, 2013-2014
Brisma Pinto-Pacheco, Undergraduate, Univ. of Puerto Rico, Río Piedras, Chemistry, summer 2012
David Allsopp, Undergraduate, Univ. of Maryland, College Park, Chemical Engineering, summer 2010
Edward Yao, Churchill High School, summer 2014, now at Duke University

Other Experience and Professional Memberships

2021	Ad hoc member, NIH 2021 ZRG1 MOSS-L Special Emphasis Panel
2019	Ad hoc member, ACS Petroleum Research Fund
2017	Ad-hoc Member, NIH 2017/10 OCDS Special Emphasis Panel
2017	Ad-hoc Member, NIH 2017/10 ZRG1 MOSS-K (57) R Special Emphasis Panel
2017	Ad-hoc Member, NIH 2017/05 OCDS Special Emphasis Panel
2016	Served as an expert for the FDI World Dental Federation, Science Committee Forum/World Oral Health Forum, Poznan, Poland
2015	Ad-hoc Member, NIH ZRG1 MOSS-U02 Special Emphasis Panel
2012	Served as an expert in Colgate-Palmolive Tech Max, New York, NY
2009-	Member of Sigma Xi, the Scientific Research Society
2006-	Member of International Association of Dental Research (IADR)
2006-	Member of American Association of Dental Research (AADR)
2002-	Member of American Chemical Society (ACS)

Honors

2006	Coates Travel Award for 231 st ACS National Meeting and Exposition, Georgia, GA
2005	Coates Travel Award for 229 th ACS National Meeting and Exposition, San Diego, CA
2003	Timothy S Evenson Award for Innovation in Polymer Science

C. Contribution to Science (Selected from 57 peer-reviewed articles, 8 patents, 6 news reports and 70 conference presentations)

1. Enhancing service life of dental resin composites and dental adhesives. The short average service life of traditional dental composite restoratives and increasing occurrence of secondary caries adjacent to composite restorations and sealants are necessitating the development of new, longer lasting dental materials. As the PI (NIH U01 DE023752), I was leading a multidisciplinary research team to make the next generation dental resin composites by incorporating novel functions into the three major components of resin composite restoratives: resin networks, fillers and additives. We invented an ether-based resin network, which is enzymatically and hydrolytically stable and make high-performance resin composites. We discovered a new photo-polymerization approach to avoid compositional drift caused by diffusion limitation during the formation of cross-linked resin network. The resin composites made with these new resins are significantly more durable than the BisGMA/TEGDMA controls. We successfully incorporated the self-healing capability into the filler system to achieve autonomous healing of micro-cracks. These cracks may lead to a failure of dental restorations but are very difficult to detect and almost impossible to repair manually. Our new self-healing dental composites increased the fatigue life up to five times comparing with the control. In this proposal, I introduce a pH sensitive antimicrobial compound that is originally designed as an additive to resin composites which will provide the needed antibacterial capability when local environment becomes acidic. We made compounds that perform as we planned. Overall, my team has made significant improvement to the service life of dental resin composites by using stable ether-based resin network, incorporating self-healing capability, and delivering environment triggered antimicrobial components.

- Wang, X., Huyang, G., Palagummi, S., Liu, X., Skrtic, D., Beauchamp, C., **Sun, J.**, High Performance Dental Resin Composites with Hydrolytically Stable Monomers *Dental Materials*, 2018, 228-237
- Yang, Y., Urbas, A., Gonzalez-Bonet, A., Sheridan, R.J., Seppala, J.E., Beers, K.L., **Sun, J.**, A composition-controlled cross-linking resin network through rapid visible-light photo-copolymerization *Polymer Chemistry*, 2016, 5023-5030.

- c. Huyang, G., Debertin, A., and **Sun, J.**, Design and development of self-healing dental composites *Journal of Materials and Design*, 2016 295-302
- d. **Sun J.**, Bowen R, Enzymatically and hydrolytically stable resins resin monomers, and resin composites for use in dental preventive and restorative applications, US Patent 9,572,753, granted in 2017

2. Nanotechnology for dentistry and tissue engineering. Many properties of materials may be changed radically when their dimension or surface features reach nano-scale. For example, TiO₂ particles have unique photo-catalytic properties when their sizes are smaller than 50 nm. Furthermore, the differentiation of stem cells may be directed by nanoscale surface features in extracellular matrix. Utilizing nanotechnology to understand biological behavior, improve performance of materials and deliver drugs has been one of my research focuses in a leading role or as a collaborator for over 10 years. I have developed a nano-imprint lithography method to make nano-patterns on different polymeric materials. I applied these nano-patterned materials to control initial cell contact with the materials and direct the cell orientation, which are important for guiding cell differentiation and improving osseointegration of implants. In addition, I have participated in development of a nano-size vehicle that can deliver drugs to the targets using magnetic forces. Intrinsic part of my on-going research on pH-sensitive antimicrobial agents, is the use of nanoparticles as carriers to enhance antimicrobial efficacy without compromising the mechanical performance. In addition, the bonding strength of dental adhesives is significantly improved by adding a very small amount of nanoparticles.

- a. **Sun, J.**, Petersen, E., Watson, S., Sims, C., Kassman, A., Frukhtbeyn, S., Skrtic, D., Ok, M., Jacobs, D., Reipa, V., Ye, Q., Nelson, B., Biophysical characterization of functionalized titania nanoparticles and their application in dental adhesives *Acta Biomaterialia*, 2017, 585-597
- b. Hollingsworth, J., Bhupathiraju, N. V. S., **Sun, J.**, Lochner, E., Vicente, M. H., and Russo, P., Preparation of Metalloporphyrin-Bound Superparamagnetic Silica Particles via "Click" Reaction *ACS Applied Materials & Interfaces*, 2016, 792-801
- c. Ding, Y., **Sun, J.**, Ro, H., Wang, Z., Zhou, J., Lin, N., Cicerone, M., Soles, C., and Lin-Gibson, S., Thermodynamic Underpinnings of Cell Alignment on Controlled Topographies *Advanced Materials*, 2011, 23, 421-425
- d. **Sun, J.**, Ding, Y., Lin, N., Zhou, J., Ro, H., Soles, C., Cicerone, M., and Lin-Gibson, S., Exploring Cellular Contact Guidance Using Gradient Nanogratings *Biomacromolecules*, 2010, 11, 3067-3072

3. Developing methodology for structure-property evaluation. Structure determines properties. Proper understanding of the structure of materials enables us to predict and confirm the properties. My extensive training in polymer chemistry and polymer physics provided me with the knowledge and capability to evaluate structure-property correlations from the molecular level to 3D macroscale. I am well-accomplished in designing, synthesizing, and characterizing organic compounds. I synthesized and characterized a series of dendrimers with high purity using multistep approaches. The self-assembly of these dendrimers was characterized from molecular level to macroscale using small angle X-ray scattering (SAXS), light scattering, SEM, TEM, and AFM and confocal microscopy. These experiences enabled me to develop new methodologies for structure-property evaluation. I have developed a 3D non-destructive method to evaluate polymerization shrinkage and map the correlated micro-leakage. My technological approaches also successfully translated into bone tissue engineering.

- a. Wang, P., Liu, X., Zhao, L., Weir, M., **Sun, J.**, Chen, W., Man, Y., Xu, H., Bone tissue engineering via human induced pluripotent, umbilical cord and bone marrow mesenchymal stem cells in rat cranium, *Acta Biomaterialia*, 2015 236-248 PMID: 25712391 PMCID: PMC4666020 DOI: 10.1016/j.actbio.2015.02.011
- b. **Sun, J.**, Fang, R., Lin, N., Eidelman, N., and Lin-Gibson, S., Nondestructive quantification of leakage at the tooth – composite interface and its correlation with material performance parameters *Biomaterials* 2009, 30, 4457-4462
- c. **Sun, J.**, Lyles, B., Yu, K., Weddell, J., Pople, J., Hetzer, M., De Kee, D. and Russo, P.S., Diffusion of dextran probes in a self-assembled fibrous gel composed of two-dimensional arborols *J. Phys. Chem. B*, 2008, 112, 29-35
- d. **Sun, J.**, Ramanathan, M., Dorman, D., Newkome, G., Moorefield, C., and Russo, P.S., Surface Properties of a series of amphiphilic dendrimers with short hydrophobic chains *Langmuir*, 2008, 24, 1858-1862

A Selection of My Published Work:

https://scholar.google.com/citations?user=DqGzP_gAAAAJ&hl=en&authuser=1

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: Xuesong He

eRA COMMONS USER NAME (credential, e.g., agency login): xuesonghe2

POSITION TITLE: 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
Peking University School of Stomatology, China	D.D.S	09/1997	Dentistry
Indiana University, Bloomington, Indiana, USA	Ph.D.	06/2006	Microbiology
University of California, Los Angeles, CA	Postdoctoral	07/2010	Microbiology

A. Personal Statement

It is with great pleasure that I commit to serving as Dr. Jeremy Elias's co-mentor for his NIH/NIDCR K25 application entitled "Three-Dimensional Hydrogel Models for Analyzing Cariogenic Bacterial and Antimicrobial Responses".

Trained as a dentist and oral microbiologist, I am fascinated by the host-associated microbial world and amazed by their significant roles in the host's health and disease. I have extensive expertise in oral microbiology, microbial pathogenesis, cariology, and microbial ecology, as well as using state-of-the-art tools, such as targeted antimicrobial peptides for microbiome modulation. The proposed project fits my interests and expertise. I have been working closely with Jeremy's mentor, Dr. Sun, on an active NIH/NIDCR-funded grant R01DE029479-01A1, which aims at developing novel antimicrobial materials for caries prevention. I am also the contact PI of an active NIH-funded R01 focusing on the characterization of Saccharibacteria, a group of newly identified obligate ultrasmall epiparasitic bacteria within the human oral cavity, which are highly associated with periodontal diseases.

Over the past 10 years, I have been actively involved in mentoring or co-mentoring predoctoral (a total of 6) and postdoctoral (a total of 11) trainees, with 3 of them receiving F award and 3 of them K award. Three of the trainees have become faculty members in research institutes, and another three have joined the biotech company. My research interests align well with Dr. Elias' training goals. I will be actively involved in providing microbiological training to Dr. Elias. For career development, I will also contribute to mentoring in lab management and grant writing. As the Associate Director of the NIDCR T90/R90 training program at Forsyth, I have extensive experience in fostering next-generation independent investigators, and my connections with the oral microbiology/microbiome community will broaden Dr. Elias's research horizons and enable him to establish networks within the field that will form the basis for future collaborations. In summary, I am confident that my passion, my knowledge, and my previous experiences will greatly contribute to Dr. Elias' training and career development.

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

NIH/NIDCR R01 DE029479A PI: Sun/He 09/01/2021 – 08/31/2026
Preventing dental caries through targeted treatment of acid-producing bacteria

NIH/NIDCR R01 DE030943 PI: He 09/01/2022 – 08/31/2027
Achieve a better understanding of the cross-kingdom trafficking of host-generated sRNA in mediating the

microbial host interaction.

NIH/NIDCR R01DE023810

PI: He/McLean

09/01/2014 – 08/31/2029

Domestication and characterization of TM7-the most elusive oral phylum

- 1) Edlund, A., Y. Tang, A. Hall, L. Guo, R. Lux, **X. He**, K. Nelson, K. Nealson, S. Yooseph, W. Shi and J. McLean 2013. A novel *in vitro* biofilm model maintaining a high species and metabolic diversity similar to the human oral microbiome. *Microbiome Journal* 1:25 PMID:PMC3971625
- 2) Guo, L., JS. McLean, Y. Yang, R. Eckert, C.W. Kaplan, P. Kyme, O. Sheikh, B. Varnum, R. Lux, W. Shi and **X. He**. 2015 A precision guided antimicrobial peptide as a targeted modulator of human microbial ecology. *Proc Natl Acad Sci USA* 112(24): 7569-7574. PMID: 26034276
- 3) Yang, Y., Reipa, V., Liu, G., Meng, Y., Wang, X., Mineart, K., Prabhu, V., Shi, W., Lin, N., **He, X.**, Sun, J. 2018 pH-sensitive compounds for selective inhibition of acid-producing bacteria. *ACS Applied Materials & Interfaces* PMID29436821 DOI:10.1021/acsami.8b01089
- 4) Zhong, Q., Liao, B., Liu, J., Shen, W., Wang, J., Ma, Y., Dong, P-T., Bor, B., McLean, J., Shi, W., Li, Y., **He, X.**, and Le, S*. 2024. Episymbiotic Saccharibacteria TM7x modulates susceptibility of its host bacteria to phage infection and promotes their co-existence. * *co-corresponding author. Proc Natl Acad Sci U S A*. PMID:PMC11032452

B. Positions and Honors**Positions and Employment**

07/2022-current	Professor, The Forsyth Institute, Cambridge, MA
01/2018-07/2022	Associate Member of Staff, The Forsyth Institute, Cambridge, MA
07/2020-07/2023	Member of Faculty, Oral Medicine, Infection and Immunity, Harvard School of Dental Medicine, MA.
07/2017-12/2017	Associate Professor, UCLA School of Dentistry, Los Angeles, CA
07/2010-07/2017	Assistant Professor, UCLA School of Dentistry, Los Angeles, CA
07/2006-07/2010	Postdoctoral Researcher, Lab of Dr. Wen Yuan Shi, UCLA School of Dentistry, Los Angeles, CA
09/2000-07/2006	Ph.D. candidate, Laboratory of Dr. Clay Fuqua, Indiana University, Bloomington, IN
09/1997-07/1999	Resident, School of Stomatology, Beijing University Dental School, Beijing, China

Other Experience and Professional Memberships**Professional Society**

2012 -- Present	American Association for Dental Research
2005 – Present	American Association for the Advancement of Science (AAAS)

Ad hoc reviewer for NIH NIDCR study section

2022-current	Oral, Dental and Craniofacial Science Study Section	NIH Standing Member
2020	NIDCR Special Grant Review: DSR Study Section June 17 th 2020	NIH Ad hoc Member
2019	NIDCR Special Grant Review: DSR Study Section Oct. 17 th 2019	NIH Ad hoc Member

2017 Oral, Dental and Craniofacial Sciences Study Section
Oct. 13th 2017

NIH
Ad hoc Member

Editorial Activities

2025-current	Editorial Board Member	mBio
2021-present	Editorial Board Member	Journal of Oral Microbiology
2021-present	Editor	Frontiers in Oral Infections and Microbes
2019-present	Editor	Frontiers in Molecular Bacterial Pathogenesis
2019-present	Editor	Frontiers in Systems Microbiology
2016-present	Editorial Board	Frontiers in Cellular and Infection Microbiology
2016-present	Editorial Board Member	Scientific Reports

Honors

2022	Harvard School of Dental Medicine/Forsyth collaborative grant awardee	Harvard School of Dental Medicine/Forsyth
2013	Dean's Faculty Seed Grant, UCLA	

C. Contributions to Science

1)“Domestication of yet-to-be cultured host-associated microbes”

One of the biggest challenges in oral microbial research is to culture those yet-to-be cultured species for detailed physiological/pathogenic analysis. By using a novel culturing method, my team successfully isolated and cultivated from the oral cavity the first TM7 strain (named TM7x), which belongs to TM7, a bacterial phylum that is omnipresent, particularly in the human oral cavity, and associated with periodontal disease. We also revealed its unique epibiotic/parasitic lifestyle with its bacterial hosts. Furthermore, TM7 belongs to Candidate Phyla Radiation (CPR), a unique class of bacteria recently revealed by metagenomics-based approach and estimated

to account for over a quarter of microbial diversity. Thus far, TM7 remains the only phylum with cultivated representatives in the CPR group. Our study has received media attention through joint press releases with NIDCR. Our study in this area was recently highlighted in a NIH review, titled “***A review of 10 years of human microbiome research activities at the US National Institutes of Health, Fiscal Year 2007-2016***”, published in a Feb. 2019 issue of *Microbiome*. Currently, we are using TM7x and their bacterial host as a model system to further study their unique lifestyle, their ecological impact, as well their role in host health and diseases. The knowledge gained will be fundamental in better understanding other CPR bacteria, which make up >25% of the bacterial domain.

- a) **He, X.**, JS. McLean, A. Edlund, S. Yooseph, A.P. Hall, SY. Liu, P. Dorrestein, E. Esquenazi, R. Hunter, G. Cheng, KE. Nelson, R. Lux and W. Shi. 2015. Cultivation of a human-associated TM7 phylotype reveals a reduced genome and epibiotic parasitic lifestyle. *Proc Natl Acad Sci USA* **112**(1):244-9. PMID:PMC4291631
- b) Bor, B., McLean, J., Foster, K., Cen, L., To, T., Serrato-Guillen, A., Dewhirst, F., Shi, W., and **He, X.** 2018 Rapid evolution of decreased host susceptibility drives a stable relationship between ultra-small parasite TM7x and its bacterial host. *Proc Natl Acad Sci U S A* **115**(48): 12277-12282 PMID:PMC6275545
- c) Chipashvili, O., Utter, D.R., Bedree, J.K., Ma, Y., Schulte, F., Mascarini, G., Alayyoubi, Y., Chouhan, D., Hardt, M., Bidlack, F., Hasturk, H., **He, X.**, McLean, J.S., and Bor, B. 2021. Ultrasmall epibiotic *Saccharibacteria* suppresses gingival inflammation and bone loss through host bacterial modulation. *Cell Host & Microbe*. PMID:PMC8595704
- d) Zhong, Q., Liao, B., Liu, J., Shen, W., Wang, J., Ma, Y., Dong, P.-T., Bor, B., McLean, J., Shi, W., Li, Y., **He, X.**, and Le, S*. 2024. Epibiotic *Saccharibacteria* TM7x modulates

susceptibility of its host bacteria to phage infection and promotes their co-existence. * co-corresponding author. *Proc Natl Acad Sci U S A*. PMID:PMC11032452

2) “Microbial-host interaction”

Microbial host interaction plays a crucial role in human health and disease. I am particularly interested in understanding the process and mechanisms, such host-derived small RNAs, in mediating host-microbial interactions.

- a) **He, X.**, Li, F., Bor, B., Koyano, K., Cen, L., Xiao, X., Shi, W., Wong, D. 2018. Human tRNA-derived small RNAs modulate host-oral microbial interactions. *J Dent Res*. PMID:PMC 6151917
- b) Chipashvili, O., Utter, D.R., Bedree, J.K., Ma, Y., Schulte, F., Mascarin, G., Alayyoubi, Y., Chouhan, D., Hardt, M., Bidlack, F., Hasturk, H., **He, X.**, McLean, J.S., and Bor, B. 2021. Ultrasmall episymbiotic *Saccharibacteria* suppresses gingival inflammation and bone loss through host bacterial modulation. *Cell Host & Microbe*. PMID:PMC8595704
- c) Yang, M., Dong, P.T., Cen, L., Shi, W., **He, X***, and Li, J*. 2023. Targeting *Fusobacterium nucleatum* through chemical modification of host-derived transfer RNA fragments. *ISME J*. * co-corresponding author
- d) Pang, Z., Cady, N., Cen, L., Schmidt, T., **He, X.**, and Li, J. 2025. Physiologically relevant co-culture model for oral microbial-host interactions, *International Journal of Oral Sciences*. PMID:40425581

3) “Understanding oral microbiome associated with health and disease, and interspecies interaction within multispecies microbial communities”

Oral cavity harbors more than 700 microbial species and is one of the most complex ecosystems ever described. While the majority of these inhabitants are considered commensal, some of them are responsible for oral infectious diseases such as dental caries, periodontitis, halitosis and stomatitis. Increasing lines of evidence suggest that these infectious diseases are often the result of the disturbed host homeostasis and imbalanced oral microbial ecology leading to overgrowth of otherwise low abundant opportunistic pathogens. One of my research interests is to better understand oral microbiome associated with health and disease, as well as investigate the impact of interspecies interaction on the physiology and virulence of key oral opportunistic pathogenic bacterial species.

- a. Agnello, M., Marques, J., Cen, L., Mittermuller, BA., Huang, A., Tran, N., Shi, W., **He*, X.**, and Schroth*, RJ. 2017. Microbiome associated with severe caries in Canadian First Nations children. *J Dent Res* 96(12):1378-1385* Co-Corresponding author
PMCID:PMC5652857
- b. Xu, H., Tian, J., Zhang, Q., Zhou, Q., Shi, W., Qin, M., **He, X***, and Chen, F. 2018. Oral microbiome shifts from caries-free to caries-affected status in 3-year-old Chinese children: Alongitudinal study. *Front Microbiol* PMID:PMC6121080 * co-corresponding author.
- c. Bedree, J.K., Bor, B., Cen, L., Edlund, A., Lux, R., McLean, J.S., Shi, W., and **He, X.** 2018. Quorum sensing modulates the epibiotic-parasitic relationship between *Actinomyces odontolyticus* and its *Saccharibacteria* epibiont, a *Nanosynbacter lyticus* strain, TM7x. *FrontMicrobiol*. PMID:PMC6166536
- d. Liu, T., Liu, J., Liu, J., Yang, R., Lu, X., **He, X.**, Shi, W. and Guo, L. 2020. Interspecies interactions between *Streptococcus* mutants and *Streptococcus agalactiae* in vitro. *Frontiers inMicrobiology* PMID: PMC7358462

4) “Establishing in vitro multispecies microbial community model system to facilitate better understanding of ecological aspects of microbiome”.

Human microbiome research revealed that every human body contains a variety of microbial communities on various mucosal surfaces that consist of thousands of different microbial species. Disturbance from host and environmental factors may alter the composition and abundance of these microbial species, leading to various polymicrobial diseases. One of my fields of interest is to study the ecology of human associated microbial

community using in vitro model system and, more importantly examine the community role and function of individual species within a complex microbial community by knocking out or knocking down one particular species with a targeted antimicrobial and then tracking the impact on the rest of the species within the same community. Using in vitro oral community and *Streptococcus mutans* as a model system, we developed a proof of concept that we could potentially modulate the microbiome structure using targeted antimicrobials, allowing insights into the key community role of specific bacterial species.

- a. Tian, Y., **X. He**, R. Lux, J. McLean, G. Yu and W. Shi 2010. Using DGGE profiling to develop anovel culture medium suitable for oral microbial communities. *Mole Oral Microbiol* 25 (5): 357- 367
- b. Edlund, A., Yang Y, Y. Shibu, A. Hall, D. Nguyen, P. Dorrestein , K. Nelson , **X. He**, R. Lux, W. Shi, JS. McLean 2015. Meta-Omics Uncover Temporal Regulation of Pathways Across Oral Microbiome Genera during in vitro sugar metabolism. *The ISME Journal* doi: 10.1038/ismej.2015.72. [Epub ahead of print]. PMID:26023872
- c. Guo, L., JS. McLean, Y. Yang, R. Eckert, C.W. Kaplan, P. Kyme, O. Sheikh, B. Varnum, R. Lux, W. Shi and **X. He**. 2015 A precision guided antimicrobial peptide as a targeted modulator of human microbial ecology. *Proc Natl Acad Sci USA* 112(24): 7569-7574. PMID: 26034276 PMID: PMC4475959
- d. Pang, Z., Cady, N., Cen, L., Schmidt, T., **He, X.**, and Li, J. 2025. Physiologically relevant co-culture model for oral microbial-host interactions, *International Journal of Oral Sciences*. PMID:40425581

5) “Developing novel therapeutics in preventing dental caries”

Collaborating with researchers at ADAF, I have been actively involved in developing novel **therapeutics**, such as acid-activated antimicrobial compounds, probiotics, prebiotics in preventing dental caries.

- a) Guo, L., JS. McLean, Y. Yang, R. Eckert, C.W. Kaplan, P. Kyme, O. Sheikh, B. Varnum, R. Lux, W. Shi and **X. He**. 2015 A precision-guided antimicrobial peptide as a targeted modulator of human microbial ecology. *Proc Natl Acad Sci USA* 112(24): 7569-7574 PMID:PMC4575959
- b) Agnello, M., Cen, L., Nini Chaichanasakul Tran, Shi, W., McLean, JS. and **He. X.** 2017. Arginine Improves pH Homeostasis via Metabolism and Microbiome Modulation. *J Dent Res* 96(8):924-930 PMID:PMC5502959
- c) Yang, Y., Reipa, V., Liu, G., Meng, Y., Wang, X., Mineart, K., Prabhu, V., Shi, W., Lin, N., **He, X.**, Sun, J. 2018 pH-sensitive compounds for selective inhibition of acid-producing bacteria. *ACS Applied Materials & Interfaces* *Co-corresponding author. PMID29436821DOI:10.1021/acsami.8b01089

Complete List of Published Work in MyBibliography:

<https://www.ncbi.nlm.nih.gov/myncbi/1I1mBfI0u98Ar/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: Felicitas B. Bidlack

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

POSITION TITLE: 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
LMU Munich, Germany	Dipl. Biol. ¹	06/1995	Biology
George Washington University, Washington D.C.	M.Phil.	06/2000	Physical Anthropology
George Washington University, Washington D.C.	Ph.D.	06/2003	Hominid Paleobiology
The Forsyth Institute, Boston, MA	Postdoctoral	06/2003	Biom mineralization

A. Personal Statement

I am fully commitment to provide continued mentorship for postdoctoral researcher Dr. Jeremy Elias and work with Drs. Jirun Sun and Xuesong He for this K25 training grant application titled "Three-Dimensional Hydrogel Models for Analyzing Cariogenic Bacterial and Antimicrobial Responses."

I am Professor and Senior Director of Research Affairs at the ADA Forsyth Institute. My research is focused on the mechanisms of tooth enamel mineralization and repair. I have more than 20 years of experience in the use of teeth as records of health history and the time resolved analysis of dental growth tracks, histology, and chemical composition. In my work, I leverage a suite of high-resolution imaging techniques to characterize the biological processes and protein-mineral interactions of tooth mineralization and the composite nature and complex structural organization of enamel and dentin. Of relevance for the proposed project and mentoring plan is my experience of imaging bacteria on different substrates and using a variety of techniques. In ongoing NIH-funded projects, I am collaborating with Dr. Xuesong He on analyses of bacterial interaction and acidogenicity using imaging of pH probes and fluorescently labelled bacteria in dual-species biofilm on tooth enamel substrates. I am working with Dr. Jirun Sun in the RM1 project on high-resolution imaging and characterization of tooth substrates and interaction with acid, self-healing resins, and dental materials.

My research has always been interdisciplinary and highly collaborative and includes oversight and mentoring of trainees, as well as instruction at ADA Forsyth, Harvard School of Dental Medicine, Harvard School of Public Health, Massachusetts General Hospital, and Dartmouth University. Since 2017, I have provided mentorship to five graduate trainees, six DMSc students, and 12 postdoctoral trainees, four of them on the T90/R90 mechanism. This rewarding mentoring experience has made me acutely aware of the different needs and goals of trainees at different stages of their career. In my role as Senior Director of Research Affairs at ADA Forsyth, I have developed with colleagues the framework and policies for research training certificates and policies to offer trainees a stimulating environment as well as a productive and fulfilling training experience. In addition, I work closely with our trainee representatives to create an environment and framework for effective training in research and science communication. My publications are under Wiedemann FB, Wiedemann-Bidlack, and Bidlack FB after 2011.

Ongoing projects that I would like to highlight include:

2024-2029	<u>Virtual Twin-powered rapid development of bioactive multifunctional dental restoratives</u> <u>NIH/NIDCR: RM1 DE034233-01</u> <u>MPI: Sun J, Bidlack FB, Mirmomen L, Zhang Y.</u>	Role: MPI
2023-2028 NCE	<u>Caries resistance mechanisms in high-risk indigenous children</u> <u>NIH/NIDCR: 1 R01 DE032834-01</u> <u>MPI: Starr, Shi, Bidlack;</u>	Role Bidlack: MPI Contact-PI

Citations:

1. Davis KA, Mountain RV, Pickett OR, Den Besten PK, **Bidlack FB**, Dunn EC. (2020) Teeth as Potential New Tool to Measure Early-Life Adversity and Subsequent Mental Health Risk: An Interdisciplinary Review and Conceptual Model. Biol Psychiatry. 2020 Mar 15;87(6):502-513. PMID: 31858984; PMCID: PMC7822497
2. Karaaslan H, Walker AR, Gil-Bona A, Depalle B, **Bidlack FB**. (2024) Posteruptive loss of proteins in porcine enamel. (2024) J Dent Res, E-Pub Dec 2024, PMID: 39725879
3. Lin JJY, Hickman R, Farmer J, Tang I, McAlaine K, Punshon T, Jackson BP, **Bidlack FB**, Bartell S, Mangano JJ, Weisskopf MG. (2025) Early Life Lead Exposure, Sensitive Periods, and Long-Term Mental Health. Accepted, JAMA Psychiatry
4. Meng Y, Yang R, Alomeir N, O'Connor TG, Rasmussen JM, **Bidlack FB**, Xiao J. (2025) Maternal salivary stress-related hormone concentration is associated with the timing of early-life tooth eruption in offspring. Accepted, Frontiers in Oral Health.

B. Positions, Scientific Appointments

Positions

2024-present	Professor, ADA Forsyth Institute, Somerville, MA
2024- present	Senior Director of Research Affairs, ADA Forsyth Institute, Cambridge, MA
2023-2024	Senior Member of Staff, ADA Forsyth Institute, Cambridge, MA
2022-2023	Assistant Professor, Harvard School of Dental Medicine, Boston MA
2020-present	Senior Science Officer, Forsyth Institute, Cambridge, MA
2016-present	Associate Member of Staff, Mineralized Tissue Biology, Forsyth Institute, Cambridge, MA
2014-present	Instructor, Harvard School of Dental Medicine, Boston MA
2013-2016	Assistant Member of Staff, Mineralized Tissue Biology, Forsyth Institute, Cambridge, MA
2012-2013	Staff Scientist, Mineralized Tissue Biology, Forsyth Institute, Cambridge, MA
2010-2012	Assistant Research Investigator, Mineralized Tissue Biology, Forsyth Institute, Cambridge, MA
2010-2020	Director, Imaging Core Facility, Forsyth Institute, Cambridge, MA
2010-2020	Director, Imaging Core Facility, Forsyth Institute, Cambridge, MA
2010-2016	Director, Histology Core Facility, Forsyth Institute, Cambridge, MA
2003-2010	Postdoctoral Research Fellow, Forsyth Institute, Boston MA

NIH and NSF Reviewer Activities

2024	NIH, NIDCR R35, Ad Hoc Reviewer
2023	NIH, NIDCR UG3, Ad Hoc Reviewer
2023	NIH, MSOS Special Emphasis Panel, Ad Hoc Reviewer
2022	NIH, INCLUDE Special Emphasis Panel, Ad Hoc Reviewer
2021	NIH, NIDCR, OCDS study section, Ad Hoc Reviewer
2021	NIH, NIEH, Special Emphasis Panel, Ad Hoc Reviewer
2010	NSF, Division of Earth Sciences; Geobiology & Low Temperature Geochemistry, Ad Hoc Reviewer

Professional Memberships

2018-2023 Member, American Society for Bone and Mineral Research
 2013-present Member, International/American Association of Dental Research (IADR and AADOCR)
 2008-present Member, American Association for the Advancement of Science

Honors

2022 Member of the External Advisory Committee for Dartmouth University Mass Spectrometry Core
 2020 Member of the Science Advisory Panel for the St. Louis Tooth Study.
 2002 Cosmos Scholar, Cosmos Club Foundation, Washington D.C.
 2000 Grantee for Exploration and Field Research, Explorers Club, Washington D.C.
 2000 Best Graduate Teaching Assistant, Dept. of Anthropology, The George Washington University, Washington D.C.

C. Contributions to Science

1. Following my interest in the mechanisms of enamel formation, I focused early in my career on *in vitro* studies to better understand how the most abundant structural matrix protein regulates enamel formation under near physiological conditions of pH and temperature. This work highlighted the effect of pH on the protein self-assembly and the importance of phosphorylation for the stabilization of an amorphous mineral phase. Please note that my pertaining publications are under my hyphenated name Wiedemann-Bidlack.
 - a. **Wiedemann-Bidlack FB**, Beniash E, Yamakoshi Y, Simmer JP, and Margolis HC. (2007) pH-triggered self-assembly of native and recombinant amelogenins under physiological pH and temperature *in vitro*. *J. Struct Biol.* 160 (1): 57-69. PMCID: PMC2375294
 - b. Kwak SY, **Wiedemann-Bidlack FB**, Beniash E, Yamakoshi Y, Simmer JP, Litman A, and Margolis HC. (2009) Role of 20kD amelogenin (P148) phosphorylation on calcium phosphate formation *in vitro*. *J Biol. Chem.* 284(28):18972-9. Epub 2009 May 14. PMCID: PMC2707234
 - c. **Wiedemann-Bidlack FB**, Kwak SY, Beniash E, Simmer JP, Margolis HC. (2011) Effects of phosphorylation on the self-assembly of native full-length porcine amelogenin and its regulation of calcium phosphate formation *in vitro*. *J Struct Biol.* 173(2): 250-260. PMCID: PMC3293703
 - d. Kwak SY, Green S, **Wiedemann-Bidlack FB**, Beniash E, Yamakoshi Y, Simmer J, Margolis HC. (2011) Regulation of calcium phosphate formation by amelogenins under physiological conditions. *Europ. J Oral Sci.* 119 (Suppl. 1): 1-9. PMCID: PMC3448280
2. The use of bone and teeth as proxy records for behavior, climate, diet, and exposures in both humans and animals has been a part of my research interests for many years, as reflected in the publications below. These studies retrieve ontogenetic information preserved in bone and teeth and depend on the discernment between processes that form the record and those that alter the record, such as during fossilization. To extract a time resolved record from tooth analyses, I have developed methods to extract and analyze micro-samples of tooth enamel for stable isotope analyses following the growth tracks in teeth, ultimately for a time resolved reconstruction of environmental variability in paleoclimate studies. This approach was foundational for time resolved analyses of tooth enamel.
 - a. **Wiedemann-Bidlack FB**, Colman A, and Fogel ML. (2008) Phosphate oxygen isotope analysis on micro-samples of bioapatite: removal of organic contamination and minimization of sample size. *Rap. Com. Mass Spec.* (22): 1807-1816. PMID: 18470876 - no PMCID available.
 - b. Dunn E, Mountain R, Davis K, Shaffer I, Smith A, Roubinov D, DenBesten P, **Bidlack FB**, Boyce WT (2022). Association between measures derived from children's primary exfoliated teeth and psychopathology symptoms: Results from a community-based study. *Frontiers in Dental Medicine*.- no PMCID available.
 - c. Gil-Bona A, Karaaslan H, Depalle B, Sulyanto R, **Bidlack FB** (2023). Proteomic Analyses Discern the Developmental Inclusion of Albumin in Pig Enamel: A New Model for Human Enamel Hypomineralization. *Int. J. Mol. Sci.* 2023, 24, 15577. PMID: 37958567; PMCID: PMC10650821

- d. Bauer JA, Punshon T, Barr MN, Jackson BP, Weisskopf MG, **Bidlack FB**, Coker MO, Peacock JL, Karagas MR (2024). Deciduous teeth from the New Hampshire Birth Cohort Study: Early life environmental and dietary predictors of dentin elements. JESEE, PMID: 38347123; PMCID: PMC11317548.
3. My ongoing research efforts focus on a mechanistic understanding of tooth enamel properties and composition, how the structural organization of tooth enamel is attained, and which mechanisms underlie healthy or disrupted enamel formation. I am especially interested to uncover how protein presence and removal during enamel formation regulates mineral composition, structural organization, and how we can leverage the interaction between organic phase and mineralization for enamel repair. I am addressing this question using a suite of high-resolution imaging techniques, proteomic analyses of enamel, and various animal models as well as human teeth in projects with different perspectives on dental health.
 - a. Green DR, Schulte F, Lee KH, Pugach MG, Hardt M, **Bidlack FB**. Mapping the tooth enamel proteome and amelogenin phosphorylation onto mineralizing porcine tooth crowns. *Frontiers in Physiology*, 2019 Jul 30; (10): 925; PMID: 31417410; PMCID: PMC6682599
 - b. Karaaslan H, Depalle B, and **Bidlack FB**. (2025) Effect of storage conditions on proteomic analyses of human tooth enamel. Accepted for publication, *Frontiers in Dental Medicine*.
 - c. Farmer JG, Specht A, Punshon T, Jackson BP, **Bidlack FB**, Bakalar CA, Mukherjee R, Davis M, Steadman DW, Weisskopf MG (2024). Lead exposures across the life span and age of death. *Sci Tot Env*. 171975 PMID: 38547974; PMCID: PMC11069331
 - d. Sarra G, Parsaei S, Gomes MP, Lin Y-C, Pedroni AC, Raval D, Hu D, Berry S, Baron R, **Bidlack FB**, and Gori F. (2025) *Sfrp4* is required for proper dental formation and stem cell regulation. Revised manuscript submitted to JDR.

Complete List of Published Work in MyBibliography:

<http://www.ncbi.nlm.nih.gov/sites/myncbi/felicitas.bidlack.1/bibliography/43608330/public/?sort=date&direction=descending>

PHS 398 OTHER SUPPORT

*Name of Individual: Sun, Jirun
Commons ID: JIRUN_SUN

ACTIVE

*Title: **Preventing Dental Caries through targeted treatment of acid-producing bacteria**

Major Goals: The proposed research focuses on prevention of dental caries through targeted treatment of acid-producing bacteria (t-TAB). Its goal will be achieved by formulating and developing a series of pH-sensitive quaternary pyridinium salts and transforming the traditional, non-pH-sensitive chlorhexidine into antimicrobial materials with t-TAB capability. The successful candidates capable of preventing acid-induced tooth damage through achieving a healthy microbial community and maintaining environmental pH above 5.5 will be identified using a multispecies biofilm model that simulates human oral microbial community and further assessed for caries prevention *in vitro* using a microbial- caries model on human enamel and *in vivo* through a well-developed mouse caries model.

Project Number: 1R01DE029479-01A1

Name of PD/PI: MPI: Sun, J. (Contact) and He, X.

*Source of Support: NIDCR

*Primary Place of Performance: Forsyth Institute

Project/Proposal Start and End Date: 07/01/2021 – 06/30/2026

*Total Award Amount (including Indirect Costs): \$4,198,108

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

Year (YYYY)	Person Months (##.##)
4. 2024 - 2025	6.00 calendar
5. 2025 - 2026	6.00 calendar

*Title: Virtual Twin-Powered Rapid Development of Bioactive Multifunctional Dental Restorative

Major Goals: Aim 1 is to create a self-improving dental adhesive that enhances its bond with the tooth substrate by facilitating mineralization at the interface. Aim 2 focuses on crafting bioactive dental composites with self-healing and antimicrobial properties, achieved by integrating innovative nanofillers. Aim 3 is to produce CAD/CAM restoratives optimized for digital dentistry, offering robust, biocompatible materials with excellent aesthetics and bonding strength to teeth. Ultimately, our integration of artificial intelligence and a virtual lab with the material development process in the physical lab will revolutionize and individualize dental care. This platform will provide materials precisely tailored to meet diverse patient needs.

Status of Support: Active

Project Number: 1RM1DE034233-01

Name of MPIs: Sun, Bidlack, Mirmomen

Source of Support: NIH/NIDCR

*Primary Place of Performance: ADA-Forsyth

Project/Proposal Start and End Date: 09/18/2024 – 06/30/2029

*Total Award Amount (including Indirect Costs): \$ 6,247,425

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

Year (YYYY)	Person Months (##.##)
1. 2024-2025	3.60 calendar
2. 2025-2026	3.60 calendar
3. 2026-2027	3.60 calendar
4. 2027-2028	3.60 calendar
5. 2028-2029	3.60 calendar

*Title: **Hydrolysis-resistant resin networks for durable and multifunctional dental restorations**

Major Goals: We will create new dental adhesives and composite materials by formulating an advanced resin network, incorporating therapeutic components and specialized additives that combat bacteria and minimize protein adherence. After conducting rigorous clinical-relevant assessments of the new materials on extracted human teeth, we aim to produce various advanced therapeutic solutions tailored to address various dental cavity challenges.

*Status of Support: Active

Project Number: R01 DE033442

Name of PD/PI: Sun, J.

*Source of Support: NIH

*Primary Place of Performance: Forsyth Institute

Project/Proposal Start and End Date: 09/2024 – 06/2029

Total Award Amount (including Indirect Costs): \$2,438,385

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

Year (YYYY)	Person Months (##.##)
1. 2024-2025	1.80 calendar
2. 2025-2026	1.80 calendar
3. 2026-2027	1.80 calendar
4. 2027-2028	1.80 calendar
5. 2028-2029	1.80 calendar

PENDING

None

IN-KIND

None

***Overlap** (summarized for each individual): No scientific or effort overlap at this time. Should pending projects be awarded prior to the completion of Dr. Sun's active commitments, his effort will be adjusted within sponsor acceptable parameters so as not to exceed 12 calendar months effort in total.

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: Jirun Sun
Jirun Sun (Jul 2, 2025 11:46 EDT)
 Email: jsun@forsyth.org

PHS398 OTHER SUPPORT

*Name of Individual: Xuesong He
 Commons ID: xuesonghe2

ACTIVE

*Title: **Impact of Saccharibacteria and their bacterial hosts in Periodontal and Inflammatory Diseases**

Major Goals: The major goal of this project is to study the pathogenic nature and eukaryotic host interaction of newly characterized ultra-small Saccharibacteria (TM7) in mouse periodontal disease model.

*Status of Support: Active

Project Number: 1R01DE031274-01

Name of PD/PI: Bor, Batbileg

*Source of Support: NIDCR

*Primary Place of Performance: ADA Forsyth Institute, Inc.

Project/Proposal Start and End Date: 01/2022 –12/2026

* Total Award Amount (including Indirect Costs): \$2,503,070

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

Year (YYYY)	Person Months (##.##)
4. 2024 - 2025	0.60 calendar
5. 2025 - 2026	0.60 calendar

*Title: **Domestication and Characterization of TM7-The Most Elusive Oral Phylum**

Major Goals: The major goal of this project is to study the ecology, evolution and pathogenesis of the first human oral TM7/Saccharibacteria isolate and only member of the Candidate Phylum Radiation cultivated to date.

*Status of Support: Active

Project Number: 2R01 DE023810-12

Name of PD/PI: MPI He (Contact PI) and McLean

*Source of Support: NIDCR

*Primary Place of Performance: ADA Forsyth Institute, Inc.

Project/Proposal Start and End Date: 09/1/2024-08/30/2029

Total Award Amount (including Indirect Costs): \$2,831,366

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

Year (YYYY)	Person Months (##.##)
12. 2024 - 2025	1.80 calendar
13. 2025 - 2026	1.80 calendar
14. 2026 - 2027	1.80 calendar
15. 2027 - 2028	1.80 calendar
16. 2028 - 2029	1.80 calendar

*Title: **Preventing dental caries through targeted treatment of acid-producing bacteria**

Major Goals: The proposed research focuses on prevention of dental caries through targeted treatment

of acid-producing bacteria(t-TAB). Its goal will be achieved by formulating and developing a series of pH-sensitive quaternary pyridinium salts and empowering the traditional, non-pH-sensitive chlorhexidine with t-TAB capability. The successful candidates capable of preventing acid-induced tooth damage through achieving a healthy microbial community and maintaining environmental pH above 5.5 will be identified using a multispecies biofilm model that simulates human oral microbial community and further assessed for caries prevention *in vitro* using a microbial-caries model on human enamel and *in vivo* through a well-developed mouse caries model.

*Status of Support: Active

Project Number: 5R01DE029479-02

Name of PD/PI: MPI Sun (Contact PI), He

*Source of Support: NIH/NIDCR

*Primary Place of Performance: ADA Forsyth Institute, Inc.

Project/Proposal Start and End Date: 09/01/2021 – 08/31/2026

* Total Award Amount (including Indirect Costs: \$3,746,349

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

Year (YYYY)	Person Months (##.##)
4. 2024 - 2025	1.80 calendar
5. 2025 - 2026	2.16 calendar

***Title: Host tRNA-derived small RNAs (tsRNAs) mediate interactions between host and oral microbes**

Major Goals: The goal of this application is two-fold: (1) To achieve mechanistic understanding of the cross-kingdom trafficking of host-derived *F. nucleatum* (Fn)-targeting tsRNAs and their modulating effect on Fn growth during NOKSI-Fn interaction, through exosome tracking and in- depth dissection of the tsRNAs transporter and intracellular targets in Fn; (2) To expand our work to profile and compare salivary tsRNAs between healthy and periodontitis subjects, with a focus on demonstrating the broad implication of host-generated tsRNAs as a conserved mechanism to achieve host-microbial homeostasis.

*Status of Support: Active

Project Number: 1R01DE030943-01A1

Name of PD/PI: He, X.

*Source of Support: NIH/NIDCR

*Primary Place of Performance: ADA Forsyth Institute, Inc.

Project/Proposal Start and End Date: 02/2022 – 01/2027

Total Award Amount (including Indirect Costs): \$2,448,988

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

Year (YYYY)	Person Months (##.##)
3. 2024 - 2025	1.80 calendar
4. 2025 - 2026	1.80 calendar
5. 2026 - 2027	1.80 calendar

***Title: Caries resistance mechanisms in high-risk Indigenous children**

Major Goals: This study investigates potential caries protective mechanisms in Indigenous children from Manitoba, focusing on the small percentage of caries free children with high load of caries causing bacteria of *Streptococcus mutans*. The Specific Aims are Aim 1. Test whether and how *Rothia* and/or other oral species may mitigate the cariogenic effects of acidogenic bacteria. Aim 2. Test whether and how tooth properties

modulate susceptibility to acid dissolution of enamel and dentin. Aim 3. Test how tooth substrate or saliva affect acidogenicity and spatial structure of biofilms.

Status of Support: Active

Project Number: 1 R01 DE032834-01

Name of PD/PI: MPI: F. Bidlack (Contact), W. Shi, J. Starr

*Source of Support: NIH/NIDCR

*Primary Place of Performance: ADA Forsyth Institute, Inc.

Project/Proposal Start and End Date: 04/2023 – 03/2028

* Total Award Amount (including Indirect Costs): \$3,813,490

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

Year (YYYY)	Person Months (##.##)
2. 2024-2025	1.08 calendar
3. 2025-2026	1.20 calendar
4. 2026-2027	1.20 calendar
5. 2027-2028	1.20 calendar

*Title: **Gut Microbiome and Salivary Gland Function: Protective Actions & Key Players**

Major Goals: To determine the impact of gut microbiome on salivary gland function, with a particular focus on the previously unexplored, salivary gland protective gut bacteria and the players and mechanisms that mediate their actions in Sjögren's syndrome.

*Status of Support: Active

Project Number: 1 R56 AI181002-01A1

Name of PD/PI: Yu, Q.

*Source of Support: NIH / NIDCR

*Primary Place of Performance: ADA Forsyth Institute, Inc.

Project/Proposal Start and End Date: 01/2024 – 12/2025 (NCE)

* Total Award Amount (including Indirect Costs): \$359,999

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

Year (YYYY)	Person Months (##.##)
1. 2024	1.40 calendar

*Title: **Design Microbiome-Based Therapies to Prevent and Ameliorate Posttraumatic Stress Disorder**

Major Goals: The overarching challenge is that we do not fully understand the PTSD-gut microbiome relationship and hence lack rational design of microbiome-based therapeutics for the prevention or amelioration of PTSD. Our central hypothesis is that microbiome-based interventions can prevent and ameliorate PTSD. Our overall objective is to initiate a unique project to evaluate the PTSD-gut microbiome relationship and develop synbiotics (a combination of prebiotics and probiotics) to prevent or ameliorate PTSD, leveraging a population-based cohort and an etiologically relevant mouse model.

Status of Support: Active

Project Number: TP220055

Name of PD/PI: Liu, Y, Koenan

*Source of Support: DoD

*Primary Place of Performance: Brigham & Women's Hospital, Inc.

Project/Proposal Start and End Date: 09/2023 – 08/2027

* Total Award Amount (including Indirect Costs): \$358,200 (Forsyth TC)

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

Year (YYYY)	Person Months
2. 2024-2025	0.96 calendar
3. 2025-2026	0.96 calendar
4. 2026-2027	0.96 calendar
5. 2027-2028	0.96 calendar

*Title: **Virtual Twin-Powered Rapid Development of Bioactive Multifunctional Dental Restorative**

Major Goals: Aim 1 is to create a self-improving dental adhesive that enhances its bond with the tooth substrate by facilitating mineralization at the interface. Aim 2 focuses on crafting bioactive dental composites with self-healing and antimicrobial properties, achieved by integrating innovative nanofillers. Aim 3 is to produce CAD/CAM restoratives optimized for digital dentistry, offering robust, biocompatible materials with excellent aesthetics and bonding strength to teeth. Ultimately, our integration of artificial intelligence and a virtual lab with the material development process in the physical lab will revolutionize and individualize dental care. This platform will provide materials precisely tailored to meet diverse patient needs.

Status of Support: Active

Project Number: 1RM1DE034233-01

Name of MPIs: Sun, Bidlack, Mimomen

Source of Support: NIH/NIDCR

*Primary Place of Performance: ADA Forsyth Institute, Inc.

Project/Proposal Start and End Date: 09/18/2024 – 06/30/2029

*Total Award Amount (including Indirect Costs): \$ 6,342,218

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

Year (YYYY)	Person Months (##.##)
1. 2024-2025	1.20 calendar
2. 2025-2026	1.20 calendar
3. 2026-2027	1.20 calendar
4. 2027-2028	1.20 calendar
5. 2028-2029	1.20 calendar

*Title: **Impact of Diabetes hyperglycemia on peri-implantitis**

Major Goals: The goals of this study are to 1) determine cellular inflammatory responses to peri-implant microbiota from normal and diabetic mice in vitro, 2) to identify and characterize peri-implant microbial changes under normal vs. DM conditions in vivo, and 3) to investigate the peri-implant inflammation and bone loss after exogenous microbial transfer in germ-free mice in vivo.

Status of Support: Active

Project Number: 1R21DE032156-01A1

Name of MPIs: Xiaozhe Han

Source of Support: NIH/NIDCR

*Primary Place of Performance: Nova Southeastern University

Project/Proposal Start and End Date: 09/2023 – 08/2025

*Total Award Amount (including Indirect Costs): \$ 53,000 (Forsyth TC)

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

Year (YYYY)	Person Months (##.##)
2. 2024-2025	0.24 calendar

*Title: **Mechanistic investigation of multispecies interactions in clear aligner induced periodontal inflammation**

*Major Goals: *Fusobacterium nucleatum*, *Actinomyces spp.* and *Saccharibacteria* (TM7) are frequently reported to have significantly increased abundance in inflammatory diseases, including clear aligner-associated gingival inflammation. This study investigates their interaction in relationship with gingival inflammation. It will provide molecular insight into these tri-species interactions and lay the foundation for therapeutic interventions against gingival inflammation.

*Status of Support: Pending

Project Number: 1R03DE034509 - 01A1

Name of PD/PI: Tingxi Wu

*Source of Support: NIH / NIDCR

*Primary Place of Performance: ADA Forsyth Institute, Inc.

Project/Proposal Start and End Date: (MM/YYYY) (if available): 07/15/2025 – 07/14/2027

* Total Award Amount (including Indirect Costs): \$398,000

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

Year (YYYY)	Person Months (##.##)
1. 2025 - 2026	0.60 Calendar
2. 2026 - 2027	0.60 Calendar

PENDING

Title: **Periodontitis Role in Inflammatory Bowel Disease**

Major Goals: This proposal investigates the causal role of periodontitis, oral bacteria, and host factors in promoting intestinal inflammation in IBD using mechanistic studies in pre-clinical models.

Status of Support: Pending

Project Number: 1R01DK140281-01A1

Name of PD/PI: Thumbigere-Math V

*Source of Support: NIH/NIDDK

*Primary Place of Performance: University of Maryland Baltimore

Project/Proposal Start and End Date: 04/2025 – 03/2030

* Total Award Amount (including Indirect Costs): \$290,720 (Forsyth TC)

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

Year (YYYY)	Person Months
1. 2025-2026	0.60 calendar
2. 2026-2027	0.60 calendar
3. 2027-2028	0.60 calendar
4. 2028-2029	0.60 calendar
5. 2029-2030	0.60 calendar

Title: Developing Chemically Modified Host-Derived Small RNAs to Target Oral Pathobionts

Major Goals: Test the hypothesis that strategic chemical modifications of *Fusobacterium nucleatum*-targeting host-derived tsRNAs will provide insights into nucleotide-specific interactions critical for bacterial protein targeting, uptake, and antimicrobial effects, thereby enabling the development of **first-of-its-kind** species-specific antimicrobials.

Status of Support: Pending

Project Number: R01

Name of PD/PI: Li, J.

*Source of Support: NIH/NIDCR

*Primary Place of Performance: University of Michigan

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

* Total Award Amount (including Indirect Costs): \$1,842,730 (Forsyth TC)

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

Year (YYYY)	Person Months
1. 2025-2026	1.80 calendar
2. 2026-2027	1.80 calendar
3. 2027-2028	1.80 calendar
4. 2028-2029	1.80 calendar
5. 2029-2030	1.80 calendar

Title: Underscoring human oral host-pathogen interactions and modulation in an immunocompetent in vitro model of early dysbiosis

*Major Goals: we aim to investigate the underlying immune-driven events that play a major role in the homeostatic balance between host and microbial communities, providing stability in healthy conditions, while contributing to immune-inflammatory progression in periodontal disease states. Leveraging our team's expertise in human in vitro modeling, microbiology, mucin biophysics, biogeography, and clinical translation, we aim to leverage a 3D human oral tissue model to provide a mechanistic framework of oral host-microbial interactions and modulation in early dysbiosis to support future therapeutic interventions

*Status of Support: Pending

Project Number: 1102

Name of PD/PI: Ghezzi, CE

*Source of Support: NIH / NIDCR

*Primary Place of Performance: University of Massachusetts, Lowell

Project/Proposal Start and End Date: 07/2025 – 06/2030

*Total Award Amount (including Indirect Costs): \$883,972 (Forsyth TC)

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


Year (YYYY)	Person Months (##.##)
1. 2025 – 2026	0.60 calendar
2. 2026 – 2027	0.60 calendar
3. 2027 – 2028	0.60 calendar
4. 2028 – 2029	0.60 calendar
5. 2029 – 2030	0.60 calendar

IN-KIND

None

***Overlap** (summarized for each individual): No scientific or effort overlap at this time. Should pending projects be awarded prior to the completion of Dr. He's active commitments his effort will be adjusted within sponsor acceptable parameters so as not to exceed 12 calendar months effort in total.

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: 
Xuesong He (Aug 7, 2025 10:19:30 EDT)

Date: Aug 7, 2025

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

UEI*: MZ9DFVC2J1B7

Budget Type*: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: ADA Forsyth Institute, Inc.

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.	Jeremy		Elias		PD/PI	100,000.00	12.00			100,000.00	26,700.00	126,700.00
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons:		File Name:									Total Senior/Key Person	126,700.00

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)							126,700.00

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

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

UEI*: MZ9DFVC2J1B7
Budget Type*: ☒ Project ☐ Subaward/Consortium
Organization: ADA Forsyth Institute, Inc.

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)	
2. Foreign Travel Costs	
Total Travel Cost	

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*: MZ9DFVC2J1B7

Budget Type*: ☒ Project ☐ Subaward/Consortium

Organization: ADA Forsyth Institute, Inc.

Start Date*: 07-01-2026

End Date*: 06-30-2027

Budget Period: 1

F. Other Direct Costs			Funds Requested (\$)*
1. Materials and Supplies			
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. program-related expenses			25,000.00
9. Data Management and Sharing Costs			0.00
Total Other Direct Costs			25,000.00

G. Direct Costs	Funds Requested (\$)*
Total Direct Costs (A thru F)	151,700.00

H. Indirect Costs			
Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
1 . MTDC	8.00	151,700.00	12,136.00
Total Indirect Costs			12,136.00
Cognizant Federal Agency		DHHS, Darryl W. Mayes, 212-264-2069	
(Agency Name, POC Name, and POC Phone Number)			

I. Total Direct and Indirect Costs	Funds Requested (\$)*
Total Direct and Indirect Institutional Costs (G + H)	163,836.00

J. Fee	Funds Requested (\$)*

K. Total Costs and Fee	Funds Requested (\$)*
	163,836.00

L. Budget Justification*	File Name: 1241-Budget Justification_11.03.25.pdf
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RESEARCH & RELATED Budget {F-K} (Funds Requested)

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

UEI*: MZ9DFVC2J1B7

Budget Type*: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: ADA Forsyth Institute, Inc.

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 .	Jeremy		Elias		PD/PI	100,000.00	12.00			100,000.00	26,700.00	126,700.00
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons:		File Name:									Total Senior/Key Person	126,700.00

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)							126,700.00

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

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

UEI*: MZ9DFVC2J1B7
Budget Type*: ☒ Project ☐ Subaward/Consortium
Organization: ADA Forsyth Institute, Inc.

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)	
2. Foreign Travel Costs	
Total Travel Cost	

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*: MZ9DFVC2J1B7

Budget Type*: ☒ Project ☐ Subaward/Consortium

Organization: ADA Forsyth Institute, Inc.

Start Date*: 07-01-2027

End Date*: 06-30-2028

Budget Period: 2

F. Other Direct Costs	Funds Requested (\$)*
1. Materials and Supplies	
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. program-related expenses	25,000.00
9. Data Management and Sharing Costs	0.00
Total Other Direct Costs	25,000.00

G. Direct Costs	Funds Requested (\$)*
Total Direct Costs (A thru F)	151,700.00

H. Indirect Costs			
Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
1 . MTDC	8.00	151,700.00	12,136.00
Total Indirect Costs			12,136.00
Cognizant Federal Agency	DHHS, Darryl W. Mayes, 212-264-2069		
(Agency Name, POC Name, and POC Phone Number)			

I. Total Direct and Indirect Costs	Funds Requested (\$)*
Total Direct and Indirect Institutional Costs (G + H)	163,836.00

J. Fee	Funds Requested (\$)*

K. Total Costs and Fee	Funds Requested (\$)*
	163,836.00

L. Budget Justification*	File Name: 1241-Budget Justification_11.03.25.pdf
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RESEARCH & RELATED Budget {F-K} (Funds Requested)

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

UEI*: MZ9DFVC2J1B7

Budget Type*: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: ADA Forsyth Institute, Inc.

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 .	Jeremy		Elias		PD/PI	100,000.00	12.00			100,000.00	26,700.00	126,700.00
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons:		File Name:									Total Senior/Key Person	126,700.00

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)							126,700.00

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

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

UEI*: MZ9DFVC2J1B7
Budget Type*: ☒ Project ☐ Subaward/Consortium
Organization: ADA Forsyth Institute, Inc.

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)	
2. Foreign Travel Costs	
Total Travel Cost	

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*: MZ9DFVC2J1B7

Budget Type*: ☒ Project ☐ Subaward/Consortium

Organization: ADA Forsyth Institute, Inc.

Start Date*: 07-01-2028

End Date*: 06-30-2029

Budget Period: 3

F. Other Direct Costs			Funds Requested (\$)*
1. Materials and Supplies			
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. program-related expenses			25,000.00
9. Data Management and Sharing Costs			0.00
Total Other Direct Costs			25,000.00

G. Direct Costs	Funds Requested (\$)*
Total Direct Costs (A thru F)	151,700.00

H. Indirect Costs			
Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
1 . MTDC	8.00	151,700.00	12,136.00
Total Indirect Costs			12,136.00
Cognizant Federal Agency		DHHS, Darryl W. Mayes, 212-264-2069	
(Agency Name, POC Name, and POC Phone Number)			

I. Total Direct and Indirect Costs	Funds Requested (\$)*
Total Direct and Indirect Institutional Costs (G + H)	163,836.00

J. Fee	Funds Requested (\$)*

K. Total Costs and Fee	Funds Requested (\$)*
	163,836.00

L. Budget Justification*	File Name: 1241-Budget Justification_11.03.25.pdf
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RESEARCH & RELATED Budget {F-K} (Funds Requested)

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

UEI*: MZ9DFVC2J1B7
Budget Type*: ☒ Project ☐ Subaward/Consortium
Enter name of Organization: ADA Forsyth Institute, Inc.

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.	Jeremy		Elias		PD/PI	100,000.00	12.00			100,000.00	26,700.00	126,700.00
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons:		File Name:									Total Senior/Key Person	126,700.00

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)	126,700.00

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

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

UEI*: MZ9DFVC2J1B7

Budget Type*: ☒ Project ☐ Subaward/Consortium

Organization: ADA Forsyth Institute, Inc.

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)

2. Foreign Travel Costs

Total Travel Cost**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*: MZ9DFVC2J1B7

Budget Type*: ☒ Project ☐ Subaward/Consortium

Organization: ADA Forsyth Institute, Inc.

Start Date*: 07-01-2029

End Date*: 06-30-2030

Budget Period: 4

F. Other Direct Costs			Funds Requested (\$)*
1. Materials and Supplies			
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. program-related expenses			25,000.00
9. Data Management and Sharing Costs			0.00
Total Other Direct Costs			25,000.00

G. Direct Costs	Funds Requested (\$)*
Total Direct Costs (A thru F)	151,700.00

H. Indirect Costs			
Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
1 . MTDC	8.00	151,700.00	12,136.00
Total Indirect Costs			12,136.00
Cognizant Federal Agency		DHHS, Darryl W. Mayes, 212-264-2069	
(Agency Name, POC Name, and POC Phone Number)			

I. Total Direct and Indirect Costs	Funds Requested (\$)*
Total Direct and Indirect Institutional Costs (G + H)	163,836.00

J. Fee	Funds Requested (\$)*

K. Total Costs and Fee	Funds Requested (\$)*
	163,836.00

L. Budget Justification*	File Name: 1241-Budget Justification_11.03.25.pdf
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RESEARCH & RELATED Budget {F-K} (Funds Requested)

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

UEI*: MZ9DFVC2J1B7
Budget Type*: ☒ Project ☐ Subaward/Consortium
Enter name of Organization: ADA Forsyth Institute, Inc.

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 .	Jeremy		Elias		PD/PI	100,000.00	12.00			100,000.00	26,700.00	126,700.00
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons:		File Name:									Total Senior/Key Person	126,700.00

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)							126,700.00

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

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

UEI*: MZ9DFVC2J1B7

Budget Type*: ☒ Project ☐ Subaward/Consortium

Organization: ADA Forsyth Institute, Inc.

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)

2. Foreign Travel Costs

Total Travel Cost**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*: MZ9DFVC2J1B7

Budget Type*: ☒ Project ☐ Subaward/Consortium

Organization: ADA Forsyth Institute, Inc.

Start Date*: 07-01-2030

End Date*: 06-30-2031

Budget Period: 5

F. Other Direct Costs			Funds Requested (\$)*
1. Materials and Supplies			
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. program-related expenses			25,000.00
9. Data Management and Sharing Costs			0.00
Total Other Direct Costs			25,000.00

G. Direct Costs	Funds Requested (\$)*
Total Direct Costs (A thru F)	151,700.00

H. Indirect Costs			
Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
1 . MTDC	8.00	151,700.00	12,136.00
Total Indirect Costs			12,136.00
Cognizant Federal Agency		DHHS, Darryl W. Mayes, 212-264-2069	
(Agency Name, POC Name, and POC Phone Number)			

I. Total Direct and Indirect Costs	Funds Requested (\$)*
Total Direct and Indirect Institutional Costs (G + H)	163,836.00

J. Fee	Funds Requested (\$)*

K. Total Costs and Fee	Funds Requested (\$)*
	163,836.00

L. Budget Justification*	File Name: 1241-Budget Justification_11.03.25.pdf
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RESEARCH & RELATED Budget {F-K} (Funds Requested)

Budget Justification

Personnel:

Jeremy Elias, PhD. Principal Investigator (12.0 CM YR01-YR05). Dr. Elias completed his Ph.D. in Materials Science and Engineering at the University of Florida in May 2022. Since August 2022, he has been under the co-mentorship of Drs. Sun, He, and Bidlack. In his current research, Dr. Elias is continuing to develop cell and bacterial culture skills and expertise, while employing advanced confocal imaging techniques for microbial study. In addition, he is continuing to employ a variety of techniques for imaging, biomaterial analysis, and mechanical evaluation, including Nanoindentation, Atomic Force Microscopy (AFM), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), micro-computed tomography (micro-CT), tensiometry, dilatometry, and the universal Instron machine. Dr. Elias will devote 100% of his time over the course of the award and will be responsible for the overall design and implementation of the experiments described in the research plan. He will receive training needed to design and execute all of the proposed experiments in the research strategy, as well as participating in all formal and informal coursework and training outlined in the career development plan.

Jirun Sun, MSc. Ph.D., Primary mentor. Dr. Sun is a Professor at AFI. He has over 20+ years of research experience in biomaterials development for dental, oral, and craniofacial applications. He is a well-established scientist and inventor. He has been awarded 9 US patents and published over 100 high-level academic papers on preparation, testing, and method development in dental materials and biomaterials. His research is mainly supported by NIH, industry, DOD, the National Institute of Standards and Technology, ADA, and the National Naval Medical Center. In his recently finished NIH cooperative grant (U01DE023752), he published 28 academic papers and was awarded 8 patents. His works have been recognized and reported by international news magazines and organizations including C&En News and Federal Dental International (World Dental International). Dr. Sun is the inventor/co-inventor of the key elements in the proposed research, e.g., Azo-QPS-C16. Dr. Sun will participate in activities associated with production, analyses, and data display to the biomaterials and bioprinting related components of this project. He will be the primary mentor for Dr. Elias. He will contribute to the project at no cost to the grant award.

Xuesong He, Ph.D., DDS, Co-mentor. Dr. He is a Professor at AFI who will serve as co-mentor on this grant. He is experienced as the leader of multiple NIH-funded research projects involving multiple PIs from different institutes. Dr. He brings the expertise of oral microbiome, microbial ecology with physiology, pathogenesis, particularly *in vitro* multispecies microbial community, oral biofilm culturing, and dental product testing, which are integral to the proposed study. Dr. He will provide oversight and offer his expertise in oral microbiome, and act to interpret results to the larger proposal team. Dr. He will participate in activities associated with the production, analyses, and data display to the public of microbiology-related components of this project. He will also play a role in advising and mentoring Dr. Elias. He will contribute to the project at no cost to the grant award.

Felicitas B Bidlack, PhD., Advisor. Dr. Bidlack is a Professor at AFI and has been an active dental enamel researcher at the Institute since 2003. Dr. Bidlack has 20 years of experience, and a unique skill set for the analysis of microstructure, histology, and chemical and proteomic composition of teeth using chemical methods and a variety of imaging techniques, including different types and modes of electron microscopy. Dr. Bidlack has ample experience of working in highly interdisciplinary research projects and team science. Dr. Bidlack will provide advice to Dr. Elias, give guidance and oversee work relating to imaging, assist in experimental design, sample processing and analyses, data interpretation, integration of results and contribute to discussion and preparation of progress reports and publications of results. In addition, in her role as Senior Science Officer, Dr. Bidlack will be closely working with the PI and mentors to facilitate the success of this project. She will contribute to the project at no cost to the grant award.

Fringe Benefits: calculated at ADA Forsyth Institute's current DHHS rate agreement, executed 05/03/2024.

7/1/24 – 12/31/29 Pred.: Employees – 29.60%; Post Docs – 26.70%

1/1/30 – 12/31/32 Prov.: Employees – 29.60%; Post Docs – 26.70%

Research and Career Development Costs (\$25,000 each year):

Supplies (\$7,500/yr): Includes reagents for sample synthesis, bacterial culture, dyes for imaging, and sample preparation resources for confocal and electron microscopy.

Core Services (\$9,000/yr first 2 years, \$11,500/year in years 3-5): Costs will include use of the ADA Forsyth Advanced Imaging Core, and the Harvard Center for Nanoscale Systems.

Publication (\$3,000/yr): Dr. Elias plans to publish one large manuscript at least once per year. \$3000 is requested to offset the publishing costs of the manuscripts.

Travel (\$3,000/yr): Dr. Elias will attend two conferences per year for networking and dissemination of research. This will include The American Association for Dental, Oral, and Craniofacial Research (AADOCR) Annual Meeting and Exhibition, which occurs annually, and a Gordon Research Conference/Seminar (GRC/GRS), where respective conferences on subject of interest occur every two years in US or international locations. The related expenses cover conference registration, accommodation, and transportation costs.

Coursework and Training (\$2,500/yr, first 2 years): Formal coursework through Harvard University will be carried out in the first two years of the proposed career development path, with estimated costs of ~\$300 per credit.

F&A Rate: 8% MTDC as allowed per PA-24-191. DHHS, Darryl W. Mayes, 212-264-2069. Exclusions: No exclusions apply to this budget.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals (\$)
Section A, Senior/Key Person	633,500.00
Section B, Other Personnel	
Total Number Other Personnel	
Total Salary, Wages and Fringe Benefits (A+B)	633,500.00
Section C, Equipment	
Section D, Travel	
1. Domestic	
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	125,000.00
1. Materials and Supplies	
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. Other 1	125,000.00
9. Other 2	0.00
10. Other 3	
11. Other 4	
12. Other 5	
13. Other 6	
14. Other 7	
15. Other 8	
16. Other 9	
17. Other 10	
Section G, Direct Costs (A thru F)	758,500.00
Section H, Indirect Costs	60,680.00
Section I, Total Direct and Indirect Costs (G + H)	819,180.00
Section J, Fee	
Section K, Total Costs and Fee (I + J)	819,180.00

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)
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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)	1242-Introduction_11.10.25.pdf
Candidate Section	
2. Candidate Information and Goals for Career Development	1243-Career Development Plan_11.03.25.pdf
Research Plan Section	
3. Specific Aims	1244-Specific Aims_11.10.25.pdf
4. Research Strategy*	1245-Research Strategy_11.10.25.pdf
5. Progress Report Publication List (for Renewal applications)	
6. Training in the Responsible Conduct of Research	1246-RCR Training_11.03.25.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)	1247-Mentors Statement and Plan_11.10.25.pdf
9. Letters of Support from Collaborators, Contributors, and Consultants	1248-Bidlack LOS_11.10.25.pdf
Environment and Institutional Commitment to Candidate Section	
10. Description of Institutional Environment	1249-Institutional Environment_11.03.25.pdf
11. Institutional Commitment to Candidate's Research Career Development	1250-Institutional support_11.10.25.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	1251-Resource Sharing_11.03.25.pdf
17. Other Plan(s)	1252-DMS Plan_11.03.25.pdf
18. Authentication of Key Biological and/or Chemical Resources	1253-Authentication_11.03.25.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: ☐

Introduction: I highly appreciate the reviewers' thoughtful feedback, including the recognition of my "strong background in materials science and biomaterials" as well as acknowledging the "well-conceived training plan to expand his expertise into printed materials containing cariogenic bacteria." In response to the concerns raised, I have made substantial revisions to the proposal. Below is a summary of my responses.

Research Plan:

1. "The absence of preliminary data further weakens the study's feasibility and rationale, raising concerns about the overall scientific rigor of the proposed research" I have generated substantial new data by integrating single-cell imaging with parallel pH and viability mapping, enabling direct 3D assessment of metabolism-triggered antimicrobial activity at a level largely unattainable with traditional assays. These promising results inspired a redesign of the specific aims, now with greater scientific rigor. In **Aim 1**, I will engineer bacteria-laden 3D printed patterns as platforms for spatiotemporal characterization of bacterial behavior. In **Aim 2**, I will quantify the metabolism-activated antimicrobial potential of a pH-responsive delivery system against a single cariogenic species. In **Aim 3**, I will probe the selective inhibition capabilities of this system within a dual-species biofilm model. The title: "Three-Dimensional Hydrogel Models for Analyzing Cariogenic Bacteria and Antimicrobial Responses" has also been updated to best reflect the updated study aims and strategies.

2. "The measurement approaches for material characterization and bacterial interactions appear underdeveloped." I have developed new measurement methodologies, leading to additional preliminary data and contributing to two major journal submissions: I am first author on a manuscript, **"Single-Cell Level Analysis of Metabolism-Activated Antibacterial Mechanisms in Customizable Hydrogels,"** submitted to *Proceedings of the National Academy of Sciences* and a key contributor to a second manuscript, **"Nano-Gatekeepers Triggered by Metabolism: From Broad-Spectrum to Precision Release,"** under review at *Nature Materials*. Detailed descriptions of these measurement approaches are presented in Sections C.1.1 and C.1.2 of the Research Plan. The completion of these publications, as well as a submitted first-author review manuscript on microbial hydrogel culture methodologies for precise analysis, have both *strengthened my publication record* and *informed the improved measures* of parallel pH labeling and spatial analysis to probe spatiotemporal microbial responses.

3. "lacks clear biological context, with an unclear justification for the use of 3D printing." The goal of this research is to deepen mechanistic insight and enable spatially targeted antimicrobial therapies. By leveraging 3D patterns that impose **precise spatial control**, coupled with single-cell analyses and parallel measurement of pH and viability, we can study spatiotemporal bacterial behavior with high resolution and localize treatment effects. The revised aims combine our expertise in hydrogel-based microbial confinement with the spatial precision of bioprinting to design precisely controlled 3D therapeutic environments, and our plan will progress from single-species to multi-species patterning to evaluate metabolism-triggered antimicrobial behavior.

4. "No back up plan for pH sensitive compounds if they do not work with polymicrobial system." Redesign alternative strategies in Aims 2 and 3 include Azo-QPS-C16 as an alternative approach to improve the selective inhibition of cariogenic bacteria. Drs. Sun and He have previously demonstrated Azo-QPS-C16 can selectively inhibit acid-producing bacteria (*ACS Appl. Mater. Interfaces* 2018, 10, 10, 8566–8573).

5. "Selection of microbes for polymicrobial studies." The modified Aim 3 provides a clearer rationale for the microbes chosen. We primarily choose a non-acid producing commensal *Rothia* species to assess the selective killing of acid-responsive antimicrobials against acid-producing *S. mutans* in a dual-species system. If time allows, we will also expand to include bacterial species with known cooperative or competitive interactions with *S. mutans* to further optimize our novel platform (C.3.3).

6. "The main limitations revolve around materials choice (acrylamide)" We chose acrylamide in our proposed design because it allows tunable pore sizes and is cost-effective. The 3D models developed can also readily be extended to alternative materials (for example, GelMA hydrogels). I have updated the "Alternative Approaches" section in Aim 1 to include evaluation of these other materials.

7. "More consideration of creating a model of tooth structures in this printing is missing." Redesign Aim 1 leverages our control over microporosity and small-scale architectures (< 5 microns) within gels to enable real-time spatiotemporal analysis in environments emulating the spatial confinement of dentin tubules, while larger-scale porosity and architecture through printed patterns considers microbial dynamics at tissue interfaces with heterogeneous bacterial geography (C.1.2-C.1.3).

Career Development:

1. "The evaluation plan lacks strong assessment metrics for tracking candidate progress and project success" I have redesigned the career development plan to incorporate measurable milestones directly tied to my evolving expertise, ensuring they closely track with the research goals. I also engaged my advisory committee to help define evaluation criteria that support both the short-term aims and my long-term career trajectory. The updated career development plan also outlines Dr. Bidlack's continued role as scientific advisor and relevant expertise in my proposed research objectives and career path.

Candidate Background

My research trajectory has been driven by a consistent fascination with materials science and its intersection with biological systems. The research opportunities I have taken part in have built a foundation that positions me to leverage my expertise in biomimetic materials synthesis while acquiring biomedical knowledge and methodologies essential for conducting independent, NIH-funded research.

My undergraduate research on ultra-high temperature ceramics for aerospace applications provided foundational expertise in materials synthesis and advanced characterization, and this skillset informed my interests and approach to engineering biomaterials in my graduate studies. During my Ph.D. at the University of Florida, I worked under the mentorship of Dr. Laurie Gower, a leader in biomimetics, focusing on nature-inspired organic-mineral composites. My thesis research explored crystal growth kinetics and mineralization strategies to replicate the hierarchical structures and mechanical properties of biological materials, such as bone-like tissues via collagen-mineral composites, and crustacean shells via chitin-based matrices. This research resulted in two first author papers ^{1,2} and a second author review paper on biomineralization principles ³. In my research characterizing bone and biomimetic scaffolds, I employed advanced techniques, including Scanning/Transmission Electron Microscopy (SEM/TEM), X-ray Diffraction (XRD), and mechanical testing, building a strong foundation in materials analysis. Beyond research, I developed my teaching and mentorship skills as a materials lab instructor and undergraduate/M.S. thesis mentor, guiding others in biomimetic synthesis and mineralization protocols. I also actively engaged with the scientific community by presenting my work at conferences, including the Gordon Research Conferences (GRC) on Biomineralization and Crystal Growth and Assembly. These experiences expanded my network and reinforced my commitment to biomimetic materials research. While my graduate work was not explicitly clinically related, it laid groundwork for regenerative medicine and tissue engineering applications. My publications demonstrated how organic matrix processing influences biological interactions, which I now plan to extend to oral microbiome and dental biomaterials in my current work.

During my postdoctoral training at the ADA Forsyth Institute, I applied my expertise in biomimetic materials to the study of mineralized tooth structures under the co-mentorship of Dr. Felicitas Bidlack, a leader in enamel research, and Dr. Jirun Sun, a pioneer in the dental materials field. My initial research investigated the dentin-enamel junction (DEJ), where I combined electron microscopy, micro-CT, and nanoindentation to elucidate sex-specific variations in mineral density, microstructure, and mechanical properties. This work revealed correlations between organic matrix development and tissue-level biomechanics, directly building on my graduate training while expanding my toolkit for biomineral characterization. During this time, I also gained experience in 3D bioprinting, designing a custom platform from a commercial bioprinter to engineer bioinks with *in situ* crosslinking capabilities. This innovation enhanced material stability for applications in bone regeneration and oral tissue models, aligning with my long-term goal of developing functional biomaterials for clinical translation.

In preparing for my transition to independent research, I have acquired new skills in bacterial culture, confocal microscopy, and oral microbiology to prepare for the study of biofilm-mediated diseases. Through literature review and collaborations with microbiologists and immunologists, I identified a critical gap at the intersection of microbial ecology and biomaterials that my materials science perspective can address. Over the past year, I have leveraged this outstanding institutional support from ADA Forsyth and the dedicated mentorship from Drs. Sun, He, and Bidlack to act toward my research goals. This effort has been highly productive, yielding substantial preliminary data and resulting in the **submission of three publications and presentations at international conferences**.

This K25 award is a significant next step in fully integrating my materials background with expertise in the discipline of microbiology. The protected time and structured training plan will allow me to gain advanced knowledge in more complex biofilm analyses and bacteria-material interactions. This guided mentorship is essential to transition my proven productivity into an independent research program uniquely positioned to analyze the spatial dynamics of infectious diseases.

Career Goals and Objectives:

My overall career goal is to become an independent researcher and senior scientist at a leading research institution, using my knowledge and expertise to advance methods and applications of biomimetic research toward the improvement of public health. My training and research in materials science has contributed greatly to this goal, as my studies have exposed me to the overall advantages of biomimetic materials and bioinspired structures for applications in biological research. The research I have carried out at ADA Forsyth has helped

me recognize a need for novel biomimetic materials and techniques for applications in oral disease modeling, treatment, and prevention, specifically in oral spaces with complex 3D architecture such as periodontal spaces and gingival tissues, where improved models or materials for repair may be crucial in improving oral health. Through the development of my existing materials research skills and study of biological research concepts, I aim to combine my expertise and acquired knowledge to advance the field of biomimetic materials for the understanding of complex microbial interactions and treatment of diseases.

Over this five-year K25 award period, my short-term goals are to:

1. Focus on the development of biomimetic material systems for the modeling of oral tissues, 3D structures, and interfaces.
2. Learn techniques for bacterial culture, manipulation, and imaging to assess bacterial behavior in 2D and 3D systems.
3. Generate data and gain understanding of cell and bacterial interactions in caries environments.
4. Continue to develop my scientific communication and presentation skills, prepare publications, and present my research at national and international conferences biannually.

By the end of year 3 of this award period, I plan to develop and submit a successful R01 grant based on compelling data generated from the use of 3D bioprinting to model bacterial interactions and responses in *in vitro* environments. The ADA Forsyth Institute provides an ideal environment to achieve these goals, providing an environment with renowned experts in mineralized tissue biology, immunology, and microbiology, as well as a variety of training and collaboration opportunities to improve my skills while also helping to advance the field of oral health.

Candidate's Plan for Career Development/Training Activities During Award Period:

My career development aims and training goals, outlined below, are designed to fill the gaps in my current knowledge of bioengineering and microbiology. This plan will include regular meetings with my mentoring team and other experts in microbiology at ADA Forsyth, formal education through courses offered by ADA Forsyth and Harvard University, additional education and training in bioimaging methodology for analysis through meetings and seminars during completion of the scientific aims, and research-based networking and presentations at scientific conferences.

1. Mentoring Team:

My mentors and supporting group have been carefully chosen based on their knowledge of my current potential and skills, expertise in areas in biological research that I plan to develop in, and willingness and ability to regularly meet and discuss scientific progress, project aims, and career goals. My primary mentor will be Dr. Jirun Sun, a leader in the field of dental materials and translational research, with a proven record of publications, patents, and multidisciplinary NIH-funded research as a PI. Dr. Sun has established strengths and leadership in both industry and academia, and his use of his materials science background toward applications for novel tissue engineering materials and cutting-edge dental research provides a strong blueprint for my own career path and acquisition of multidisciplinary skills. I will continue the strong mentorship pattern and plans that Dr. Sun and I have already managed to establish in my time at ADA Forsyth through weekly one-on-one meetings where we will discuss research projects, methods and career plans based on my progression through the aims of the proposed project. I will also continue to participate in bi-weekly meetings with the Sun lab research group, engaging in presentations and discussions of multidisciplinary research with group members who have expertise in engineering and dental clinical methods and practices.

Dr. Xuesong He will be my co-mentor, adding to the mentorship provided with his established record in dental microbiology and its variety of applications and impacts for human health. Dr. He is also a leader in physiological analysis regarding pathogens and microbes related to oral diseases. His work isolating and analyzing TM7x from the oral cavity has helped to advance knowledge and understanding of parasitic interactions and infections in oral tissue, as evidenced by an extensive record of well-referenced and cited publications and multiple NIH R grants. As a trained dentist, Dr. He brings essential clinical and translational expertise, which is valuable in helping to effectively bridge *in vitro* materials research and microbiology innovations with real-world oral health applications. Through biweekly meetings with Dr. He, I will continue to gain knowledge and training in methods of microbial manipulation and analysis relevant to my proposed projects and research aims. I will also continue to participate and present in biweekly joint group meetings with

the research groups of Dr. Sun and Dr. He, along with the groups of Dr. Batblieg Bor and Dr. Mary Ellen Davey, experts in the study of pathogens and understanding of oral diseases. These regular meetings with a multidisciplinary team of faculty, postdoctoral researchers and other leaders in oral research will assist greatly in expanding my knowledge of various concepts of oral biological research, and I will be able to acquire valuable feedback and knowledge to implement into my own projects and progress.

Dr. Felicitas Bidlack continues to be a personal and professional mentor and will serve as an additional scientific advisor on my mentoring team to assist with my proposed research and career development. Dr. Bidlack is an expert in mineralized tissue research and tooth development, and her research on the formation, structure, and properties of tooth enamel is highly relevant to my proposed research regarding the emulation of tooth structures and microenvironments. Dr. Bidlack also possesses extensive experience in electron microscopy and advanced imaging methods, providing a valuable resource to bridge my current experience and knowledge to bioimaging and analysis of biologically relevant tissue structures. Since joining ADA Forsyth, I have already been able to carry out valuable research with Dr. Bidlack and the Bidlack lab, resulting in a publication on enamel and dentin-enamel junction development, properties and defects. I will build on my experiences and the knowledge I have developed through bi-weekly meetings with Dr. Bidlack to discuss my projects and analysis methods, as well as weekly meetings with the Bidlack lab, where I can gain additional experience and knowledge from other researchers in the group on tooth formation and properties.

Table 1: Mentoring Group, Meetings, and Expertise

Meeting / Group	Frequency	Role and Expertise
Jirun Sun, PhD	Weekly – 1 hour	Primary Mentor: Materials science, bioengineering, translational research and career mentoring
Xuesong He, DDS, PhD	Biweekly – 1 hour	Secondary Mentor: Microbiology concepts and methods, oral microbe interactions
Felicitas B. Bidlack, PhD	Biweekly – 0.5 hour	Internal Advisor: Advanced Microscopy, bioimaging and career mentoring.
Sun Lab Meetings	Biweekly – 1.5 hours	Presentation and discussion of research with materials focus
Microbiology Group Meetings	Biweekly – 2 hours	Presentation and discussion of research with a microbiology focus
Bidlack Lab Meetings	Weekly – 2 hours	Discussion of analysis methods and additional feedback

2. Training in Biological Study and Bioengineering:

Goal 1: Gain proficiency in advanced hydrogel bioprinting and imaging techniques. Through my PhD and postdoctoral training, I have developed expertise in biomaterial processing and 3D bioprinting, with a focus on designing multiscale architectures for biological applications such as biomineralization. As part of Aim 1, I will build on this foundation by pursuing structured training in biomaterial fabrication, microbial bioprinting, and advanced imaging techniques critical for bridging materials science with microbial studies. Under the mentorship of Dr. Sun, I will enhance our bioprinting system with specialized modules (e.g., cell mixers, coaxial nozzles) to enable precise bacterial manipulation. Concurrently, I will work closely with Dr. He to complete targeted coursework in microbiology (Year 1) to optimize printed architectures for bacterial viability and function. To rigorously evaluate bacteria-material interactions, I will receive hands-on training in the use of additional dyes and preparation for live-cell imaging (Advanced Microscopy Core) for dynamic biofilm analysis, and biomaterial and microbe preparation and imaging methods for high-resolution electron microscopy (Dr. Bidlack, Harvard's Center for Nanoscale Systems) for ultrastructural characterization. This integrated skill set will directly inform my subsequent aims, ensuring that my biomaterial platforms are both functionally and biologically relevant.

Goal 2: Develop experience in real-time and endpoint analysis of bacterial function and behavior. To achieve the objectives of Aims 2 and 3, I will leverage the world-class microbiology resources at ADA Forsyth, including expertise in microbial biogeography and cutting-edge *in situ* imaging platforms. These resources are critical for elucidating bacterial function and behavior at both **community and single-cell resolution**. My structured training will include hands-on training in fluorescent protein (FP) tagging and fluorescent *in situ* hybridization (FISH) through the Advanced Imaging Core and Microbiology Group in years 3-5, enabling high-resolution spatial and temporal analysis of bacteria-material and interspecies interactions. Under the guidance of Dr. He, I will also master bacterial transcriptomics to complement our real-time spatiotemporal models with

endpoint molecular validation. To enhance my quantitative analysis of bacterial behavior and function, I will receive formal training in biostatistics (via the Biostatistics Core) and advanced application of R and SAS (Years 3–4) to rigorously analyze complex microbial datasets.

Goal 3: Develop professional and leadership skills for continued interdisciplinary collaboration.

Leveraging the exceptional training environment and collaborative networks available through ADA Forsyth, the Forsyth Dental Clinic, and Harvard School of Dental Medicine, I will pursue a structured professional development plan to transition toward research independence. I will engage in internal skill-building by delivering quarterly research presentations to the Forsyth Microbiology Group to refine my scientific communication skills and receive critical feedback from leaders in the field, while fostering new interdisciplinary collaborations. This will extend to external engagement by attending key Gordon Research Conferences on Additive Manufacturing and Bacterial Cell Development during Years 3-4 to disseminate my findings to top experts and identify critical collaborations. I will synthesize these insights to develop and submit a competitive R01 proposal by the end of Year 4, positioning me for a successful transition to an independent investigator role.

3. Professional Responsibilities and Activities:

During the award period, I also plan to take advantage of a variety of resources offered through the ADA Forsyth Institute, the Broad Institute of MIT and Harvard, and other seminars and course offerings from Forsyth collaborators. These courses in a variety of scientific and professional development topics will assist in the completion of compelling publications and presentations for my research, as well as developing leadership and mentoring skills for transition into independent research. I will also attend and present at multiple conferences each year to build a strong network of peers and colleagues in oral and biological research.

a. Scientific Meetings and Conferences: Attending and presenting at national and international conferences related to my field of study has helped greatly with networking, presentation skills, and introduction to new knowledge, methods, and opportunities for collaboration. I plan to continue developing in these areas by attending at least two conferences yearly relating to my research. The American Association for Dental, Oral, and Craniofacial Research (AADOCR) Annual Meeting and Exhibition is a preferred yearly conference as it gathers top researchers and clinicians from across the nation and covers a wide range of oral research topics, including oral microbiology and novel dental materials. The Materials Research Society (MRS) Meeting & Exhibit, and Gordon Research Conferences (GRC) on Additive Manufacturing of Soft Materials, Bioinspired Materials, and Biomaterials and Tissue Engineering are other conferences that I plan on attending during this grant period. The GRC meetings are also significant because of their associated GRS Seminars, which focus on centering doctoral and post-doctoral researchers, giving unique opportunities to form peer groups and interact with mentors and trainees in smaller settings.

b. Manuscripts and Publications: In conjunction with the presentation of my research at conferences and meetings, I plan to complete at least two first-author publications per year, on either the findings and conclusions from my projects or the novel methods established through progression of this study.

c. Science Communication: I have taken a 7-week science communication course through ADA Forsyth, taught by Dr. Dennis Mangan, Director of the Chalk Talk Science Project. This course has helped greatly in developing and refreshing oral presentation skills, and I plan to take additional courses on communication and presentation skills in the future. ADA Forsyth also offers a variety of learning opportunities and courses each quarter, including courses on presentation and leadership skills that I will continue to take advantage of as part of my development. I will also use other internal resources offered by ADA Forsyth, including weekly hour-long brainstorming sessions and trainee seminars for discussion of research progress and feedback, which are attended by ADA Forsyth faculty, clinicians, and other researchers, and I plan to give annual presentations and utilize the breadth of experience and knowledge that ADA Forsyth members possess.

d. Responsible Conduct of Research: ADA Forsyth offers an annual 10-week Responsible Conduct of Research course taught by Forsyth faculty. This in-person course covers topics of safe research environments, collaborative research and data acquisition, and I will attend this course in the coming year in conjunction with the responsible conduct of research course offered through CITI that I have completed this year to gain additional instruction.

Table 2: Timeline of Training Goals

Milestones:	Pre-Award	Y1	Y2	Y3	Y4	Y5
Aim 1: To optimize architectural control of 3D bacterial hydrogels via bioprinting.						
<u>Didactic Coursework</u>						
BIOS E-1AX: Introduction to Molecular and Cellular Biology		✓				
BIOS E-10: Introduction to Biochemistry		✓				
Science Communication	✓					
Responsible Conduct of Research	✓					
<u>Laboratory and Experimental Training</u>						
Bacterial culture: Successfully culture multiple <i>Streptococcus spp.</i> in bulk and printed hydrogel patterns	✓	✓				
Bacterial Imaging: Completion of <i>S. mutans</i> live imaging experiments tracking chemical gradients		✓				
Biomaterials: Proficiency in 3D bioprinting methods (coaxial extrusion, inkjet bioprinting)			✓	✓		
Aim 2: To evaluate spatiotemporal antimicrobial performance in 3D bacterial microenvironments.						
<u>Didactic Coursework</u>						
BIOS E-240: Biochemical and Physiological Adaptation of Microbes			✓			
BIOS E-12: Principles and Techniques of Molecular Biology			✓			
<u>Laboratory and Experimental Training</u>						
Microbiology: Proficiency in conventional antimicrobial testing against <i>S. mutans</i> (inhibition zone, broth dilution)		✓				
Biomaterials: Manipulate acrylamide and gelatin pore architecture to tune Azo-QPS-MSN release			✓			
Biomaterials: Proficiency in alternative hydrogel pore generation techniques				✓	✓	
Biostatistics: Successful transcriptome analysis and statistical analysis of <i>S. mutans</i> assays					✓	✓
Aim 3: To evaluate the efficacy of metabolism-triggered antimicrobial activity in a dual-species 3D system.						
<u>Didactic Coursework</u>						
BIOS E-155: Medical Microbiology			✓			
<u>Laboratory and Experimental Training</u>						
Bacterial Culture: Successfully culture <i>R. dentocariosa</i> and maintain multi-species cultures				✓		
Bacterial Imaging: Proficiency in Fluorescent Protein and FISH imaging methods for multi-species and biofilm evaluation					✓	✓
<u>Dissemination and Grant Preparation</u>						
Dissemination of Results		✓	✓	✓	✓	✓
R01 Grant Preparation and Submission				✓	✓	
<u>Seminars and Meetings</u>						
Forsyth Brainstorming and Weekly Seminars, Weekly Lab Group Meetings, Broad Institute Seminars, Harvard Catalyst Courses						
<u>Conferences</u>						
American Association for Dental, Oral, and Craniofacial Research, Materials Research Society, Gordon Research Conferences, Gordon Research Seminars						

Specific Aims: My long-term goal is to advance the study of infectious disease by creating precise models of host-microbe-drug interactions. The goal of my proposed research is to establish a platform that **integrates three-dimensional (3D) bacterial positioning with spatiotemporal imaging** and analysis to evaluate bacterial activity and antimicrobial efficacy in single- and dual-species oral biofilm models. To accomplish this, I plan to integrate ratiometric fluorescence imaging with 3D bioprinting techniques, enabling real-time, single-cell-resolution analysis of bacterial dynamics and antimicrobial actions. This combination facilitates continuous monitoring of pH gradients, bacterial viability, and drug performance, offering insights into the spatial and temporal aspects of antimicrobial effectiveness that traditional methods cannot provide. Building upon our laboratory's development of 3D bacteria-laden hydrogels, I will assess the spatiotemporal performance of pH-responsive antimicrobial drug delivery systems, which have demonstrated metabolism-triggered inhibition of cariogenic bacteria such as *Streptococcus mutans* (*S. mutans*). Further investigations will focus on evaluating the selective inhibition capabilities of these stimuli-responsive drug delivery systems within an experimental dual-species environment. I will achieve my goal with three specific aims:

Aim 1: To optimize architectural control of 3D bacterial hydrogels via bioprinting. Objective: Develop and optimize hydrogel-based culture platforms that allow precise spatial control of bacteria by manipulating hydrogel properties and 3D architecture via 3D bioprinting. At the ADA Forsyth Institute, I established a 3D acrylamide-based hydrogel system that sustains *S. mutans* viability (>70%) and demonstrated that pore size directly influences bacterial proliferation, distribution, and acid production. By integrating this hydrogel with in-situ curing bioprinting (ISCB), I achieved multi-scale control over hydrogel architecture and bacterial positioning. Building on these promising initial results, I will engineer 3D hydrogels with defined pore sizes, architecture, and stiffness, and characterize them using SEM and rheology to correlate material properties with bacterial behavior. I will then quantify bacterial metabolism (e.g., lactic acid production) and biofilm dynamics through 3D confocal imaging, viability assays, and spatially resolved pH mapping using ratiometric imaging. This aim will establish a tunable *in vitro* platform to reveal how spatial confinement and microenvironment architecture influence cariogenic biofilms. This work will inform spatiotemporal studies and dual-species oral models proposed in Aims 2 and 3.

Aim 2: To evaluate spatiotemporal antimicrobial performance in 3D bacterial microenvironments. Objective: Evaluate the spatiotemporal interplay between antimicrobial release, bacterial response, and pH modulation using pH-responsive delivery systems. Traditional antimicrobial assays lack the spatial precision and dynamic context needed to assess spatiotemporal performance. By leveraging our 3D hydrogel platform, we will precisely monitor antimicrobial release and bacterial response within tunable architectures. Our preliminary work shows that the pH-responsive nanocarrier Azo-QPS-MSN delivers chlorhexidine selectively under acidic, acid-producing conditions—killing *S. mutans* as confirmed via live–dead fluorescence imaging and ratiometric pH mapping by confocal microscopy. Building on this foundation, I will systematically vary hydrogel pore architecture, Azo-QPS-MSN concentration, and chlorhexidine loading in *S. mutans* single-species models. Real-time pH mapping will quantify how drug release correlates with bacterial metabolism, while 3D confocal imaging will determine killing efficiency relative to biofilm microarchitecture. This aim will elucidate the spatiotemporal dynamics of pH-triggered antimicrobial delivery, laying the groundwork for dual-species selectivity studies in Aim 3.

Aim 3: To evaluate the efficacy of metabolism-triggered antimicrobial activity in a dual-species 3D system. Objective: Assess selective elimination of *S. mutans* within a dual-species system. To enhance complexity, I will introduce commensal bacterial species alongside *S. mutans* to promote cooperation or competition in our hydrogel platform. Spatial arrangement will be controlled using pore confinement and 3D-printed architectures. I hypothesize that chlorhexidine-loaded Azo-QPS-MSN will preferentially kill cariogenic *S. mutans* under acidic conditions while sparing commensals due to its metabolism-triggered release profile. I will quantify species-specific growth and spatial distribution via fluorescent labeling, and map acid gradients across micro- and macro-scale biofilms throughout developmental stages. These analyses will elucidate how spatial patterning and microenvironmental architecture influence microbial dynamics and underpin targeted treatments for cariogenic communities.

Successful completion of these aims will deepen our understanding of the selective inhibition of cariogenic bacteria by acid-responsive antimicrobials. This research will also establish advanced 3D *in vitro* models for probing bacterial activity and antimicrobial efficacy at the cellular level, critical for investigating complex spatiotemporal bacterial interactions. These platforms will serve as a foundational framework for future studies involving dual-species human oral microbiome models. Ultimately, the data generated will directly support the preparation and submission of a competitive R-series grant focused on innovative antimicrobial strategies.

A. Significance:

Infectious diseases remain a significant public health and economic burden ¹⁻³, necessitating research into more effective treatment efforts and precise therapies to mitigate threats of antimicrobial resistance ^{4,5}. Within this effort, precision antimicrobial strategies have represented a critical advantage in combating infectious diseases, offering a powerful alternative to broad-spectrum approaches that may exacerbate antimicrobial resistance and disrupt protective host microbiota ⁶. Tailored treatments for infectious diseases, which have thus far been enabled by advancements in genomic analyses ⁷, also require improved understanding of pathogen behaviors and the specialized environments they inhabit ^{8,9}, which are still lacking in some conventional *in vitro* models of diseases ¹⁰⁻¹³. My long-term goal is to bridge this gap and advance precision medicine for infectious diseases by developing platforms that precisely manipulate microbial communities and drug interactions. The objective of this proposal is to directly advance that goal by establishing a tunable, 3D hydrogel-based model to dissect the pathogenesis of and treatment strategies for oral biofilm-mediated diseases, using dental caries as a primary model.

Conventional antimicrobial testing, often conducted alongside traditional therapies, typically relies on planktonic or two-dimensional cultures. These simplified models limit the ability to advance novel therapies with increased precision, as they often fail to capture critical aspects of biofilm biology ^{14,15} such as spatial confinement ¹⁶, temporal development ¹⁷, localized pH gradients ¹⁸⁻²⁰, and interspecies interactions ²¹⁻²⁵. Additionally, these culture environments do not adequately replicate tissue porosity or structural changes caused by decay, both of which influence biofilm architecture and treatment response ^{18,26}. As a result, these models provide limited insight into targeted intervention strategies. The need for tunable, precise culture and imaging platforms to study spatiotemporal biofilm dynamics and antimicrobial efficacy represents a major gap in oral health research, one that has become increasingly apparent through my investigations into caries treatment. Addressing this need motivates my pursuit of advanced biomaterials to better understand biofilm-microenvironment interactions and to accelerate the development of precision therapies.

To address this critical gap, I propose a novel approach using 3D-bioprinted hydrogel models that enable precise control over bacterial spatial organization, biofilm architecture, and microenvironmental conditions. This platform uniquely integrates advanced material characterization with single-cell-level bacterial analysis to quantify how hydrogel microarchitecture and chemical gradients influence biofilm formation and behavior. I will employ 3D confocal imaging combined with ratiometric fluorescence techniques to track the spatial dynamics of bacterial aggregation, acid production, and antimicrobial penetration in real time at cellular resolution. Together, these capabilities will establish a powerful platform for high-resolution analysis of caries-associated biofilm dynamics, and the evaluation of metabolism-targeted antimicrobials under tunable single- and multi-species conditions. By bridging the gap between conventional *in vitro* models ²⁷⁻³⁰ and advances in biomaterials engineering, this work will support the development of precision therapies that selectively disrupt cariogenic communities while preserving the healthy oral microbiome.

The proposed research plan builds on the skills and insights I gained while working on my mentors' R01 grant (DE029479A; Co-PIs: Drs. He and Sun). Through the K25 award, I will integrate my materials science expertise developed during my Ph.D. and postdoctoral training with focused microbiology instruction, which will substantially strengthen my foundation for establishing an independent academic research program.

B. Innovation:

Successful completion of the proposed research will create a transformative platform that combines tunable hydrogel architecture and 3D patterning to **precisely control bacterial spatial organization**, biofilm architecture, and microenvironmental conditions. By manipulating hydrogel properties (e.g., pore size and chemical composition) and using advanced analysis tools including high-resolution 3D confocal imaging, ratiometric labeling techniques, and electron microscopy, I can directly correlate material parameters with bacterial behavior, including proliferation dynamics, acid production, and interspecies interactions, at single-cell resolution. This platform overcomes critical limitations of conventional cultures by replicating the confined, heterogeneous microenvironments that drive biofilm virulence in caries.

I will integrate this platform with a newly developed stimuli-responsive drug delivery system, Azo-QPS-MSN, which enhances the functionality of conventional broad-spectrum antimicrobials, such as chlorhexidine, by enabling metabolism-triggered antimicrobial activity (MTAA). Azo-QPS-MSNs are mesoporous silica nanoparticles (MSNs) functionalized with Azo-QPS, a pH-responsive, azo derivative of quaternary pyridinium salt (QPS) compounds developed in Dr. Sun's laboratory (U.S. Patents: 11,104,647 and 10,836,726) ^{31,32}. Using the proposed 3D hydrogel platform, I have demonstrated that MTAA can be achieved with chlorhexidine-loaded Azo-QPS-MSNs (Azo-QPS-MSN + CHX) (Section C.2.1). This system allows for the investigation of spatiotemporal drug release and bacterial response dynamics, which cannot be captured using traditional planktonic models or 2D substrates. The proposed research will leverage our tunable 3D hydrogel system, uniquely capable of real-time, high-resolution monitoring within defined micro- and macro-architectures, to reveal spatiotemporal dynamics of antimicrobial activity in both mono- and multi-species biofilms.

A key innovation of this project is the extension of our platform to *dual-species analyses*, evaluating selective inhibition of cariogenic bacteria within complex communities. By leveraging the platform's ability to control bacterial spatial organization, pH gradients, and drug diffusion, I will investigate if MTAA strategies can spare commensal species while targeting acid-producing pathogens, a critical step toward informing precision antimicrobial therapies. This work will help bridge gaps between biomaterials design and translational dentistry, while further developing my expertise in microbial ecology, 3D imaging, and antimicrobial delivery systems.

C. Approach:

Throughout this proposal, we will take advantage of our expertise in materials development and microbiology to test the hypotheses. Methods, reagents, and protocols to be used are established in Drs He and Sun's labs, and the results obtained from their uses are shown in our preliminary results and throughout this section. Due to space limitations, a general description of methods is mostly given below. **For scientific reproducibility, rigor, and biological variables**, all studies will be repeated in triplicate. **Power analysis** (Power = 0.80, relative effect size 0.75, and $\alpha = 0.05$ for a two-sided test) will determine the number of samples needed for reliable statistical judgment. **Statistical significance** will be calculated by the two-sided Student's t-test (p-value < 0.01) after validation of the sample distribution by the Shapiro-Wilk normality test³³. The use of proper controls is always carefully described in our research articles.

C.1 - Aim 1: To optimize architectural control of 3D bacterial hydrogels via bioprinting.

Objective: Develop and optimize hydrogel-based culture platforms that allow precise spatial control of bacteria by manipulating hydrogel properties and 3D architecture via 3D bioprinting.

Hypothesis: 3D hydrogel architectures will enable precise control of *S. mutans* spatial organization and metabolism through tunable pore geometries, with lattice patterns promoting distinct bacterial aggregation and pH gradients compared to bulk gels.

Rationale: This Aim is grounded in our preliminary data: we have established a hydrogel platform in which *S. mutans* proliferation and aggregate formation can be tuned via modification of hydrogel microstructure and architecture (Section C.1.1). Because biofilm formation in the oral cavity is strongly influenced both by bacterial aggregation and by the three-dimensional geometry of the surrounding environment, we have developed a tunable 3-D hydrogel platform that mimics the physiological constraints of structural niches³⁴ such as the tubule network of dentin. This approach mimics the spatial confinement³⁵ and aggregated environments bacteria utilize to initiate biofilms and penetrate intra-tubular spaces^{36,37}, offering a more relevant model than traditional 2-D culture systems. We have shown that altering parameters such as pore size and spatial confinement impacted bacterial clustering and aggregate growth (Section C.1.1). By leveraging tunable hydrogel bio-inks together with 3D bioprinting, we can precisely manipulate spatial confinement and microstructure in three dimensions. Coupled with advanced nanotechnology and stimuli-responsive drug-release tools, this approach will enable the construction of complex, multi-scale structures spanning from single-cell arrays to polymicrobial community networks embedded within defined architectures^{38,39}. In doing so, this integrated hydrogel/bioprinting/drug-release approach will enable us to (i) directly probe how structural confinement and micro-environmental architecture influence bacterial aggregation and biofilm initiation; (ii) model dual-species colonization dynamics under controlled 3D architectural conditions; and (iii) test interventions (e.g., targeted drug release) in physiologically relevant 3D microenvironments of confinement. Together, these capabilities create a powerful platform that positions us to address fundamental questions about oral biofilm initiation and progression and to develop novel strategies for inhibition or modulation of biofilm formation in clinically relevant settings.

C.1.1 - Preliminary Data: 3D Hydrogel Platform for *S. mutans* Viability, Spatial & pH Mapping:

We have established a tunable and reproducible hydrogel platform to investigate the growth dynamics and spatial organization of *S. mutans*. *S. mutans* strain UA140, a widely used strain found in the oral cavity and caries environments, was chosen for analysis in this preliminary research and for all proposed *S. mutans* analyses. This cost-effective system employs an acrylamide-based hydrogel composed of poly(acrylamide-co-sodium acrylate), in which microstructural parameters such as pore size can be tailored by varying the acrylamide mass fraction and sodium acrylate concentration^{40,41}. Both expanding (200–300% volumetric swelling after 24 hours in aqueous media) and non-expanding hydrogel formulations were utilized to examine bacterial spatial behavior. Brain Heart Infusion (BHI) medium was incorporated into the hydrogel matrix to support *S. mutans* proliferation. *S. mutans* were embedded within the hydrogel precursor solution, crosslinked in custom-fabricated molds to ensure consistent bulk geometry, and incubated in BHI for either 1 or 24 hours. Post-

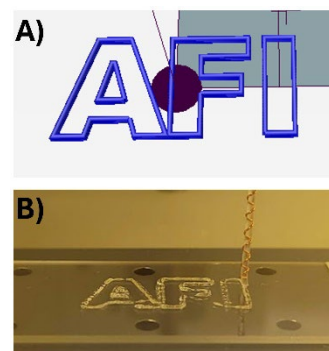


Figure 1. A) Programmed design of "AFI" logo and B) printed acrylamide gel filaments following the design for demonstration of spatial patterning and control.

incubation, bacterial viability and distribution were assessed using live/dead and pH assays. The hydrogel platform is also compatible with a modified bioprinter that enables *in situ* crosslinking⁴², facilitating the fabrication of stable, spatially patterned macrostructures (Figure 1).

Our experimental design uses parallel imaging workflows to interrogate *S. mutans* viability, spatial localization, and local microenvironmental pH using fluorescent staining and confocal microscopy. Samples are initially incubated with the ratiometric pH indicator C-SNARF-4, which enables quantitative pH mapping by virtue of its dual emission peaks (excitation 488 nm; emission maxima at ~580 and ~640 nm)⁴³. Calibration was performed using pH-adjusted buffer standards spanning pH 4.2 to 7.4. Thereafter, samples were counterstained with DAPI (a nucleic acid stain) and SYTOX Green (a dead-cell indicator), permitting co-registered imaging of pH and bacterial viability in identical fields of view. In our hydrogel systems, *S. mutans* viability remains high (> 70 %) over 48 h. Supplementation with 4 % sucrose promotes bacterial aggregation between 1 h and 24 h, consistent with active proliferation within the gel and correlated with microenvironmental pH heterogeneity (Figure 2a, 2b). Following confocal imaging, hydrogels were fixed, sectioned, and examined by scanning electron microscopy (SEM), which reveals distinct microstructural features. Image analysis confirms the ability to replicate confinement of dentinal microenvironments, revealing hydrogel pore architectures of 1–5 μm that effectively mimic the spatial confinement of dentin tubules. Ultrastructural analysis also demonstrates the platform's customizability, confirming sucrose-induced cultures in expanding gels exhibit larger bacterial aggregates and enhanced extracellular polymeric substance depositions compared to non-induced controls (Figure 2c). Together, these data confirm that (1) hydrogel porosity can be modulated via precursor composition, mimicking the size and diameter of dentinal tubules (1-5 μm), and (2) *S. mutans* develops metabolically active, matrix-enclosed aggregates under permissive conditions, validating our 3D hydrogel platform as a tractable model for studying biofilm-like growth in well-controlled environments.

C.1.2: Design and Patterning of *S. mutans*-laden 3D Bioinks.

Fabrication of bioinks: Based on the success of our preliminary work, we will control the microarchitecture of *S. mutans*-laden hydrogels by adjusting average pore size and cross-linker concentration to simulate degrees of spatial confinement reflective of oral microenvironments. Using a baseline formulation of 5 wt% acrylamide and 0.2 wt% N,N'-methylenebis(acrylamide) (bis) with 0.02 wt% lithium phenyl(2,4,6-trimethylbenzoyl) phosphinate (LAP) in BHI medium, we will vary the acrylamide mass fraction and the bis:acrylamide ratio to tune pore size and network architecture. Prior to incorporation into the hydrogel precursor, *S. mutans* UA140 will be cultured in BHI for 48 h at 37 °C, concentrated by centrifugation, and resuspended in fresh BHI; the bacterial suspension will be quantified by optical density at 600 nm (OD_{600}) and adjusted to an OD_{600} of 3.0 as the initial concentration in the bioinks. **Bioprinting with in-situ crosslinking:** Acrylamide gel suspensions with/without bacteria will be loaded into syringes with a light-permeable silicone nozzle, 3 cm in length and 0.5 mm in diameter, to allow for in-situ crosslinking of bioinks. Bioprinting will be performed on a Cellink BIOX printer. An OmniCure S1500 spot UV curing lamp (wavelength > 400 nm) will be used for photo crosslinking of the bioinks during extrusion, with the light focused on the light-permeable portion of the printing nozzle using a collimating lens.

Design and control of bio-printed 3D architecture: To investigate how multiscale hydrogel architecture influences bacterial dynamics, we will engineer both microscale pore size (modulated via cross-linking density; see Figure 3a) and macroscale structural patterns (via extrusion-based bioprinting). Using our in-situ bioprinting system, we will fabricate two distinct pattern types:

Pattern 1 – Alternating filament arrays: Linear arrays of parallel filaments (each 5 mm in length) comprising alternating *S. mutans*-laden and drug-laden filaments, with inter-filament spacings of 0 μm (co-printed *S. mutans* and drug), 500 μm (one filament diameter) and 1000 μm (two filament diameters) (Figure 3b). **Pattern 2 – Two-Layer Lattice:** A 1 × 1 cm² structure composed of two stacked layers of the linear arrays from Pattern 1 (500 μm spacing), with the second layer aligned at 90° relative to the first to create defined macropores and a broader range of spatial separation between *S. mutans*-laden and drug-laden filaments (Figure 3c).

Aim 1 develops the core bioprinting platform of alternating filaments, Aim 2 integrates pH-responsive antimicrobials (Azo-QPS-MSN+CHX and positive controls) into these filaments to test targeted therapy, and Aim 3 introduces commensal bacteria into the filaments to study interspecies dynamics. The diameter of the

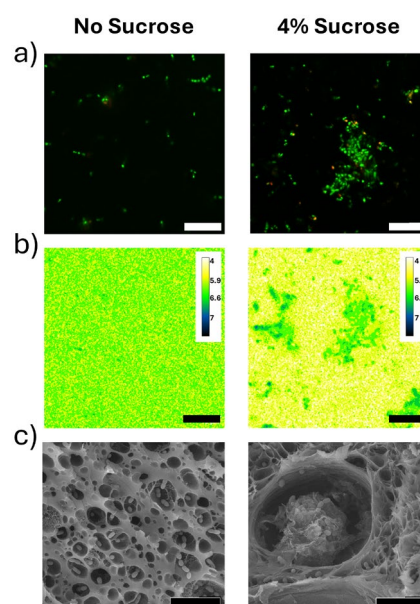


Figure 2. Confocal viability imaging (a) pH maps (b) and SEM images (c) of *S. mutans* cultures in hydrogels after 48h, alone (left) or with 4% sucrose in culture media (right) (all scale bars: 5 μm)

printed hydrogel filaments will be determined by the nozzle size and the swelling behavior of the hydrogels. We will use 500 μm -diameter silicone nozzles and employ two hydrogel formulations with distinct swelling properties: a non-swelling formulation to represent minimal biofilm formation conditions, and a formulation that swells approximately 2x to promote biofilm formation. This design will allow us to systematically assess the impact of microporous and macro-spatial architecture on bacterial aggregation and biofilm development.

C.1.3: Assessment of architecture-bacterial behavior relationships.

Triplicate cultures of hydrogel patterns will be incubated for 1, 12, 24, and 48 hours to assess bacterial proliferation, viability, spatial distribution, and local pH both within the hydrogels and in the surrounding medium. At the end of each incubation period, hydrogel patterns will be stained with 50 μM C-SNARF-4 after a 30-minute incubation in culture medium and imaged using a Zeiss LSM 980 confocal microscope at the ADA Forsyth Advanced Microscopy Core. Single images and confocal z-stacks will be collected, and simulated three-dimensional images will be generated using Zeiss Zen software. Ratiometric pH quantification will be performed using dual-emission images according to established protocols for bacterial biofilms⁴⁴ and analyzed in ImageJ, calibrated against standards spanning pH 4.2 to pH 7.4. Bacterial growth, viability, and biofilm formation will be evaluated within the same imaging regions via live/dead staining using DAPI to label all cells and SYTOX Green to identify dead cells. Viability percentages will be calculated from cell counts across at least five representative fields per sample. For real-time pH monitoring, initial data from the predefined time points will first identify the critical window during which the pH drop occurs in sucrose-supplemented *S. mutans* cultures. Once this window is established (e.g., between 12-24h), we will perform continuous live imaging using a microscope-equipped 37°C environmental chamber. During these sessions, ratiometric C-SNARF-4 images will be acquired at 10-minute intervals to precisely resolve the kinetics of the pH transition, enabling time-resolved correlation with subsequent antimicrobial release and bacterial response.

For ultrastructural analysis, hydrogels will be fixed in 10% Neutral Buffered Formalin (24h, 4°C), post-fixed with ruthenium red in cacodylate buffer for 2 hours to enhance contrast and serially dehydrated in ethanol. Critical-point drying will be used to preserve native architecture, followed by Pt-Pd sputter-coating to prevent charging. Samples will be imaged using a Zeiss Gemini 360 SEM (Harvard Center for Nanoscale Systems), with pore size and bacterial positioning quantified from at least 5 cross-sections per condition using Fiji/ImageJ (2.16.0/1.54p).

C.1.4 - Expected outcomes, potential problems, and alternative strategies:

Milestones: 1a) Y1: Bioprinted hydrogel patterns with > 70% viability up to 48 h. 1b) Y2: Live-imaging quantification of pH gradients at different time points in both patterns at < 5 μm resolution

Successful completion of Aim 1 will assist in the development of stronger design principles for Aims 2 and 3, which will sequentially introduce antimicrobial agents and additional bacterial species into the hydrogel patterns. Given our robust preliminary data, we do not anticipate significant challenges in achieving the proposed milestones and overall objectives. In the current imaging approach, pH and bacterial viability are assessed sequentially. As an alternative strategy, we will explore additional dyes and imaging methods, such as the use of alternative live/dead staining methods or GFP-labeled *S. mutans*⁴⁵, which will enable simultaneous evaluation of pH, viability, and proliferation within the same imaging field.

The proposed research utilizes extrusion bioprinting with *in-situ* crosslinking^{42,46} as the primary fabrication method due to its current optimization in our preliminary work and proven post-printing cell viability alongside precise patterning. To ensure the project's success and enhance resolution capabilities, we have also established an alternative strategy using drop-on-demand piezoelectric inkjet bioprinting. By adapting a commercially available inkjet printing system, we can achieve microbial patterning with resolution to the tens of microns⁴⁷, as well as the printing of defined numbers of bacterial droplets to facilitate further single-cell resolution studies of bacterial behavior and multispecies interactions in tissue-like landscapes⁴⁸. The adaptability of our platform and core hydrogel chemistry to multiple patterning techniques ensures that we can achieve our goals with increased rigor.

C.2 - Aim 2: To evaluate spatiotemporal antimicrobial performance in 3D bacterial microenvironments.

Objective: To understand the spatiotemporal interplay between antimicrobial release, bacterial response, and pH modulation in three dimensions using pH-responsive delivery systems.

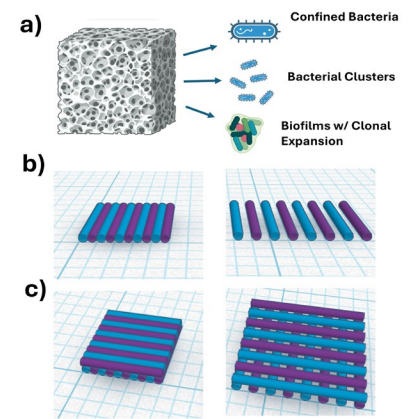


Figure 3: A) Hydrogel pores permitting analysis of confined bacteria, small clusters or large aggregates dependent on pore conditions. B) Pattern 1: Alternating filament arrays, with filaments in contact (left) or spaced at 500 μm (right). C) 2-layer lattice patterns with adjacent (left) or spaced (right) macroporous architectures.

Hypothesis: I hypothesize that the stimuli-responsive drug-delivery vehicle, Azo-QPS-MSN, incorporated into the bioprinted hydrogel patterns will achieve high spatiotemporal specificity of antimicrobial treatment by releasing its payload in response to local triggers, e.g., acidic pH and elevated metabolic activity.

Rationale: This Aim leverages our novel finding of metabolism-triggered antimicrobial activity (MTAA) using the Azo-QPS-MSN + CHX system and our understanding that acidified micro-environments on tooth surfaces exhibit high spatial specificity¹⁸. Specifically, when exposed to sucrose, bacteria activate metabolic pathways and produce acid, thereby triggering the release of CHX from Azo-QPS-MSN + CHX system. In our CHX release kinetic studies, over 90% of CHX was released within 6 h at pH 4.0, whereas less than 1% was released at pH 7.5. Furthermore, stepwise pH-switching experiments demonstrated on-demand control: CHX release ceased immediately when the environment shifted from acidic (pH 4.0) to basic (pH 8.0). In contrast, standard 2-D or planktonic antimicrobial assays fail to capture such localized, stimuli-responsive behavior. Therefore, in this Aim I will combine 3D bioprinting with Azo-QPS-MSN + CHX to enable real-time, spatially resolved assessment of antimicrobial dynamics under physiologically relevant conditions.

C.2.1 - Preliminary Data: Evaluation of MTAA at single-cell level in 3D: MTAA was achieved by delivering CHX, a broad-spectrum antimicrobial, using pH-responsive Azo-QPS-MSNs. CHX was loaded in Azo-QPS-MSNs particles at a mass fraction of 21%. The pH-responsive release of CHX was achieved through a combination of acid-base interactions between the basic CHX and the acidic functional groups on Azo-QPS, along with physical adsorption within the nanopores. The Azo-QPS-MSN+CHX particles were incorporated into *S. mutans*-laden hydrogels and incubated at 37°C. Chlorhexidine gluconate (CHX-G), a traditional oral rinse drug and a salt form of CHX, was used as a positive control and loaded in *S. mutans*-laden hydrogels, while hydrogels without antimicrobials served as the negative control. Specimens were cultured with or without 4% sucrose to distinguish between metabolic states based on carbohydrate consumption, which results in acid production.

After 48 hours, pH, total bacterial count, and viability were sequentially assessed in the same imaging field via confocal microscopy (63X oil immersion), using C-SNARF-4 for pH mapping, DAPI for total cells, and SYTOX Green for dead cells. In the negative controls (Figure 4a), *S. mutans* grew modestly without sucrose, while notable aggregation occurred with sucrose. In both conditions, pH dropped (Table 1), falling below 5.5 in the presence of sucrose—indicative of enhanced metabolic activity. Conversely, the positive control showed low bacterial viability regardless of sucrose presence, with the pH value remaining above 6. Notably, Azo-QPS-MSN+CHX exhibited MTAA behavior: bacterial growth resembled the negative control in the absence of sucrose but resulted in low viability, like the positive control, when sucrose was added. These results establish proof-of-concept for evaluating the spatiotemporal performance of MTAA by Azo-QPS-MSN+CHX in the bioprinted hydrogel architecture proposed in Aims 2 and 3.

C.2.2 - Assessment of spatial specificity of MTAA. We will evaluate the pH-responsive antimicrobial efficacy of Azo-QPS-MSN + CHX in 3D patterns discussed in Aim 1 (Figure 3). Antimicrobials will be introduced through co-printing with *S. mutans* within the same filaments or in alternating filaments as designed in Patterns 1 and 2. Azo-QPS-MSN +CHX particles will be dispersed in hydrogel precursor solutions via sonication bath for 15 minutes to ensure homogeneity before loading onto the 3D printer. The Azo-QPS-MSN+CHX concentration will be systematically optimized to achieve a chlorhexidine (CHX) payload equivalent to a 0.2 wt% (CHX-G) solution. Based on promising preliminary data demonstrating effective bacterial killing with a 1 wt% concentration, this will serve as our initial baseline with subsequent adjustments according to measured antimicrobial performance. We will initially use non-swelling hydrogels to optimize bioprinting protocols and

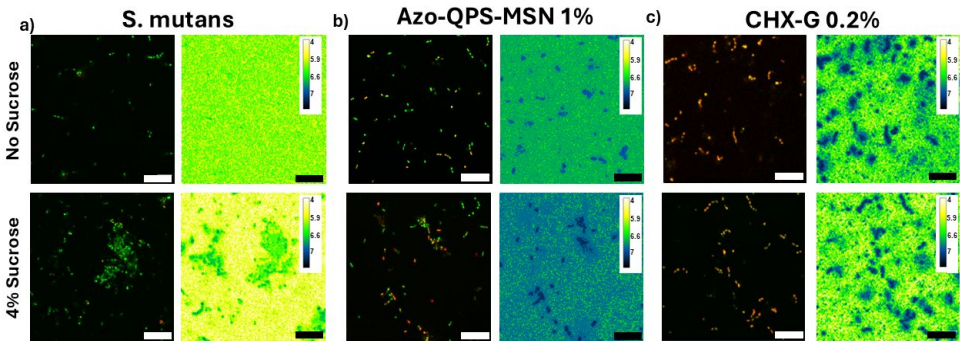


Figure 4. Parallel viability images (left) and pH maps (right) of *S. mutans* hydrogel cultures at 48h alone (a), with added Azo-QPS-MSN + CHX (b) or CHX-G (c) added to cultures, without sucrose (top) or with 4% sucrose (bottom) added to culture media (scale bars: 5µm)

Table 1: pH and viability statistics of hydrogel cultures at 48h

		pH	Viability (%)
<i>S. mutans</i>	No Sucrose	6.9 ± 0.1	84 ± 4
	Sucrose	4.6 ± 0.2	78 ± 5
Azo-QPS-MSN 1% w/v	No Sucrose	7.0 ± 0.1	85 ± 6
	Sucrose	6.9 ± 0.2	12 ± 9
CPC 0.2%	No Sucrose	6.2 ± 0.4	8 ± 5
	Sucrose	6.7 ± 0.4	1 ± 2

establish imaging workflows at multiple time points (1 h, 12 h, 24 h, 48 h). The final hydrogel composition and filament spacing will be determined based on bacterial proliferation and aggregate formation determined in Aim 1, with the goal of selecting formulations that are both optimal for bioprinting and support aggregate formation within 48 hours. Metabolic activity will be stimulated by supplementing BHI with 4% sucrose, with sucrose-free BHI serving as the control.

Spatiotemporal pH dynamics will be monitored in real time using ratiometric C-SNARF-4 imaging, utilizing confocal microscopy to image labeled biological triplicates at the specified time points or via live-imaging under the controlled incubation conditions outlined in C.1.3, while antimicrobial efficacy will be quantified by comparative treatment with Azo-QPS-MSN + CHX (1% w/v) vs. CHX-G (0.2% w/v) as a clinical control, and viability mapping via live/dead staining (SYTOX Green/DAPI) correlated with pre-/post-treatment pH profiles. Diffusion of CHX or CHX-G from hydrogel structures will be quantified through absorbance measurements, using previously obtained calibration curves of CHX and CHX-G in media and hydrogels to correlate drug distribution, release, and bacterial killing to local pH gradients. Post-treatment, hydrogels will be processed for SEM/TEM to resolve ultrastructural relationships between antimicrobial distribution, bacterial clusters, and hydrogel geometry. This approach will define how 3D architectural features and microenvironmental acidity synergistically govern the *targeted release kinetics* and *spatial killing efficiency* of Azo-QPS-MSNs at single-cell-level resolution.

C.2.3 - Expected outcomes, potential problems, and alternative strategies:

Milestones: 2a) Y2: Establish bioprinting protocols of patterns 1 and 2. 2b) Y3-Y4: Establish spatiotemporal correlation of MTAA in Patterns 1 and 2 and identify optimal time points for evaluating MTAA performance to inform Aim 3.

This integrated approach in Aim 2 will elucidate how 3D architectural features and MTAA in microenvironmental synergistically regulate bacterial activities, providing spatiotemporal insights into antimicrobial performance within complex biofilm environments. This will generate new knowledge that cannot be obtained through traditional antimicrobial assays. Supported by strong preliminary results, we expect these studies to advance our understanding of MTAA mechanisms and provide improved feedback for dual-species evaluations in Aim 3.

As an alternative strategy, we will test MTAA activities of (E)-1-hexadecyl-4-((4-(methacryloyloxy) phenyl) diazenyl)-pyridinium bromide (Azo-QPS-C16)⁴⁹, in Patterns 1 and 2. Azo-QPS-C16 has shown selective inhibition of acid-producing bacteria in a multi-species model⁴⁹, providing a promising alternative compound for the evaluation of spatiotemporal efficacy in the single-species models of this Aim as well as the dual-species antimicrobial analyses proposed in Aim 3 (C.3.2).

C.3 - Aim 3: To evaluate the efficacy of metabolism-triggered antimicrobial activity in a dual-species 3D system.

Objective: To achieve selective elimination of *S. mutans* within a dual-species system.

Hypothesis: When integrated into dual-species cultures containing acid-producing *S. mutans* and non-acid producing commensal bacteria, Azo-QPS-MSN+CHX particles will selectively release antimicrobial agents in responding to acid-production by *S. mutans*, enabling targeted killing of pathogenic bacteria while preserving commensal populations.

Rationale: This hypothesis is grounded in our preliminary data demonstrating metabolism-triggered inhibition of *S. mutans* by Azo-QPS-MSN + CHX and established literature documenting spatially heterogeneous pH gradients and pathogen-enriched architectures in multispecies cariogenic biofilms. To test our hypothesis, we will employ our engineered 3D hydrogel platform, capable of real-time, high-resolution monitoring of pH dynamics and bacteria viability, to quantify the spatiotemporal specificity of Azo-QPS-MSN + CHX activity. We will model dual-species biofilm communities by co-culturing *S. mutans* with *Rothia dentocariosa*, commensal bacteria often found in dental plaque⁵⁰. Integrating our 3D patterns, optimized in Aims 1 and 2, will allow us to analyze both interspecies behavior and antimicrobial activity under controlled 3D environment. By analyzing how Azo-QPS-MSN + CHX exposure alters species-specific viability and pH progression, these studies will inform the optimization of microenvironment-responsive antimicrobial design (Aim 2), while advancing understanding of targeted therapies in polymicrobial contexts.

C.3.1 - Evaluation of biofilm-forming behavior through multi-species co-culture in 3D. Building on the optimized single-species parameters from preliminary data discussed in Aims 1 and 2, we will advance our 3D platform to incorporate *multispecies* bacterial communities, which exhibit enhanced resilience and physiologically relevant responses to antimicrobial exposure⁵¹. The initial portion of this aim will focus on *S. mutans* and *R. dentocariosa*, a non-acidogenic commensal species contributing to the diversity of dental biofilms⁵², to dissect how spatial organization influences pH dynamics, biofilm formation, and treatment efficacy in complex microbiomes. Specifically, *R. dentocariosa* ATCC 14190 will be used. Under *in vitro*

conditions in previous studies, *R. dentocariosa* ATCC 14190 is non-acidogenic, as confirmed by phenol red assay, in contrast to the pronounced acid production and local pH reduction by *S. mutans* UA140 (Figure 5).

To establish robust co-culture conditions, *S. mutans* UA140 and *R. dentocariosa* will be cultured separately for 48 h in BHI at 37 °C under 5% CO₂ to mid-log phase. Bacteria will be harvested and combined in acrylamide hydrogel precursors at varying inoculation ratios (e.g., 1:1, 2:1, and 1:2 *S. mutans* to *R. dentocariosa*). These will be fabricated into bulk gels and cultured for analysis at the time points optimized in Aim 2. Parallel single-species hydrogels will serve as controls to distinguish species-specific growth dynamics from interspecies effects and identify oxygen-dependent interactions. Leveraging these optimized co-culture ratios, we will fabricate multi-species patterns based on alternating filament arrays (Pattern 1, C.1.2). The three main configurations will consist of mixed filaments with species printed within the same hydrogel filament, adjacent filaments with species printed in separate, directly adjacent filaments (0 µm spacing) and species printed with 500 µm and 1000 µm spacing. These bioprinted constructs will be cultured in BHI, with or without the addition of 4% sucrose, and imaged using our sequential imaging pipeline to quantify local pH, viability, and microbial growth in overall cultures (mixed filaments) compared with individual species effects (separate filaments).

Following confocal imaging, select constructs will be fixed, dehydrated, and critically point-dried (C.1.3) for SEM. This will allow direct correlation of the live functional data with ultrastructural features, specifically quantifying EPS matrix density and biofilm morphology associated with different spatial organizations.

C.3.2 - Real-time assessment of MTAA in 3D dual-species communities. Building on the dual-species spatial arrangements optimized in C.3.1, we will evaluate the MTAA of Azo-QPS-MSN + CHX in complex biofilms, leveraging insights from C.2.2. To evaluate antimicrobial specificity within defined spatial contexts, we will integrate the pH-responsive Azo-QPS-MSN + CHX or a CHX-G positive control directly into the engineered multi-species architectures. Two delivery strategies will be employed: 1) homogeneous distribution, uniformly incorporating the antimicrobials into each bioink prior to printing and enabling assessment of triggered release within mixed-species, adjacent-filament, and spaced filament patterns, and 2) Lattice distribution, printing antimicrobials in an alternate layer (Pattern 2, C.1.2), allowing for analysis of the efficacy and spatial range of MTAA across specific inter-species distances.

Metabolic activity will be stimulated by supplementing BHI with 4% sucrose, with sucrose-free BHI serving as the control. Spatiotemporal viability and pH dynamics will be monitored through parallel live/dead staining and ratiometric C-SNARF-4 imaging (C.1.3), correlating antimicrobial activity with localized acidity. SEM of sectioned samples will also be performed to quantify antimicrobial localization and biofilm dynamics/disruption relative to bacterial clusters and EPS matrices, using the methods established from C.1.3.

C.3.3 - Expected outcomes, potential problems, and alternative strategies:

Milestones: 3a) Y2: *S. mutans* and *R. dentocariosa* single-species and combined cultures in bulk and printed constructs with >70% viability at 48 h. 3b) Y3: Confocal and SEM quantification of local pH, viability, and biofilm morphology at <5µm resolution as a function of interspecies distance. 3c) Y4: Bactericidal Azo-QPS-MSN behavior in areas of high *S. mutans* acid production (<20% viability) while preserving commensals (>70% viability)

We anticipate that our optimized 3D biofilm platform will demonstrate MTAA, with Azo-QPS-MSN + CHX showing localized drug release specifically in *S. mutans*-rich microdomains (validated by correlative pH mapping/viability staining), and significantly greater preservation of commensal viability (>70% survival) compared to conventional antimicrobials. To enhance the precision of our targeted antimicrobial strategy, we will implement alternative optimization approaches informed by the data from our initial aims. We will employ Azo-QPS-C16, a variant from our lab with proven selectivity for acid-producing bacteria within polymicrobial oral biofilms⁴⁹.

If time allows, we will also engineer additional dual-species communities featuring 1) *Veillonella parvula* that displays synergistic interaction with *S. mutans*, and is known to enhance caries pathogenesis through metabolic cross-feeding^{53,54}; and 2) Commensal species *Streptococcus gordonii*, which has been shown to engage in antagonistic interaction with *S. mutans*⁵⁵. This will allow us to further optimize this novel platform and set the foundation for future applications of this system for studying more complex polymicrobial communities. Our transition to high-resolution inkjet bioprinting (C.1.4) will allow us to pattern these complex communities into intricate architectures, establishing design rules for antimicrobials that selectively suppress pathogens while preserving a healthy microbiome.

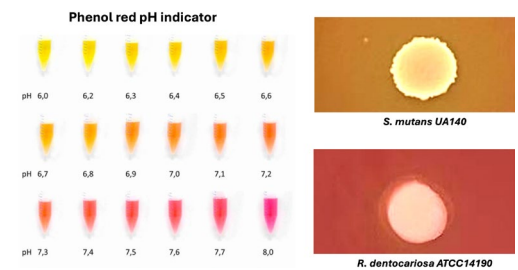


Figure 5: Phenol Red Assays displaying *S. mutans* acid generation (top) vs neutral/basic *R. dentocariosa* environments (bottom) after sucrose exposure.

Plan for Training in the Responsible Conduct of Research:

During my time at the ADA Forsyth Institute, as a requirement for postdoctoral researchers and trainees, I completed the Collaborative Institutional Training Initiative (CITI) responsible conduct of research training in 2022 and took the course again in 2025 to refresh my knowledge and fulfill the ADA Forsyth institutional requirements. ADA Forsyth also offers biannual responsible conduct of research refresher courses for staff and trainees to attend, which I will take advantage of as they occur.

ADA Forsyth currently offers an annual NIH compliant in-person RCR training course, in conjunction with the CITI courses and refreshers, which I will enroll in and attend if I receive this K25 award. This is a 10-week course with 90-minute weekly meetings, covering topics that include the following:

- Safe laboratory practices
- Safe research environments
- Conflict of interest
- Secure, ethical data use
- Collaborative research
- Peer review
- Handling of misconduct
- Mentor/Mentee relationships
- Responsible authorship and publication
- Data acquisition and management
- Recordkeeping practices

Further formal training will be obtained through Harvard Catalyst courses available to researchers in the community, and further informal training will be facilitated by Drs. Xuesong He, Felicitas Bidlack, and Jirun Sun through regular meetings and discussion of research throughout this award period.



October 27, 2025

K25 Award Committee

National Institute of Dental and Craniofacial Research (NIDCR)

National Institutes of Health (NIH)

Mentor Plan and Statement

Dear Members of the NIH Review Committee,

I am pleased to serve as a mentor to Dr. Jeremy Elias for his NIH/NIDCR K25 application, titled "Three-Dimensional Hydrogel Models for Analyzing Cariogenic Bacterial and Antimicrobial Responses." As a materials scientist with a research focus on translating innovative technologies into clinical applications, I bring extensive expertise in biomaterials development, nanotechnology, and tissue engineering. My contributions in these fields are reflected in 9 U.S. patents and over 70 publications in leading academic journals, including *Advanced Materials*, *Bone Research*, *Biomaterials*, *Journal of Dental Research*, and *Dental Materials*. My research has garnered significant recognition, with over 1,700 citations in the past five years according to Google Scholar. My work has also been highlighted by respected organizations such as the Federal Dental International, the Canadian Dental Association, and *C&EN News*, the leading magazine for chemists and chemical engineers. Over the past decade, I have mentored more than 30 trainees, including undergraduate and graduate students, postdoctoral fellows, and dentists. Many of them have gone on to pursue careers as dentists or independent principal investigators. I am committed to providing Dr. Elias with guidance that draws from my extensive experience in both fundamental research and its clinical translation.

Jeremy joined my research group as a postdoctoral fellow in August 2022, supported by the diversity supplement of my R01 grant (R01 DE029479A). Dr. Elias, an African American U.S. citizen, completed his PhD in Materials Science and Engineering in April 2022 under the mentorship of Professor Laurie Gower at the University of Florida. He is a distinguished scholar, having received both the UF Graduate Student Preeminence Award (2017) and the McKnight Dissertation Fellowship (2021), and demonstrates strong potential for an independent research career. In the past two years, Dr. Elias has made substantial contributions to the parent grant, building a solid foundation for his future as an independent investigator. His exceptional work has been recognized with the AADOCR Bloc Travel Award, and he has actively disseminated his research through numerous presentations, submitted papers, and ongoing projects. I am deeply grateful to NIDCR for their invaluable support of Dr. Elias, and I am pleased to report the excellent training he has received and the significant contributions he has made to my R01-funded projects. Dr. Elias is well-positioned for his next career move, and I am confident in his potential to excel as an independent researcher. Below is a summary of his achievements since joining the lab on August 1, 2022.:

1. Obtained high-level training through programs at Harvard Catalyst, Harvard CNS, and ADA Forsyth. These trainings include Clinical Investigation Training, Grant Writing, and instrumentation.
2. Established new collaborations with scientists at ADA Forsyth and different universities including Boston University and Harvard School of Dental Medicine.
3. Provided training to dental residents at HSDM.
4. Established new projects to prepare him to become an independent principal investigator.
5. Built a new 3D printer for the parent R01 project and his future K25 project.
6. Won an award from AADOCR: 2024 and 2025 AADOCR Bloc Travel Award.
7. Publications and Presentations:
 - a. Gower, L., Elias, J., 2022. Colloid assembly and transformation (CAT): The relationship of PILP to biomineralization. *Journal of Structural Biology*: X, 6, 100059.
 - b. Elias, J., Angelini, T., Martindale, M.Q., Gower, L., 2022. Assessment of Optimal Conditions for Marine

Invertebrate Cell-Mediated Mineralization of Organic Matrices. Biomimetics, 7, 86.

- c. Elias, J., Matheson, B.-A., Gower, L., 2023. Influence of Crosslinking Methods on Biomimetically Mineralized Collagen Matrices for Bone-like Biomaterials. Polymers, 15, 1981.
- d. Elias, J., Kattinanon, R., Kraemer, S., Depalle, B., Sun, J., Bidlack, F.B., 2024. Collagen Point-Mutation and Altered Enamel Matrix Change Dentin-Enamel Junction Properties. J Dent Res Vol #103, B.
- e. Elias, J., Kattinanon, R., Kraemer, S., Depalle, B., Sun, J., Bidlack, F.B., 2025 (in review) Collagen mutation or enamel matrix disruptions have sex-specific effects on dentin-enamel junction and beyond. Acta Biomaterialia
- f. Elias, J., Sun, J., 2025 (in progress) 3D printing of biomaterials and biofilms: techniques and applications for simulated microenvironments.
- g. Elias, J., Dentin and Enamel Organic Matrix Disruptions: Effects Beyond the DEJ, IADR March 2024 (Poster)
- h. Elias, J., "Tech Showcase Vol. 1: 3D Printer Modifications for Versatile Bioprinting". Seminar presented at ADA Forsyth Institute, May 2, 2024 (Oral)
- i. Elias, J., Dentin and Enamel Organic Matrix Disruptions: Effects Beyond the DEJ, AADOCR Boston Section 2024 Spring Meeting, May 2024 (Poster)

I am thrilled to contribute to this interdisciplinary research and to support Jeremy in his transition to becoming an independent scientist. I will provide guidance as he applies his expertise in biomaterials development, ensuring he maximizes his potential. Having been trained as a chemist and successfully transitioned into biomedical research, I am uniquely positioned to offer Jeremy valuable insights and advice on navigating his own career transition. Additionally, Dr. He, an expert in microbiology, will offer crucial support for Jeremy's project, specifically in developing the 3D bacteria-laden model. The interdisciplinary collaboration and knowledge gained through working on the R01 grant have already laid a strong foundation for Dr. Elias in the biomedical field. Dr. Bidlack, in her role as Senior Science Officer, contributes to creating this institutional framework and is dedicated to fostering Dr. Elias' growth. She will help sharpen his ideas through interactions with leading scientists, supporting him in achieving his individual career development goals.

Beyond our regular weekly group meetings, Dr. Elias will actively engage in Forsyth Trainee activities, which include training in the responsible conduct of research, presentation skills, grant writing workshops, journal clubs, trainee presentations in our research seminar series, brainstorming sessions, and participation in institute-wide research seminars and symposiums within the Forsyth community.

ADA Forsyth Institute is the leading institution in oral microbiology and immunology in oral and craniofacial research. ADA Forsyth institute also has an active T90/R90 NIDCR training center to foster the next generation of independent investigators. Dr. He is the Associate Director and plays an important role in the training center. Both Dr Bidlack and I play an important role in the training center as mentors. We will guide Dr. Elias and provide valuable advice for his next career steps.

Sincerely,



Jirun Sun, MS, PhD
Associate Member of Staff
ADA Forsyth Institute



Nov. 10, 2025
K25 Award Committee
National Institute of Dental and Craniofacial Research (NIDCR)
National Institutes of Health (NIH)

Dear Members of the NIH Review Committee,

It is with great pleasure that I commit to serving as Dr. Jeremy Elias's co-mentor for his NIH/NIDCR K25 application entitled "Three-Dimensional Hydrogel Models for Analyzing Cariogenic Bacteria and Antimicrobial Responses".

I met Jeremy when his mentor, Dr. Sun, and my lab began collaborating on a NIH-funded research project aiming at developing novel antimicrobials, such as acid-activated antimicrobial compounds, in preventing dental caries by targeted removal of cariogenic bacteria. Jeremy swiftly proved to be an invaluable team member, playing a critical role in participating in the execution of key experiments and applying his knowledge to assist with multiple projects and techniques including material synthesis and 3D printing. Notably, he demonstrated exceptional teamwork while collaborating with members of my lab, displaying a proactive approach to expediting workflow and rapidly acquiring technical skills in a discipline that was previously unfamiliar to him. His pre-doctoral training, followed by two years of mentorship under Dr. Sun has paved the way for his independent academic career. Furthermore, his application's exceptional interdisciplinary research and training plan will reinforce this trajectory.

Trained as a dentist and microbiologist, my involvement in oral microbiology research spans more than 17 years. I have accumulated vast expertise in bacterial inter-species interactions, biofilm, bacterial genetics, microbial ecology, microbiome, and microbial-host interaction throughout my career. Mainly, I am interested in achieving a comprehensive understanding of microbial ecology in the human oral cavity and its role in health and diseases. I have consistently utilized state-of-the-art technologies and employed multi-omics approaches to unravel the complexities of microbial host interactions.

Dental caries is one of the most widespread and costly infections in humans. A better understanding of the physiological, ecological, and pathological behavior of cariogenic bacteria may provide critical knowledge in developing preventive and therapeutic measures against caries. A recent new concept in microbiome research, i.e. biogeography, emphasizes the importance of spatial arrangement of bacterial consortium within the multispecies communities in impacting the physiology of individual bacterial species and the community-level function. However, the traditional *in vitro* planktonic and biofilm model, either in mono- or multispecies communities, cannot capture the *in vivo* spatial structure arrangement of cariogenic bacteria and its microbial neighbors, as recently revealed by spectrum imaging. As part of this exciting proposed project, Jeremy will establish a bioprinted bacteria-laden hydrogel model to enhance the understanding of bacterial interspecies interaction. Specifically, through the bioprinting of hydrogels, Jeremy will fabricate biomimetic architecture for the precise patterning of bacteria to generate models with customizable three-dimensional architectures for studying oral microbial interaction and antimicrobial dynamics.

This proposal aligns perfectly with the research endeavors in my lab. I am pleased to support Jeremy in his proposed project. I will meet with him regularly throughout the experiment design and implementation stages. In my lab, I will provide hands-on techniques that Jeremy proposes in this application, including but not limited to spatial investigation of the microbiome. Along with his laboratory training, Jeremy will expand his theoretical microbiology knowledge by taking courses at Harvard Medical School. I will also actively engage in discussions about his results

and work closely with him to understand their implications.

During Jeremy's postdoc training period, I have regularly met with him to discuss his individual development plans, evaluated his progress and provided support to guide him in his next steps. Therefore, I have already been actively involved in fostering Jeremy's professional growth in academia. I am genuinely delighted to continue to mentor him through his research training and support him as he establishes his path to independence.

Very truly yours,

A handwritten signature in black ink, appearing to be 'Xuesong He', with a stylized, flowing script.

Xuesong He, D.D.S, PhD.

Senior Member of Staff
The Forsyth Institute
245 First Street, Cambridge, MA 02142
Email: xhe@forsyth.org



Review Panel for K25 Award Applications
National Institute of Dental and Craniofacial Research (NIDCR)
National Institutes of Health

July 11, 2025

Dear Members of the Review Committee,

It is with great pleasure that I fully commit to provide mentorship and support for Dr. Jeremy Elias and his proposed research project for this K25 grant application titled "Three-Dimensional Hydrogel Models for Analyzing Cariogenic Bacteria and Antimicrobial Responses."

I am Senior Director of Research Affairs and Professor of Staff in the Mineralized Tissue Biology and Bioengineering division at the ADA Forsyth Institute. In this role, I facilitate opportunities for trainees to present their research in formal settings. I meet monthly with the trainee representatives at ADA Forsyth Institute, organize monthly gatherings for informal interactions between PIs and trainees, and provide a framework to foster collaborative research, project initiatives, and a safe environment for the exchange of ideas at ADA Forsyth.

My research focuses on the formation, structural organization, and repair of tooth enamel, and the use of teeth as biomarkers for the reconstruction of health histories and prediction of health risks. In addition, I have extensive experience in electron microscopy and light microscopy techniques for the analysis of tooth microstructure and the high-resolution imaging of microbial species and their interaction (Bor et al., JDR 2020). A key area of my ongoing research projects is focused on the analyses of oral microbes on tooth substrates, including visualization of pH changes and biofilm architecture.

I have previously mentored postdoctoral researchers with engineering background as they charted new territory in working on tooth mineralization and learned to appreciate the structural organization of enamel and dentin and the work with teeth. I am thrilled to provide support and guidance for Jeremy as a co-mentor along with Drs. Sun and He who have been close collaborators for several years in NIH funded projects.

Jeremy joined Forsyth in August 2022 as postdoctoral researcher with Dr. Sun and me as joint mentors. This mentoring constellation has proven excellent for leveraging our complementary research expertise to provide comprehensive guidance for the research and career development of Jeremy. Since his arrival, Jeremy has been a great team member and demonstrated his aptitude for efficiently transferring his skillset from biomineralization studies using polymer induced liquid precursor (PILP) for *in vitro* collagen mineralization to analyses of the dentin-enamel interface and comparison between effects of mutations that alter the dentin and enamel matrix. Jeremy has successfully integrated his understanding of *in vitro* collagen mineralization and analyses with new knowledge on enamel mineralization and structural organization of both dentin and enamel. His productivity is reflected in two conference presentations in 2024 (IADR and AADOCR Boston Chapter), a published abstract, (Elias et al. J Dent Res Vol #103, A) and a manuscript "Collagen mutation or enamel matrix disruptions have sex-specific effects on dentin-enamel junction and beyond" with revisions in response to reviewer comments submitted in April to Materialia.

Jeremy has taken full advantage of the training opportunities provided at ADA Forsyth. He has shown his professional growth in trainee centered activities and by leveraging technical training to move his research project forward. Specifically, he has presented his work progress in laboratory meetings and in the ADA Forsyth trainee seminar series; he has expanded his technical and analytical skillset through courses and technical training in electron microscopy, nanoindentation and mechanical testing at Harvard CNS. Most importantly, Jeremy has shown that he is ready for the next step in his career and will be able to successfully execute the proposed project. In a technical seminar given in September 2024 at ADA Forsyth, Jeremy shared his expertise on the application of the 3D printer that he custom built for the project proposed in this K25 proposal. Since then, he has optimized the 3D printing protocols and use of pH sensitive fluorescent dyes in his microbe-seeded experimental hydrogels.

I am delighted to be involved as an advisor in Jeremy's proposed project as he applies his engineering background to fundamental questions in oral biology and leverages his skillset to address mechanisms of microbial interactions and acid production that are of fundamental importance for oral health. I am committed to advise Jeremy on electron microscopy and the application of light microscopy for analyses of dental microstructure, or the imaging analyses of microbes and microbial interaction. I am looking forward to Jeremy's continued attendance in our weekly lab meetings, his active participation in group discussions, presentation of project challenges as well as advances and findings, and his journal club presentations and involvement in team activities.

Jeremy is an excellent candidate for this K award mechanism. He is ideally positioned and ready for this K25 award to build on his training and embark on the proposed interdisciplinary research project and his steep upward career trajectory. Without hesitation, I am lending my full support within ADA-Forsyth and my expertise and guidance as a mentor to Jeremy in his next steps towards independence.

Best regards,



Felicitas Bidlack
Senior Director of Research Affairs, Professor
Mineralized Tissue Biology and Bioengineering

Email: fbidlack@forsyth.org

Institutional Environment:

The proposed research and most of the outlined career development and training activities will be performed at the ADA Forsyth Institute, a renowned independent, nonprofit research institute that is a leader in oral health research and education in biomedical sciences. On an institutional level, ADA Forsyth is committed to the support and development of its network of trainees and researchers, with an established roster of multiple current and previous NIH Career and Research Development Awardees. To facilitate this support, ADA Forsyth provides a variety of resources and training opportunities to its researchers, including Advanced Microscopy, MicroCT, Microbiome, Bioinformatics and Biostatistics Core facilities, as well as numerous courses and career development activities to assist early-career researchers outside of the lab.

My research and professional development will be carried out with guidance from a strong group of mentors at ADA Forsyth who have wide-ranging experience and expertise. This research will primarily be performed in the labs of Dr. Jirun Sun and Dr. Xuesong He, who are respective leaders in the fields of bioengineering and microbiology. Dr. Sun has a variety of expertise in academic fields as well as industry, combined with scientific expertise in the fields of dental materials that complement my current skills and knowledge in materials science. The Sun lab also consists of expert researchers and clinicians, providing a strong support network for sharing ideas and information to assist in successful research. Dr. He provides expertise in dental microbiology and has helped advance the understanding of oral infections and microbiome interactions, providing an optimal mentoring environment for my transition into biological research. As a trained dentist, Dr. He also offers invaluable clinical perspective, ensuring my work remains translationally focused. His expertise bridges critical gaps between in vitro models and patient-centered outcomes, guiding the development of clinically relevant solutions. The He lab provides opportunities for collaboration and training by many experts in microbiology and immunology, presenting a substantial resource for my development in bioengineering research and techniques.

Dr. Felicitas Bidlack will also serve as a mentor and scientific advisor, providing expertise in advanced imaging methods as well as offering valuable scientific and professional advice, building on our positive co-mentoring and research connections over the past 3 years.

Drs. Sun, He, and Bidlack are established leaders in their respective fields of dental research, providing a range of valuable connections and networking opportunities locally, nationally, and internationally at conferences and meetings that they attend or organize with their labs. Being a part of these labs and research environments provides additional opportunities for training, dissemination of research, and building networking skills throughout the larger dental research community. My mentors are situated locally at the ADA Forsyth Institute, which gives frequent availability and access as needed to their expertise and knowledge for my research and development.

In addition to this, the position of ADA Forsyth in Somerville, Massachusetts provides close proximity to state-of-the-art resources and equipment in the Boston area including Harvard and Boston University, as well as close connections and collaborations with nearby institutions such as the Broad Institute, Massachusetts General Hospital, and the Massachusetts Institute of Technology. Combined with the advanced laboratory spaces, equipment, and clinic at the ADA Forsyth facility, this provides great support for training and research efforts. Outside of ADA Forsyth, the Harvard Center for Nanoscale Systems provides a diverse range of state-of-the-art instruments and resources for preparation and analysis of samples, as well as many faculties that are committed to training and offering information on materials and systems analysis.

The ADA Forsyth Institute is a valuable resource for the development of early-career researchers, as evidenced by a strong record of success of current and previous Forsyth researchers, as well as my achievement and development in my short time at this institution. It is evident that my training and mentorship under a group of leaders in the field of dental research and oral health will contribute greatly to my future development and success as an independent researcher.



November 10, 2025

Institutional Commitment to Candidate's Research Career Development

Dear Members of the Review Section:

On behalf of ADA Forsyth Institute, I write to offer our full institutional support for the training and research plan described in the NIH K25 grant application entitled “**Three-Dimensional Hydrogel Models for Analyzing Cariogenic Bacterial and Antimicrobial Responses**” from Dr. Jeremy Elias.

During his postdoctoral fellowship at the ADA Forsyth Institute, Dr. Jeremy Elias has made significant contributions to the NIH-funded R01 (DE029479A) project led by Drs. Xuesong He and Jirun Sun (Co-Investigator Dr. Felicitas Bidlack). Under their mentorship, Dr. Elias has developed innovative measurement methodologies that have already yielded two high-impact manuscripts. The first, “*Single-Cell Level Analysis of Metabolism-Activated Antibacterial Mechanisms in Customizable Hydrogels*” (submitting to Proceedings of the National Academy of Sciences, first author), and the second, “*Nano-Gatekeepers Triggered by Metabolism: From Broad-Spectrum to Precision Release*” (under review at Nature Materials, second author), demonstrate his technical creativity and growing leadership in biomaterials and microbial analysis.

The proposed K25 award will provide an outstanding opportunity for Dr. Elias to further advance his expertise in materials engineering, quantitative imaging, and advanced analytical techniques. By integrating these skills with his expanding knowledge of microbiology and biomaterial manipulation, he is poised to generate transformative insights into oral health. Dr. Elias’s proposal aligns seamlessly with the ADA Forsyth Institute’s mission to accelerate innovation in oral and craniofacial health through cutting-edge research in microbiology, biomaterials, and immunology. His plan to employ hydrogel patterning and bioprinting to model oral microbial behavior in three dimensions represents an exciting frontier; using biomimetic materials to modulate bacterial interactions offers the potential to uncover new mechanisms underlying disease development and therapeutic response in complex oral environments.

I can assure you that ADA Forsyth Institute will provide the essential resources to facilitate this project, including dedicated office and laboratory space, computing infrastructure, microscopy and microbiome core facilities, as well as support for travel, training and education, and administrative assistance to ensure efficient execution and attainment of the project’s goals. Under the mentorship of Drs Sun and He, Dr. Elias will be immersed in an optimal training environment to refine his expertise in bacterial research and biomaterials. In addition, the collaboration with nearby premier institutions—Massachusetts General Hospital, Massachusetts Institute of Technology, and Broad Institute—provides complementary resources and enhanced career-development opportunities to support Dr. Elias’s transition into bioengineering and biological research.

Sincerely,

A handwritten signature in black ink that reads "Benjamin".

Benjamin Wu, DDS, PhD
Chief Scientific Officer,
Chief Operating Officer, ADA Forsyth Institute

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

Delayed Onset Studies

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

Resource Sharing Plan:

All research resources will be made freely available to all researchers in the scientific community. All investigators participating in this project will agree to the ADA Forsyth Institute's adherence to the NIH sharing policies, as well as guidelines regarding NIH-funded research resources. For the research community to benefit from any tools or resources generated by this project, ADA Forsyth will transfer materials under a Material Transfer Agreement (MTA). Such MTAs will be made with no more restrictive terms than the Simple Letter Agreement (SLA) to non-profit institutions or the Uniform Biological Material Transfer Agreement (UMBTA) for profit purposes.

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 Resources and/or Chemical Resources:

We will adhere to the following methods for analysis and verification of the key chemical and biological resources listed in this project:

Key Chemical Resources:

Acquired and synthesized compounds and reagents used for this project will be authenticated for both identity and purity following the guidelines of the American Chemical Society for characterization of compounds. We will continue to employ our currently used techniques of NMR, UV/Visible and IR spectroscopy, and elemental analysis. We will add additional techniques as needed including particle size and zeta potential. Acquired compounds will be purchased from certified chemical vendors of the highest quality available, and records on batch information will be kept ensuring consistency.

Key Biological Resources:

Bacteria: *S. mutans*, *R. mucilaginosa*, and *V. parvula* bacteria will be obtained from the ADA Forsyth laboratory collection and the He laboratory. Each strain will be authenticated using real-time quantitative polymerase chain reaction (PCR). Bacteria strains will be stored in a -80 freezer when not being cultured and reisolated periodically and checked for characteristic phenotypic markers.