

Electronic Cover Sheet																													
PI: Makkar, Hardik	Title: Probing the Mechanical Regulation of Inflammation in Periodontal Health and Disease																												
Received: 11/11/2025	Opportunity: PAR-25-333	Council: 05/2026																											
Competition ID: FORMS-I	FOA Title: NIDCR Dual Degree Dentist Scientist Pathway to Independence Award (K99/R00 Clinical Trial Not Allowed)																												
1K99DE035581-01A1	Dual: AI	Accession Number: 5219508																											
IPF: 6463801	Organization: UNIVERSITY OF PENNSYLVANIA																												
Former Number: 1K99DE035581-01	Department: 5106 - Preventive and Restorative Sciences																												
IRG/SRG: ZRG1 MSOS-F (22)S	AIDS: N	Expedited: N																											
<u>Subtotal Direct Costs</u> <u>(excludes consortium F&A)</u> Year 1: 129,824 Year 2: 129,824 Year 3: 249,000 Year 4: 249,000 Year 5: 249,000	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N HFT: N Special Topics: Data Management Sharing	New Investigator: Early Stage Investigator:																											
<table border="1"> <thead> <tr> <th><i>Senior/Key Personnel:</i></th> <th><i>Organization:</i></th> <th><i>Role Category:</i></th> </tr> </thead> <tbody> <tr> <td>HARDIK MAKKAR</td> <td>TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE</td> <td>PD/PI</td> </tr> <tr> <td>GEORGIOS HAJISHENGALLIS</td> <td>TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE</td> <td>Other (Specify)-Advisory Committee Member</td> </tr> <tr> <td>HYUN KOO</td> <td>TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE</td> <td>Other (Specify)-Advisory Committee Member</td> </tr> <tr> <td>KATHLEEN STEBE</td> <td>TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE</td> <td>Other (Specify)-Advisory Committee Member</td> </tr> <tr> <td>KANG KO</td> <td>TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE</td> <td>Other (Specify)-Advisory Committee Member</td> </tr> <tr> <td>MICHAEL ABT PhD</td> <td>TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE</td> <td>Other (Specify)-Advisory Committee Member</td> </tr> <tr> <td>REBECCA WELLS</td> <td>TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE</td> <td>Other (Specify)-Co-Mentor</td> </tr> <tr> <td>KYLE VINING</td> <td>TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE</td> <td>Other (Specify)-Mentor</td> </tr> </tbody> </table>			<i>Senior/Key Personnel:</i>	<i>Organization:</i>	<i>Role Category:</i>	HARDIK MAKKAR	TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE	PD/PI	GEORGIOS HAJISHENGALLIS	TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE	Other (Specify)-Advisory Committee Member	HYUN KOO	TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE	Other (Specify)-Advisory Committee Member	KATHLEEN STEBE	TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE	Other (Specify)-Advisory Committee Member	KANG KO	TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE	Other (Specify)-Advisory Committee Member	MICHAEL ABT PhD	TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE	Other (Specify)-Advisory Committee Member	REBECCA WELLS	TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE	Other (Specify)-Co-Mentor	KYLE VINING	TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE	Other (Specify)-Mentor
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KYLE VINING	TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE	Other (Specify)-Mentor																											

Reference Letters

Chwee Lim	National University of Singapore	11/11/2025
Vinicius Rosa	National University of Singapore	11/11/2025

Reference Letters

Marco Bottino	University of Michigan School of Dentistry	11/11/2025
Gopu Sriram	National University of Singapore	11/11/2025
Nagihan Bostanci	Karolinska Institutet	11/11/2025

Additions for Review

Accepted Publication	Post-Submission Materials
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APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

3. DATE RECEIVED BY STATE		State Application Identifier
1. TYPE OF SUBMISSION*		4.a. Federal Identifier DE035581
<input type="radio"/> Pre-application <input checked="" type="radio"/> Application <input type="radio"/> Changed/Corrected Application		b. Agency Routing Number
2. DATE SUBMITTED 2025-11-10	Application Identifier 10107262	c. Previous Grants.gov Tracking Number
5. APPLICANT INFORMATION UEI*: GM1XX56LEP58		
Legal Name*: TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE		
Department: 5106 - Preventive and Restorative Sciences		
Division: 5100 - School of Dental Medicine		
Street1*: Office of Research Services		
Street2: 3451 Walnut Street, 5th Floor		
City*: Philadelphia		
County: Philadelphia		
State*: PA: Pennsylvania		
Province:		
Country*: USA: UNITED STATES		
ZIP / Postal Code*: 19104-6205		
Person to be contacted on matters involving this application		
Prefix: First Name*: ELIZABETH Middle Name: D Last Name*: PELOSO Suffix:		
Position/Title: AssocVicePres/AssocViceProvost for Research		
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County:		
State*: PA: Pennsylvania		
Province:		
Country*: USA: UNITED STATES		
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Phone Number*: 2157460234 Fax Number: 2158989708 Email: PennAORs@lists.upenn.edu		
6. EMPLOYER IDENTIFICATION NUMBER (EIN) or (TIN)*		1231352685A1
7. TYPE OF APPLICANT*		O: Private 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"/> A. Increase Award <input type="radio"/> B. Decrease Award <input type="radio"/> C. Increase Duration
<input type="radio"/> Renewal <input type="radio"/> Continuation <input type="radio"/> Revision		<input type="radio"/> D. Decrease Duration <input type="radio"/> E. Other (specify):
Is this application being submitted to other agencies?* <input type="radio"/> Yes <input checked="" type="radio"/> No What other Agencies?		
9. NAME OF FEDERAL AGENCY* National Institutes of Health		10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER TITLE:
11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT* Probing the Mechanical Regulation of Inflammation in Periodontal Health and Disease		
12. PROPOSED PROJECT Start Date* Ending Date* 07/01/2026 06/30/2031		13. CONGRESSIONAL DISTRICTS OF APPLICANT PA-003

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

Prefix: First Name*: HARDIK Middle Name: Last Name*: MAKKAR Suffix:

Position/Title: Postdoctoral Fellow

Organization Name*: TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE

Department: 5106 - Preventive and Restorative Sciences

Division: 5100 - School of Dental Medicine

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State*: PA: Pennsylvania

Province:

Country*: USA: UNITED STATES

ZIP / Postal Code*: 19104-6030

Phone Number*: 267-764-6688 Fax Number: Email*: makkarh@upenn.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested* \$1,027,420.00

b. Total Non-Federal Funds* \$0.00

c. Total Federal & Non-Federal Funds* \$1,027,420.00

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*: BETH Middle Name: M Last Name*: ALIOTO Suffix:

Position/Title*: Associate Director

Organization Name*: TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE

Department: 8760 - Research Services

Division:

Street1*: 3451 Walnut Street

Street2: Franklin Building, 5th floor

City*: PHILADELPHIA

County:

State*: PA: Pennsylvania

Province:

Country*: USA: UNITED STATES

ZIP / Postal Code*: 19104-6205

Phone Number*: 2158987269 Fax Number: 2158989708 Email*: PennAORs@lists.upenn.edu

Signature of Authorized Representative*
BETH M ALIOTO

Date Signed*
11/11/2025

20. PRE-APPLICATION File Name:**21. COVER LETTER ATTACHMENT** File Name: Cover Letter _ 11062025.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: TRUSTEES OF THE UNIVERSITY OF
PENNSYLVANIA, THE
UEI: GM1XX56LEP58
Street1*: 240 South 40th Street
Street2: Penn Dental Medicine
City*: Philadelphia
County: Philadelphia
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 19104-6030
Project/Performance Site Congressional District*: PA-003

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: <input type="text"/> 1 <input type="text"/> 2 <input type="text"/> 3 <input type="text"/> 4 <input type="text"/> 5 <input type="text"/> 6 <input type="text"/> 7 <input type="text"/> 8	
If NO, is the IRB review Pending? <input type="radio"/> Yes <input type="radio"/> No	
IRB Approval Date:	
Human Subject Assurance Number	
2. Are Vertebrate Animals Used?* <input checked="" type="radio"/> Yes <input type="radio"/> No	
2.a. If YES to Vertebrate Animals	
Is the IACUC review Pending? <input checked="" type="radio"/> Yes <input type="radio"/> No	
IACUC Approval Date:	
Animal Welfare Assurance Number A3079-01	
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 Project Summary_11102025.pdf
8. Project Narrative*	Project Narrative.pdf
9. Bibliography & References Cited	References_11062025.pdf
10. Facilities & Other Resources	1102025_FACILITIES RESOURCES .pdf
11. Equipment	EQUIPMENT.pdf

PROJECT SUMMARY

This award will train Dr. Hardik Makkar, a dentist-scientist, at the intersection of tissue mechanics and mechanobiology in periodontal health and disease, supporting his transition to an independent career developing mechano-immune therapeutics for periodontitis. While microbial dysbiosis and immune responses are well characterized, the role of gingival tissue mechanics in immune regulation remains poorly understood. Periodontitis involves an exaggerated host response in which bacterial and host proteases degrade the extracellular matrix (ECM), weakening tissue integrity and disrupting immune homeostasis. Current therapies target bacterial biofilms but fail to restore gingival mechanics. Gingival fibroblasts (GFs) sense ECM stiffness and coordinate stromal-immune interactions that sustain homeostasis. I hypothesize that ECM degradation reprograms GF chromatin, driving persistent proinflammatory activation. This project will define how mechanical cues regulate GF mechano-epigenetic signaling and identify ECM stiffness-dependent drivers of inflammation, revealing new therapeutic targets to restore tissue and immune balance. Preliminary data show that GFs in stiffer matrices downregulate inflammatory cytokines and matrix metalloproteinases, with enrichment of matrix-related and epigenome regulation pathways. Stiffness-dependent inflammatory responses of GFs are mediated through nuclear organization and DNA methylation. GFs in stiff matrices promote differentiation of myeloid progenitors into activated antigen-presenting cells, and ECM crosslinking in human gingival explants enhances mechanical properties and reduces IL-6 secretion. I hypothesize that reduced tissue stiffness in periodontal disease impairs fibroblast-mediated matrix and immune homeostasis via epigenetic regulation. To test this, I will use gingival ECM-mimicking hydrogels and a mouse model of periodontitis to address two aims: (1) determine how ECM stiffness regulates GF epigenetic state, and (2) examine how ECM stiffness influences stromal-myeloid crosstalk. During the K99 phase, I will gain expertise in tissue mechanics, super-resolution microscopy, ATAC-seq, spatial transcriptomics, and bioinformatics, while strengthening teaching and grant-writing skills. The highly experienced mentoring team provides complementary expertise in periodontology, immunology, bioengineering, and mechanobiology. Drs. Kyle Vining (mentor) and Rebecca Wells (co-mentor) will guide training, research, and career transition; Drs. George Hajishengallis and Michael Abt will advise on the mouse model, mucosal immunology, and spatial transcriptomics; Dr. Kang Ko will contribute clinical expertise in fibroblast biology; and NIDCR R90 program directors Drs. Michel Koo and Kathleen Stebe will support cross-disciplinary training. Drs. Kai Tan and Su Chin Heo will provide expertise in bioinformatics analysis of high-throughput sequencing and super-resolution microscopy, respectively. In the R00 phase, the Makkar lab will investigate ECM stiffness-dependent fibroblast-myeloid crosstalk in vitro and in vivo to elucidate the ECM's role in periodontal inflammation and guide ECM-targeted therapy development.

PROJECT NARRATIVE

Periodontitis is one of the most prevalent chronic inflammatory conditions, affecting over one billion people worldwide. We found that stiff gingival tissue promotes tissue homeostasis which is reversed in periodontitis with concomitant microscale ECM degradation with altered mechanics. This study aims to dissect the regulation matrix stiffness dependent immunological homeostasis in periodontal health and disease.

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FACILITIES & RESOURCES

The proposed research and career development program will be conducted across biomedical research and engineering laboratories, as well as core facilities at the University of Pennsylvania. Below are key resources supporting this initiative:

Institutional Support: The University of Pennsylvania has a strong tradition of nurturing early-career researchers and fostering collaborative, interdisciplinary research. Penn has committed substantial institutional resources to ensure the success of Dr. Hardik Makkar as an emerging investigator. Dr. Makkar is currently a postdoctoral trainee in the NIDCR-funded T90/R90 Training Program, *Advanced Training at the Interface of Engineering and Oral-Craniofacial Sciences*, offered through the inter-school Center for Innovation & Precision Dentistry (CiPD), a collaboration between Penn Dental Medicine and Penn Engineering. The T90/R90 program aims to train a new generation of cross-disciplinary experts in oral health and engineering.

A key component of this program is the Career Mentoring Committee (CMC), which comprises leading scientists and clinicians who provide tailored, hands-on mentorship. The CMC functions as a “research vision and career development committee,” regularly assessing progress and offering actionable guidance on both research and career trajectories. In addition, Penn’s Biomedical Postdoctoral Program (BPP) offers a range of career development resources, including workshops on Responsible Conduct of Research, scientific writing, public speaking, and career planning. Institutional funding opportunities, such as the CiPD IDEA Award, Seed Funds, the Penn Health Tech Award, and various internal pilot grants from Penn Dental and Penn Engineering, further enhance Dr. Makkar’s ability to pursue innovative research.

Hardik Makkar (PI):

- **Laboratory:** The Principal Investigator has access to Vining Lab (primary mentor). This has approximately 900 square feet of laboratory space equipped for the safe use of chemicals and biological materials (BSL-2 level) and molecular analyses in the Levy Center for Oral Health Research (Levy Building Rooms 249 and 248) at the University of Pennsylvania-School of Dental Medicine/Penn Dental Medicine (PDM).
- **Office Space:** Dedicated office space (approximately 100 square feet) adjacent to Vining Lab at Penn Dental Medicine is available to Dr. Makkar, with access to a personal computer and a networked laser color printer. Meeting rooms equipped with high-quality wireless presentation system and teleconferencing equipment are readily available. As a current NIDCR CiPD T90R90 Postdoctoral Trainee, Dr. Makkar has full access to a new CiPD Innovation Hub that provides home base for integrated multidisciplinary collaboration and training activities. A center IT support team is also available for maintaining the computer/network systems and providing IT services.
- **Computer:** Dr. Makkar will have access to lab computers in Dr. Vining’s laboratory that is equipped with 2 personal computers (PC) capable of data processing and statistical and graphical analysis (such as GraphPad and JMP/SAS software programs), flow cytometry analysis (FlowJo), and image analysis (Imaris, MATLAB, ImageJ). The PC is networked into the central facility to aid in analysis, storage and retrieval of data. Dr. Makkar will also have full access to the online resources (e.g., online full-text articles; license for professional software such as the Adobe packages) provided by the University of Pennsylvania. School-based librarians and IT technicians are available to aid in complex search schemes, data retrieval, and research related IT services as needed. High Performance Computing (Penn HPC) environment is also readily available when more computing power is needed to process large datasets. The local area network provides e-mail services and access to biomedical databases, and retrieval of on-line full-text articles from the leading biomedical journals. A bioinformatics specialist is available at Penn Dental to aid in complex search schemes and data retrieval as needed. All computers are connected into the central network to facilitate the management, analysis, and storage of data (e.g., via PennBox, LabArchives).
- **Animal Facility:** The proposed animal research is performed in the Animal Facilities of the University of Pennsylvania. All protocols involving animals for this project are reviewed and approved by the University of Pennsylvania Institutional Animal Care and Use Committee (IACUC). The University Laboratory Animal Resources (ULAR) provides vivarium services, diagnostic and veterinary care, ordering, transfer, and quarantine services. The IACUC provides oversight of this facility. The University of Pennsylvania personnel involved in the animal care and use program for research and education are experienced and devoted to the

care of animals, with regard to the physical environment, housing, and maintenance of good health. Animals required for the proposed studies will be housed and maintained according to the prescribed standards for these species, under the supervision of professional veterinarians. An attending veterinarian is on-call 24 hours per day. The animal vivarium is AAALAC approved.

- **Core facilities:** The multidisciplinary research at Penn's 12 schools is supported by numerous core facilities available across the Penn campus, offering shared resources to investigators and providing access to state-of-the-art research equipment, technology, and technical support. Relevant facilities include:
 - i. **Penn Dental Medicine Core Facilities:** The Levy, Evans, and Schattner Buildings house PDM. Levy is devoted primarily to basic science research. Levy building has a **Histology Core** for tissue processing and cutting paraffin and frozen sections, and a **Live Cell Imaging Core** that includes a Nikon A1R microscope equipped with CO2 and temperature control probes and chamber for live cell imaging. Penn Dental also has a newly established **Spatial Transcriptomics Core** which features the 10X Genomics Xenium Analyzer for highly multiplexed in situ RNA imaging at a single cell level. The core is open to the Penn community. Users will have access to pre-experiment consultation, specimen sourcing, sectioning assistance, Xenium workflow assistance with a dedicated core staff member, and bioinformatics support for data analysis.
 - ii. **The Center for Engineering MechanoBiology (Co-Director: Dr. Rebecca G. Wells, Co-Mentor for Hardik Makkar):** The Center for Engineering MechanoBiology (CEMB) is a multi-institution Science and Technology Center funded by the National Science Foundation (NSF). The CEMB is committed to nurturing interdisciplinary integrated mechanobiology research on ECM-cellular interaction, nuclear mechanobiology and tissue mechanics in 4D. The center provides expertise in studying tissue mechanics, super resolution imaging, biomechanical assays. It also provides funding opportunities for new projects, collaborations and to develop preliminary and/or feasibility data for investigators.
 - iii. **Multiphoton Core Facility:** The PennVet Imaging Core (PVIC) offers cutting-edge multi-photon technology and equipment to acquire and analyze high-quality confocal images. The facility is equipped with Coherent Chameleon Ultra II, Ti:Sapphire pulse laser (680-1080nm) and a Leica SP5 spectral imaging confocal/multiphoton microscope equipped with custom-built optics and software for image analysis and 3D renderings.
 - iv. **Children's Hospital of Philadelphia (CHOP) Center for single cell biology (CSCB):** The CSCB (Director: Kai Tan, Consultant for Dr. Hardik Makkar) provides a unified research infrastructure for the single-cell research community at CHOP and UPenn. This center is equipped with state of the art assays- **Single-cell transcriptomics** (10x Genomics Connect), **Single-cell ATAC-Seq** (10x Genomics Chromium), **Spatial transcriptomics** (10x Genomics Visium; Nanostring GeoMx) and **Multiplexed immunohistochemistry** (Akoya Biosciences CODEX). The core has experienced research assistants and bioinformaticians to offer in-house experimental and computational support.
 - v. **The flow cytometry core (Director: Florin Tuluc)** is dedicated to providing all investigators at the University of Pennsylvania access to high quality, cost-effective flow cytometry services as well as the scientific expertise necessary to use this technology in their research efforts. Both staff-performed and investigator-performed services are available utilizing a full array of state-of the art instrumentation. The core is equipped with Cytex Aurora analyzers and sorters for spectral flow cytometry. An active training and consultation program allows investigators to create a customized approach that will take full advantage of the technology
 - vi. **The Biostatistics Analysis Center (BAC)** is a University of Pennsylvania service center offered by the Perelman School of Medicine's Center for Clinical Epidemiology and Biostatistics. The BAC is staffed by professionally trained biostatisticians, biostatistical programmers and data managers, and provides a wide range of biostatistical consulting services to the University's biomedical research community and externally.
- **Administrative Support:** Secretarial, business, and grant management supports are readily available through CiPD, Penn Dental Medicine, and Penn Engineering.

EQUIPMENT

Major equipment from laboratories at Penn Dental Medicine, Penn Medicine and CEMB will be used to perform the assays described in the proposal.

Vining Lab (Penn Dental Medicine): HR 30 Discovery Hybrid Rheometer (TA Instruments – Waters Inc.) with DHR smart swap advanced peltier plate, air cooled peltier circulator, hard anodized aluminum solvent trap, and DHR UV light guide accessory, low-inertia Advanced Drag Cup Motor; Omnicure S-2000 UV light source with curing box; MilliporeSigma Milli-Q IQ 7000 Ultrapure Water System with Q-POD Remote Dispenser for Ultrapure Water; Countess 3 FL Automated Cell Counter with fluorescence light cubes; 2 Thermo CO2 incubators; 2 BSL2 biosafety cabinets; EVOS M5000 Imaging System; 2 chemical fume hoods; 1 chromatography fridge for materials preparation and dialysis; Harvard Apparatus Pump 33 DDS (Dual Drive System) syringe pump; Mettler Toledo Excellence XSR Analytical Balance; Heidolph Multi Reax Vortex Mixer; BioRad PCR and real-time PCR systems; Amaxa Nucleofactor II; Eppendorf 5810R Swinging Bucket Centrifuge, refrigerated; microfuges, vortexers, and orbital plate and microtube shakers; Minus 80 and minus 20 freezers.

Wells Lab (Penn Medicine):

The lab has two dedicated perfusion stations and all necessary equipment for rodent dissection, perfusion, and primary cell isolation, including hepatocytes. The lab owns an inhaled anesthesia delivery device, and others are also found in the surgical suites of the John Morgan vivarium. The lab has a Leica DM 4000M microscope which is particularly useful for immunohistochemistry. For histological studies, The Wells and Vining group has access to the **Pathology Core Laboratories** at the Children's Hospital of Philadelphia (CHOP), which carries out specialized staining on a fee-for-service basis. Through the **Center for Engineering MechanoBiology**, the lab has access to a custom microindentation device for meso-scale measurements in defined tissue areas, and to several rheometers including a shear rheometer (Kinexus) with rSpace software.

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator				
Prefix:	First Name*: HARDIK	Middle Name	Last Name*: MAKKAR	Suffix:
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PROFILE - Senior/Key Person				
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PROFILE - Senior/Key Person				
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PROFILE - Senior/Key Person				
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PROFILE - Senior/Key Person				
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PROFILE - Senior/Key Person				
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PROFILE - Senior/Key Person				
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County:				
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Credential, e.g., agency login: KSTEBE				
Project Role*: Other (Specify)		Other Project Role Category: Advisory Committee Member		
Degree Type: PHD		Degree Year: 1989		
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Attach Current & Pending Support:		File Name:		

BIOGRAPHICAL SKETCH

NAME: Makkar, Hardik

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

POSITION TITLE: NIH (NIDCR) R90 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)	END DATE MM/YYYY	FIELD OF STUDY
JSS Dental College & Hospital, Mysore, India	BDS	08/2013	Dentistry
KIIT University, Bhubaneswar, India	MDS	06/2017	Conservative Dentistry & Endodontics
Faculty of Dentistry, National University of Singapore, Singapore, Singapore	PHD	10/2023	Oral Sciences and Bioengineering
School of Dental Medicine, University of Pennsylvania, Philadelphia, Pennsylvania	Postdoctoral Fellow	present	Mechanobiology

A. Personal Statement

As a dentist-scientist trained in Bioengineering and Microbiology, I am committed to establishing an independent career in basic and translational research to understand the pathogenesis of periodontal diseases and to develop next-generation mechano-immune therapeutic strategies to treat this debilitating condition. Currently, I am a postdoctoral fellow in the **NIDCR- T90/R90 Training Program, "Advanced Training at the Interface of Engineering and Oral-Craniofacial Sciences,"** at the Center for Innovation & Precision Dentistry (CiPD), a collaborative research center between Penn Dental Medicine and Penn Engineering.

My current research focuses on the role of gingival extracellular matrix (ECM) stiffness in periodontal health and disease. Specifically, I am investigating how changes in tissue mechanics, caused by ECM degradation in periodontitis, influence host immune responses. Using a gingival ECM-mimicking hydrogel model as a mechanobiological tool, I have demonstrated that stiffer ECM, resembling healthy tissue, downregulates pro-inflammatory immune responses, while softer ECM, associated with disease, exacerbates inflammation. These findings highlight the critical role of changing tissue mechanics in periodontal disease pathogenesis.

I bring over 11 years of experience in biomaterials, bioengineering/tissue engineering, and host-microbe interaction research in periodontology. My foundational dental education from JSS Dental College and Hospital (India), followed by specialized training in Endodontics at KIIT University (India), earned me honors including the **University Gold Medal** and the **Pierre Fauchard International Senior Student Merit Award**. My post-graduate thesis, which combined clinical work and research in endodontic biomaterials, led to several publications and a project funded by the **Biotechnology Ignition Grant**, Government of India. Additionally, I gained training in life science entrepreneurship (**BIRAC-Ignite Fellowship, University of Cambridge**), acquiring skills for the successful commercialization of technologies.

To build a rigorous foundation for an independent academic research career, I pursued a Ph.D. in Oral Sciences and Bioengineering at the **National University of Singapore (NUS)** as a **President's Graduate Fellow**. There I developed 3D micro-vascularized models and microfluidic organ-on-chip systems to study periodontal host-microbe interactions. These engineered systems allowed me to investigate long-term periodontal host-microbe interactions, tissue and vascular invasion by periodontal pathogens, and the immunomodulatory effects of gingival crevicular fluid (GCF) on gingival fibroblasts. Developing these animal-alternative technologies offered significant ethical advantages, reduced experimental timelines, and produced more human-relevant results. I published several first-author articles in *Advanced Healthcare Materials*, *Journal of Tissue Engineering*, *Biofabrication*, and *Lab on a Chip*, and received the **IADR Hatton Award** and **NUS Travel Award**.

My work has also led to several collaborative research projects spanning oral soft tissue regeneration and targeted drug delivery. In one effort, I contributed to biofabrication and biomaterials strategies for engineering oral mucosal tissue constructs. In separate collaborations, I developed advanced biomaterials and targeted therapeutic platforms, including shape-memory cryogels optimized for load-bearing tissue engineering applications and hydroxyapatite-targeting lipid nanoparticles for localized delivery of STAT3-siRNA to modulate periodontal inflammation.

The objective of my K99 proposal is to position me as a leading dentist-scientist in mechanobiology and oral immunology, with the interdisciplinary expertise and vision required to advance dental, oral, and craniofacial sciences. The K99 career development plan will provide critical training and mentorship to help me transition to an independent investigator. Specialized training in tissue mechanics, spatial biology, super-resolution microscopy, and epigenetics will equip me with the technical expertise to lead mechanistically driven research to study periodontal pathogenesis and develop next generation of biomaterial-based therapeutics. Mentorship from **Dr. Kyle Vining** (primary mentor), **Dr. Rebecca Wells** (co-mentor), and my advisory committee will foster my growth in both research and career development, preparing me to manage my own research program. This training period is critical for acquiring technical skillset, research independence, and collaborative network required to secure a tenure track faculty position and launch a successful research program focused on developing mechano-immune therapeutic strategies to improve patient care in oral health.

I have not published or created research products under any other name.

1. **Makkar H**, Tran N, Chen Yu-Chang, Ko IK, Wells RG, Vining KH. Matrix Stiffness Governs Fibroblast-Driven Immune Homeostasis in Gingival Tissues. 2025 October. DOI: 10.1101/2025.10.20.683155
2. **Makkar H**, Sriram G. Advances in modeling periodontal host-microbe interactions: insights from organotypic and organ-on-chip systems. Lab Chip. 2025 Feb 25;25(5):1342-1371. PubMed Central PMCID: PMC11833442.
3. **Makkar H**, Lim CT, Tan KS, Sriram G. Modeling periodontal host-microbe interactions using vascularized gingival connective tissue equivalents. Biofabrication. 2023 Aug 2;15(4) PubMed PMID: 37473752.
4. **Makkar H**, Zhou Y, Tan KS, Lim CT, Sriram G. Modeling Crevicular Fluid Flow and Host-Oral Microbiome Interactions in a Gingival Crevice-on-Chip. Adv Healthc Mater. 2023 Jan;12(6):e2202376. PubMed PMID: 36398428.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2025 -	AADOCR NSRG Regional Representative - Ohio Valley Region, AADOCR National Student Research Group
2024 -	Postdoctoral Fellow, Center for Innovation and Precision Dentistry, School of Dental Medicine, University of Pennsylvania, Philadelphia, PA
2022 - 2023	Graduate Research and Development Intern, Umami Bioworks, Singapore
2019 - 2020	Director of Student Affairs, National University of Singapore, Graduate Student Society, Singapore
2018 - 2019	Project Leader - NIDHI Prayas, Department of Science & Technology (DST), Government of India, India
2018 - 2019	Mentor - Social innovation and Immersion Program, Biotechnology Industry Research Assistance Council, India
2018 - 2018	BIRAC-IGNITE Fellow, Judge Business School, University of Cambridge, Cambridge
2017 - 2019	Project Lead & Scientist - BIG Grant, Biotechnology Industry Research Assistance Council, India
2015 - 2017	Teaching Assistant, KIIT University, Department of Conservative Dentistry and Endodontics, India

Honors

2024	NIDCR R90 Postdoctoral Fellowship, Center for Innovation and Precision Dentistry, School of Dental Medicine, University of Pennsylvania, Philadelphia, PA
2023	NUS President's Graduate Fellowship Travel Award 2023, National University of Singapore
2023	IADR Hatton Award (Senior Category), IADR General Session 2022, International Association of Dental Research - SEA Division
2019 - 2023	NUS President's Graduate Fellowship, National University of Singapore
2018	BIRAC Ignite Award and Scholarship, 2018, Biotechnology Industry Research Assistance Council
2017	Rising Star Award, 2017, Indian Association of Conservative Dentistry & Endodontics
2016	IACDE Student Research Grant, Indian Association of Conservative Dentistry & Endodontics
2013	Pierre Fauchard International Senior Student Merit Award, Pierre Fauchard Academy
2013	Student Merit Award, Indian Orthodontic Society
2013	Student Merit Award, Indian Society of Pedodontics and Preventive Dentistry
2012	CJ Raju Gold Medal, JSS University
2012	JSS University Gold Medal, JSS University
2012	Indian Dental Association-Colgate Scholarship, Indian Dental Association

C. Contributions to Science

1. **ECM Mechanobiology in Periodontal Health and Disease-** As an NIDCR R90 Postdoctoral Fellow in the Vining Lab at the University of Pennsylvania, under the mentorship of Dr. Kyle Vining and Dr. Rebecca Wells, I investigate how extracellular matrix (ECM) mechanics regulate gingival fibroblast–myeloid cell crosstalk in the context of periodontal disease. Healthy gingival tissue features a highly crosslinked ECM predominantly composed of collagen type I, which confers stiffness and structural resilience. During periodontitis, proteolytic activity from both host and microbial sources degrades the ECM, reducing tissue stiffness and disrupting the mechanical microenvironment that governs immune regulation. To elucidate these mechano-immune interactions, I combined second harmonic generation (SHG) imaging to visualize collagen degradation in diseased gingival tissues with mechanical characterization of fresh human gingival biopsies. To model these biomechanical changes, I developed a mechanically tunable gingival ECM hydrogel system composed of interpenetrating networks of collagen type-1 and alginate, allowing precise control of stiffness independent of ligand density, mesh size, and collagen fiber architecture. This bioengineered platform recapitulates the viscoelastic and structural features of native gingiva, providing a powerful system to dissect how ECM stiffness modulates cellular function. Gingival fibroblasts encapsulated within stiff matrices exhibited suppressed toll-like receptor–mediated inflammatory signaling relative to those in soft matrices, driven in part by nuclear epigenetic organization. Extending these findings to multicellular contexts, I co-cultured human gingival fibroblasts with myeloid progenitors in stiffness-tuned hydrogels and observed stiffness-dependent differentiation toward immunomodulatory dendritic cell phenotypes. Consistent with these in vitro results, ex vivo crosslinking of human gingival explants increased matrix stiffness and attenuated inflammatory cytokine expression. Collectively, this work uncovers a mechanobiological framework through which ECM stiffness shapes periodontal immune responses—an underexplored dimension of disease pathogenesis that holds promise for the development of new biomaterials-based therapies for periodontitis.
 - a. **Makkar H**, Tran N, Chen Yu-Chang, Ko IK, Wells RG, Vining KH. Matrix Stiffness Governs Fibroblast-Driven Immune Homeostasis in Gingival Tissues. 2025 October. DOI: 10.1101/2025.10.20.683155
 - b. Lin Y, **Makkar H**, Zhang S, Chen B, Zhan C, Vining K. Mechanical cues orchestrate monocyte behavior in immune regulation and disease. APL Bioeng. 2025 Jun;9(2):021506. PubMed Central PMCID: PMC12205964.
 - c. **Makkar H**, Sriram G. Advances in modeling periodontal host-microbe interactions: insights from organotypic and organ-on-chip systems. Lab Chip. 2025 Feb 25;25(5):1342-1371. PubMed Central PMCID: PMC11833442.
2. **Animal Alternatives for Studying Periodontal Host-Microbe Interactions-** During my PhD at the National University of Singapore, under the mentorship of Dr. Sriram Gopu and Dr. Chwee Teck Lim, I developed tissue-engineered organotypic models and organ-on-chip platforms to study host-microbe interactions in

periodontal health and disease. These advanced models overcome the limitations of traditional monolayer cell cultures by recapitulating the native tissue microarchitecture, epithelial and vascular barriers, and interstitial fluid flow (gingival crevicular fluid) properties. In the first phase of my research, I biofabricated connective tissue equivalents and characterized their innate immune responses to various microbial challenges, including both planktonic and biofilm forms. To enhance the cellular complexity of the tissues, I integrated microvasculature and investigated regional fibroblast heterogeneity, macrophage polarization, and the spatiotemporal dynamics of oral microbial colonization and vascular invasion. This approach opens new avenues to explore the systemic effects of oral infections—an area that remains underexplored with current in vitro models. In subsequent work, I contributed to understanding the role of gingival crevicular fluid (GCF) in host protection, specifically through cellular mechanotransduction and microbial clearance. To study this, I developed an organ-on-chip system that mimicked the gingival crevicular microenvironment, recapitulating its cellular, structural, fluid transport, and pathophysiological properties. Gingival connective tissue equivalents were cultured on-chip with continuous interstitial flow to simulate GCF. This dynamic system allowed us to study bacterial colonization, long-term host-microbe coexistence, and the protective effects of GCF in attenuating immune responses following microbial clearance. The advantages of this approach over traditional in vitro methods lie in its ability to more accurately record immune responses, providing deeper insights into the pathophysiology of periodontal diseases. This work has the potential to significantly advance our understanding of periodontal health and disease at a cellular and systemic level.

- a. Rahimnejad M, **Makkar H**, Dal-Fabbro R, Malda J, Sriram G, Bottino MC. Biofabrication Strategies for Oral Soft Tissue Regeneration. *Adv Healthc Mater.* 2024 Jul;13(18):e2304537. PubMed Central PMCID: PMC11254569.
- b. **Makkar H**, Lim CT, Tan KS, Sriram G. Modeling periodontal host-microbe interactions using vascularized gingival connective tissue equivalents. *Biofabrication.* 2023 Aug 2;15(4) PubMed PMID: 37473752.
- c. **Makkar H**, Zhou Y, Tan KS, Lim CT, Sriram G. Modeling Crevicular Fluid Flow and Host-Oral Microbiome Interactions in a Gingival Crevice-on-Chip. *Adv Healthc Mater.* 2023 Jan;12(6):e2202376. PubMed PMID: 36398428.
- d. **Makkar H**, Atkuru S, Tang YL, Sethi T, Lim CT, Tan KS, Sriram G. Differential immune responses of 3D gingival and periodontal connective tissue equivalents to microbial colonization. *J Tissue Eng.* 2022 Jan-Dec;13:20417314221111650. PubMed Central PMCID: PMC9340411.

3. **Designing and Synthesizing Advanced Biomaterials and Targeted Nanotherapeutics for Dental, Oral and Craniofacial Applications** - My research has focused on designing and synthesizing advanced biomaterials and delivery systems to improve the mechanical performance, biological compatibility, and therapeutic potential of materials used in dental and craniofacial applications. The development of novel biomaterials is critical for improving the longevity and functionality of dental restorations, while innovative manufacturing and drug delivery strategies can expand accessibility and therapeutic efficacy.

During my Endodontics Residency and subsequent research training, I collaborated with the Infection Biology Laboratory and the Materials Science Department at KIIT University, India, to develop a nano-calcium aluminosilicate root repair material using high-energy ball milling. This approach enables low-cost, room-temperature synthesis of nanomaterials with tunable mechanical properties and microstructures that enhance clinical performance. To evaluate biocompatibility, I helped establish the zebrafish as a vertebrate model for studying dental biomaterials, leveraging its low maintenance cost, minimal ethical constraints, and capacity for high-throughput, multistage analysis. Using this model, we assessed the biological safety of bioceramics commonly used in restorative dentistry and endodontics and explored mechanistic pathways of material-induced toxicity. Additionally, I contributed to developing antimicrobial coatings for root-filling materials and lipid nanoparticle-based drug delivery systems targeting oral infections, such as herpetic gingivostomatitis.

During my postdoctoral training in the Vining Lab, I contributed to a project focused on developing hydroxyapatite-targeting lipid nanoparticles (HA-LNP) for the localized delivery of STAT3-siRNA to gingival tissues, aiming to control periodontal inflammation and alveolar bone resorption. By incorporating piperazine-linked bisphosphonate ionizable lipids to enhance mineral binding, these nanoparticles achieved >90% STAT3 gene knockdown in gingival fibroblasts and attenuated inflammatory responses. This work provides proof of concept for localized gene therapy in periodontitis and advances the understanding of STAT3-mediated inflammation in oral tissues. Collectively, these studies establish versatile biomaterial and drug

delivery platforms that bridge material science, biology, and precision therapeutics to address unmet needs in oral health care.

- a. Rath PP, **Makkar H**, Agarwalla SV, Sriram G, Rosa V. Stearic acid nanoparticles increase acyclovir absorption by oral epithelial cells. *Dent Mater*. 2024 Nov;40(11):1703-1709. PubMed PMID: 39112293.
 - b. **Makkar H**, Verma SK, Panda PK, Jha E, Das B, Mukherjee K, Suar M. In Vivo Molecular Toxicity Profile of Dental Bioceramics in Embryonic Zebrafish (*Danio rerio*). *Chem Res Toxicol*. 2018 Sep 17;31(9):914-923. PubMed PMID: 30058326.
 - c. **Makkar H**, Verma SK, Panda PK, Pramanik N, Jha E, Suar M. Molecular insight to size and dose-dependent cellular toxicity exhibited by a green synthesized bioceramic nanohybrid with macrophages for dental applications. *Toxicol Res (Camb)*. 2018 Sep 1;7(5):959-969. PubMed Central PMCID: PMC6116807.
 - d. Verma SK, Jha E, Panda PK, Mukherjee M, Thirumurugan A, **Makkar H**, Das B, Parashar SKS, Suar M. Mechanistic insight into ROS and neutral lipid alteration induced toxicity in the human model with fins (*Danio rerio*) by industrially synthesized titanium dioxide nanoparticles. *Toxicol Res (Camb)*. 2018 Mar 1;7(2):244-257. PubMed Central PMCID: PMC6061716.
4. **Engineering shape-memory cryogels for high load-bearing tissue engineering applications-** I contributed towards the development of novel shape-memory collagen cryogel scaffolds capable of sustaining >90% compressive strain, mimicking dynamic physiological environments. We developed and optimized a glutaraldehyde cross-linking strategy combined with mechanical predensification, which imparts superior elasticity, fatigue resistance, and robust shape recovery after hydration to the scaffolds, addressing key limitations of conventional biopolymer hydrogels for load-bearing applications. Through microstructural analysis, we elucidated mechanisms, such as fiber alignment and strain-induced stiffening, that underlie these mechanical properties. I led cell encapsulation studies that demonstrated high cell viability and alignment under repeated mechanical compression, thereby validating the cytocompatibility and mechanobiological relevance of the system. These scaffolds provide a versatile platform for tissue engineering in high load-bearing regions (e.g., cartilage, bone, tendon) and for mechanobiology research, establishing a new paradigm for designing biocompatible materials with dynamic mechanical resilience.
- a. Luo Y, **Makkar H**, Hu Y, Chen K, Purohit PK, Vining KH. Shape Memory Collagen Scaffolds Sustain Large-Scale Cyclic Loading. *ACS Mater Lett*. 2025 Aug 11;7(9):3150-3158. doi: 10.1021/acsmaterialslett.5c00817. PMID: 40909108; PMCID: PMC12406248
5. **Biomaterials to study the mechanobiology of bone marrow fibrosis-** I contributed to advancing the understanding of how extracellular matrix (ECM) mechanical properties direct the fate of bone marrow mesenchymal stromal cells (BM-MSCs) and their crosstalk with hematopoietic stem cells. The bone marrow is viscoelastic—healthy tissue dissipates stress rapidly, whereas fibrotic marrow exhibits increased stiffness and slower stress relaxation, contributing to inflammation and hematopoietic dysfunction. I contributed towards the development of a dynamically tunable hydrogel model composed of an interpenetrating network (IPN) formed by ionically crosslinked tetrazine-functionalized alginate (Tz-VLVG) and self-assembled collagen type I, which could be further stiffened by introducing norbornene-functionalized polyethylene glycol (PEG-Nb) crosslinkers. This on-demand stiffening strategy enables a tenfold increase in storage modulus (G') and markedly slower stress relaxation, recapitulating the mechanical transition from healthy to fibrotic bone marrow tissue in situ. BM-MSCs encapsulated in tunable 3D hydrogels exhibited stiffness-dependent spreading and secretion of IL-6, IL-8, and CCL2. Matrix stiffening activated actomyosin contractility, integrin β 1, and FAK signaling, driving a pro-inflammatory stromal phenotype. Pharmacologic inhibition of these pathways suppressed cytokine release and restored quiescent morphology. These findings establish ECM stiffening as a key regulator of stromal inflammatory activation, positioning this dynamic hydrogel platform as a physiologically relevant model for dissecting bone marrow fibrosis and testing anti-fibrotic therapies.
- a. Lin Y, **Makkar H**, Zhang S, Chen B, Zhan C, Vining K. Mechanical cues orchestrate monocyte behavior in immune regulation and disease. *APL Bioeng*. 2025 Jun;9(2):021506. PubMed Central PMCID: PMC12205964.

My Bibliography: <https://www.ncbi.nlm.nih.gov/myncbi/hardik.makkar.1/bibliography/public/>

BIOGRAPHICAL SKETCH

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

NAME: Vining, Kyle Holmberg (formerly Holmberg, Kyle Vining)

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

POSITION TITLE: Assistant 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
Northwestern University, Evanston, IL	B.S.	06/2009	Biomedical Engineering
University of Minnesota, Minneapolis, MN	D.D.S.	05/2014	Dentistry
Harvard University, Cambridge, MA	Ph.D.	01/2020	Bioengineering
Dana Farber Cancer Institute, Boston, MA	Post-doctoral	06/2022	Immuno-Oncology

A. Personal Statement – My research lab at Penn Dental Medicine and Penn Engineering investigates biomaterials science in diverse mechanical and biological settings. Broadly, my research aims to improve fundamental understanding of how biological systems interact with synthetic and natural materials to discover new knowledge, develop new materials technologies, and ultimately improve human health. My research and clinical work as a dentist intersect to focus on bridging the gaps between the latest advances in biomaterials, immunology, cancer biology, and regenerative medicine with the practical needs and shortfalls in oral, craniofacial, and dental healthcare.

I have 19 years of experience since 2006 in both materials science and bioengineering. I developed expertise in materials chemistry and synthesis in undergraduate and pre-doctoral research, as well as a background in cell and developmental biology at the National Institutes of Health. I gained expertise in biomaterials and mechanobiology through my Ph.D. with Prof. David Mooney at Harvard University, where I developed a collagen-alginate extracellular matrix (ECM) hydrogel that mimics native fibrillar architecture with independently tunable rigidity and fluid-like or solid-like properties (Vining et al., 2019). I use ECM hydrogels to model the bone marrow niche and investigate how immune fates are impacted. I determined how immunomodulation of mesenchymal stromal cells is regulated by stiffness and viscoelasticity (Lim et al., 2024). I gained expertise in cancer immunology and immuno-oncology through my post-doctoral training at the Dana-Farber Cancer Institute with oncologist F. Stephen Hodi and immunologist Kai Wucherpfennig on the mechanical regulation of monocytes in fibrotic ECM (Vining et al., 2022). I apply these findings to investigate new mechanisms of immuno-mechanobiology to understand how innate inflammation is regulated by biophysical cues of tissue ECM. (Adu-Berchie et al., 2023) My current research develops new biomaterial-based methods for studying myeloid cells and T cells. Together, these approaches will uncover new mechanisms that regulate immune cell fate and function.

I previously published as Kyle Vining Holmberg (Holmberg, KV) in years 2014 and earlier.

I am very enthusiastic to support Dr. Makkar's highly innovative and impactful K99 proposal on investigating the nuclear mechanobiology of gingival fibroblast inflammatory responses. His work is distinct and does not overlap with my existing projects. I am privileged to be able to help Dr. Makkar launch his independent career.

Publications Highlighted:

- a. **Vining K.H.**, Stafford A, Mooney DJ. Sequential modes of crosslinking tune viscoelasticity of cell-instructive hydrogels. *Biomaterials*. 2019 Jan;188:187-197. PMID: PMC6279497.

- b. Lim JJ, **Vining K.H.**, Mooney DJ, Blencowe BJ. Matrix stiffness-dependent regulation of immunomodulatory genes in human MSCs is associated with the lncRNA CYTOR. Proc Natl Acad Sci U S A. 2024 Aug 6;121(32):e2404146121. PMID: PMC11317610.
- c. **Vining, K.H.**, Marneth AE, Adu-Berchie K, Grolman JM, Tringides CM, Liu Y, Wong WJ, Pozdnyakova O, Severgnini M, Stafford A, Duda GN, Hodi FS, Mullally A, Wucherpennig KW, Mooney DJ. Mechanical checkpoint regulates monocyte differentiation in fibrotic niches. Nat Mater. 2022 Aug;21(8):939-950. PMID: PMC10197159.
- d. Adu-Berchie K, Liu Y, Zhang DKY, Freedman BR, Brockman JM, **Vining, K.H.**, Nerger BA, Garmilla A, Mooney DJ. Generation of functionally distinct T-cell populations by altering the viscoelasticity of their extracellular matrix. Nat Biomed Eng. 2023 Nov;7(11):1374-1391. PMID: PMC10749992

Ongoing and recently completed projects:

NSF/MRSEC DMR 2309043

Stach (PI); Vining and Purohit (Roles: Seed Grant Recipient)

6/1/2025-5/31/2027

IRG1 Seed Grant: Adaptive strain-responsive biopolymer networks

NIH/NIGMS R35 GM157079

Vining (PI)

1/01/25-12/31/29

Immuno-mechanical regulation of monocytes in fibrotic niches

NIH/NIDCR R00 DE030084

Vining (PI)

07/01/22-06/30/26 (NCE)

Targeting Mechanical Regulation of Monocyte Fate in Head and Neck Cancer

B. Positions, Scientific Appointments, and Honors

Positions

- 2022-Present Assistant Professor of Preventive and Restorative Sciences, School of Dental Medicine
Assistant Professor of Materials Science and Engineering, School of Engineering and Applied Science, University of Pennsylvania, Philadelphia, PA (full-time)
- 2022-Present General Dentist, Penn Dental Family Practice, Philadelphia, PA (part-time)
- 2022-Present Dental License, Pennsylvania State Board of Dentistry
- 2021-22 Scientist I, Dana-Farber Cancer Institute, Boston, MA (full-time)
- 2016-21 Bioengineering Fellow, Harvard University, Cambridge, MA (full-time)
- 2014-24 Dental License, Massachusetts Board of Registration in Dentistry
- 2014-22 General Dentist, Brookline Dental Specialists, Brookline, MA (part-time)
- 2014-16 Graduate Research Assistant, Harvard University, Cambridge, MA (full-time)
- 2012-13 Research Trainee, NIH Medical Research Scholars Program, Bethesda, MD (full-time)
- 2010-12 Research Assistant, University of Minnesota School of Dentistry, Minneapolis, MN (part-time)

Scientific Appointments

- 2025-Present Ad-hoc Study Section Member, Musculoskeletal, Skin and Oral Sciences (MSOS), NIH
- 2025-Present Vice President, Oral and Maxillofacial Surgery Group, IADR (voluntary)
- 2025-Present Standing Study Section Member, American Cancer Society Postdoctoral Fellowship, Cancer Biology and Immunology
- 2025-Present Member, Penn Cell and Molecular Biology Graduate Group
- 2023-Present Member, Penn Mechanical Engineering and Applied Mechanics Graduate Group
- 2022-Present Member, Penn Bioengineering Graduate Group
- 2022-Present Member, Penn Materials Science and Engineering Graduate Group
- 2022-Present Full Member, Center for Innovation & Precision Dentistry, University of Pennsylvania
- 2022-Present Full Member, Abramson Cancer Center, Philadelphia, PA
- 2022-Present Full Member, Penn Center for Musculoskeletal Disorders, University of Pennsylvania

2022-Present Affiliate Member, Center for Engineering Mechanobiology, Philadelphia, PA
 2022-Present Member, Penn Institute for Regenerative Medicine (IRM), University of Pennsylvania
 2021-Present Member, American Chemical Society (ACS)
 2021-Present Member, Biomedical Engineering Society (BMES)
 2021-2024 Councilor, Oral and Maxillofacial Surgery Group, IADR (voluntary)
 2021 Science First Task Force, AADOCR (voluntary)
 2018-2020 Mentor, MDS/American Student Dental Association Mentor Program (voluntary)
 2016-Present Member, Society for Biological Engineering
 2016-2020 Judge, National Collegiate Research Conference, Harvard University (voluntary)
 2013-2014 Vice President, AADR National Student Research Group (voluntary)
 2013-2014 Student Ambassador, University of Minnesota School of Dentistry (voluntary)
 2013-2014 Evidence-Based Dentistry Task Force, Minnesota Dental Association (voluntary)
 2012-2013 Vice President-elect, AADR National Student Research Group (voluntary)
 2011-Present Member, American Association for Dental, Oral and Craniofacial Research (AADOCR)
 2011-Present Member, International Association for Dental Research (IADR)
 2011-2012 Vice President, Minnesota Dental Student Research Group (voluntary)
 2009-Present Member, American Dental Association (ADA)

Honors

2025 RESTORE Prize, Center for Innovation and Precision Dentistry and Schoenleber Fund
 2025 Institute of Regenerative Medicine Collaborative Research Grant, University of Pennsylvania
 2024 Individual Biomedical Research Award, Hartwell Foundation
 2024 Joseph and Josephine Rabinowitz Award for Excellence in Research, Penn Dental Medicine
 2024 Research Scholar Grant, American Cancer Society
 2023 IDEA Prize, Center for Innovation and Precision Dentistry and Penn Health-Tech
 2021 American Society of Hematology Abstract Achievement Award
 2021 NIH/NIDCR K99 Pathway to Independence Award
 2021 First Place, AADOCR Hatton Competition, Senior Category
 2021 Second Place, AADOCR Boston Section Research Award, Boston, MA
 2016 NIH/NIDCR K08 Mentored Clinician Scientist Career Development Award
 2016 Second Place, Emerging Technologies Competition, Royal Society of Chemistry, London, UK
 2014-2015 Anne and Marcus Wedner Graduate Research Fellowship, Harvard University
 2014 Great Lakes National Scholarship
 2014 Henry M. Thornton/SCADA Fellowship Award
 2014 ADEA Scholarship for Predoctoral Dental Students Pursuing Academic Careers
 2014 Omicron Kappa Upsilon National Dental Honor Society
 2014 Undergraduate Certificate of Merit, Pierre Fauchard Academy
 2013-2014 George S. Monson Scholarship, Saint Paul District Dental Society
 2013-2014 Fellow, ADEAGies/AADR Academic Dental Careers Fellowship Program
 2013-2014 Academic Dental Careers Fellowship Program, ADEA
 2013 First Place, AADR Hatton Awards Competition, Junior Category, Seattle, WA
 2013 First Place, IADR Hatton Awards Competition, Junior Category, Seattle, WA
 2013 Wrigley Clinical Salivary Research Award for Dental Students, Seattle, WA
 2012 Third Place Poster, American Student Dental Association Annual Session, Minneapolis, MN
 2012 Rapid Fire Poster Award, World Biomaterials Congress, Chengdu, China
 2012 Student Research Fellowship, Implantology Research Group, IADR
 2011 ADA/Dentsply Award, University of Minnesota School of Dentistry
 2009 Best Research Award, Biomedical Engineering, Northwestern University
 2008 Sigma Xi Student Travel Grant, Northwestern University
 2007 First Place Poster, Minnesota Materials Research Science and Engineering Center
 2007 NSF Research Experience for Undergraduates Summer Research Fellowship

C. Contributions to Science

1. I investigate the impact of viscoelasticity on inflammation in fibrotic tissues and develop new immunotherapies in cancer. I study the role of monocytes, which impact the local immune responses by producing cytokines and differentiating into phagocytic and antigen-presenting cells, like macrophages and dendritic cells. I utilize a collagen-alginate ECM hydrogel to dissect a mechanical checkpoint of monocyte fate and develop strategies to target myeloid inflammation in vivo. I first investigated this concept in hematopoietic malignancies that result in myelofibrosis, which was published in *Nature Materials* (Vining et al., 2022). A major impact of my work was revealing that changes of viscoelasticity in diseases associated with myelofibrosis, more than changes in stiffness alone, regulate inflammation and differentiation of myeloid cells, and are targetable by a PI3K-gamma inhibitor. This work has a potential impact more broadly for fibrotic and inflammatory diseases. My K99/R00 research examines the mechanical regulation of myeloid cells in head and neck cancer (Li, et al., 2025). I was recently awarded an **American Cancer Society Research Scholar Grant** and **Hartwell Foundation Individual Biomedical Research Award** in 2024 for investigating the in vivo mechanism of inflammatory monocytes and CAR T cells in myelofibrosis and bone marrow malignancies (Adu-Berchie, et al., 2023).
 - a. **Vining, K.H.**, Marneth AE, Adu-Berchie K, Grolman JM, Tringides CM, Liu Y, Wong WJ, Pozdnyakova O, Severgnini M, Stafford A, Duda GN, Hodi FS, Mullally A, Wucherpennig KW, Mooney DJ. Mechanical checkpoint regulates monocyte differentiation in fibrotic niches. *Nat Mater*. 2022 Aug;21(8):939-950. PMID: PMC10197159.
 - b. Adu-Berchie K, Liu Y, Zhang DKY, Freedman BR, Brockman JM, **Vining, K.H.**, Neger BA, Garmilla A, Mooney DJ. Generation of functionally distinct T-cell populations by altering the viscoelasticity of their extracellular matrix. *Nat Biomed Eng*. 2023 Nov;7(11):1374-1391. PMID: PMC10749992
 - c. Zecheng Li, Yifei Ren, Sharvari Kemkar, Paul Mollenkopf, Jakub Kochanowski, Paul A Janmey, Prashant K Purohit, Ravi Radhakrishnan, **Kyle H Vining**. Modeling tumor transport and growth with poroelastic biopolymer networks. 2025 September. *BioRxiv* [preprint]. DOI: 10.1101/2025.09.23.678021.
2. I contributed to the understanding of how ECM mechanical properties direct the fate of bone marrow cells. The immune system develops in the bone marrow, which is viscoelastic, exhibiting properties of both a solid and a fluid. Healthy bone marrow is normally viscous and can be measured by its stress relaxation; stress dissipates rapidly over time as a static deformation is applied (Vining & Mooney, 2017). In diseases associated with bone marrow fibrosis (myelofibrosis), inflammation can result in bone marrow failure and poor patient survival. However, the direct impact of mechanical cues on immune cell fate remains poorly understood. I developed artificial systems with human cells to study how the mechanical resistance of ECM can direct the immune fate of mesenchymal stromal cells (MSCs) (Vining et al., 2019; Lim et al., 2024). An artificial fibrillar ECM was fabricated with interpenetrating networks of type-I collagen and chemically modified polysaccharides. Viscoelasticity was specifically tuned independently of other material properties across a physiologic range of bone marrow stiffness (Zhang et al., 2025). A more fluid-like, viscous matrix was associated with immunomodulatory expression of MSCs, which is consistent with homeostasis in healthy bone marrow. **I was recently awarded a NIGMS R35 MIRA award for this project.**
 - a. **Vining, K.H.**, Mooney, D.J. (2017). Mechanical forces direct stem cell behavior in development and regeneration. *Nature Reviews Molecular Cell Biology* 18, 728-742. PMID: PMC5803560
 - b. **Vining, K.H.**, Stafford, A., Mooney, D.J. (2019) Sequential modes of crosslinking tune viscoelasticity of cell-instructive hydrogels. *Biomaterials*, 188, 187-197. PMID: 30366219
 - c. Lim JJ, **Vining K.H.**, Mooney DJ, Blencowe BJ. Matrix stiffness-dependent regulation of immunomodulatory genes in human MSCs is associated with the lncRNA CYTOR. *Proc Natl Acad Sci U S A*. 2024 Aug 6;121(32):e2404146121. PMID: PMC11317610.
 - d. Zhang, K., Li, Z., Chen, Y.C., Yoon, I., Graham, A., **Vining, K.H.** Tunable Compressive Stiffening of Dual-Cross-Linked Alginate Hydrogels. *ACS Applied Bio Materials*, 2025. 10.1021/acsabm.5c00094
3. I also made contributions focused on designing and synthesizing new biomaterials. Developing new material-based therapies will require expanding our repertoire of materials and chemistries to enable new technologies. At Harvard University, during my Ph.D. in bioengineering, I examined the impact of the chemistry of an underlying synthetic substrate on the cell behavior of dental pulp stem cells (DPSCs). We

screened a diverse library of >100 synthetic polymers with a polymeric microarray for DPSC adhesion in serum-free media conditions. Cells only remained adhered and spread on polymers of triacrylates. We scaled-up triacrylates into light-curable bulk polymeric materials, which could be used similar to low-viscosity dental resins, and showed they supported differentiation and proliferation of DPSCs for regenerative dentistry applications. These works resulted in a first-author publication in *Advanced Materials* (Vining et al., 2018), a U.S. patent (11,224,679), and second place in the 2016 Emerging Technologies Competition of the Royal Society of Chemistry in London, UK, which was featured by the Washington Post, CBS News, Scientific American, Newsweek, Popular Science, and Dentistry Today. Currently, we are developing resin-based biocompatible materials for regenerative dentistry (Yan et al., 2025) and investigating the materials properties of the developing dentition (Jiang et al., 2025). In collaboration with chemical engineering Prof. Michael Mitchell at Penn, we recently developed new piperazine-linked bisphosphonate ionizable lipids to target lipid nanoparticle delivery to mineralized tissues (Yoon et al., 2024). Based on this work, I was recently awarded an **Institute of Regenerative Medicine Collaborative Research Grant** to develop lipid nanoparticles for local bone regeneration, as well as a **RESTORE Prize** to develop tumor exosome-lipid nanoparticle fusions for oral cancer immunotherapy.

- a. **Vining, K.H.**, Scherba, J., Bever, A., Alexander, M., Celiz, A.D., Mooney, D.J. Synthetic Light-Curable Polymeric Materials Provide a Supportive Niche for Dental Pulp Stem Cells. *Advanced Materials*. 2018. 30, 1704486. PMCID: PMC5788014
 - b. Luo Y, Zhang C, Fulco S, Liu J, Chen K, Hu Y, Jiang Y, Xu R, Rakesh L, Fusun O, Tertuliano O, Turner K, **Vining KH**. Biocompatible Multifunctional Polymeric Material for Mineralized Tissue Adhesion. *Adv Healthc Mater*. 2025 Aug 18:e01993. doi: 10.1002/adhm.202501993. Epub ahead of print. PMID: 40823909.
 - c. Jiang Y, Katsura KA, Badt NZ, Didziokas M, Dougherty S, Bhoj EJ, **Vining KH**. Multimodal Characterization of Rodent Dental Development. *ACS Appl Mater Interfaces*. 2025 Jun 11;17(23):33745-33755. doi: 10.1021/acsami.5c08408. Epub 2025 May 28. PMID: 40436377; PMCID: PMC12164827.
 - d. Yoon, I., Xue, L., Chen, Q., Liu, J., Xu, J., Siddiqui, Z., Kim, D., Chen, B., Shi, Q., Ruiz, M.C., **Vining, K.H.*** & Mitchell, M.J. Piperazine-derived bisphosphonate-based ionizable lipid nanoparticles enhance mRNA delivery to the bone microenvironment. *Angewandte Chemie*. 2024. doi/10.1002/anie.202415389, *Co-corresponding
4. Another important contribution I made was in stem cell biology and regenerative medicine. I completed a one-year pre-doctoral fellowship training in the **NIH Medical Research Scholars Program with Dr. Matthew Hoffman**. Our research goal was to develop a cell-based regenerative therapy to reverse irradiation-induced xerostomia in cancer patients by functionally restoring salivary glands. I demonstrated that factors involved in embryonic gland development can be used to improve adult progenitor cell maintenance and expansion in vitro, and that a neurotrophic growth factor-laden laminin hydrogel supported innervation and development of adult progenitor cells into branching epithelium (Vining et al., 2019). Ongoing research is working towards translating these findings in animal models to regenerate damaged salivary glands. I also investigated mRNA transfection (Ledo et al., 2020) and growth factor delivery (Vidovic-Zdrilic et al., 2018) using hydrogel systems for promoting musculoskeletal and dental pulp tissue regeneration, respectively.
- a. **Vining, K.H.**, Lombaert, I.M., et al. (2019). Neurturin-containing laminin matrices support innervated branching epithelium from adult epithelial salispheres. *Biomaterials*, 216, 119245. PMCID: PMC6720117
 - b. Ledo, A.M., **Vining, K.H.**, Alonso, M.J., Garcia-Fuentes, M., Mooney, D.J. (2020) Extracellular matrix mechanics regulate transfection and SOX9-directed differentiation of mesenchymal stem cells. *Acta Biomaterialia*, 110, 153-163. PMCID: PMC7291356.
 - c. Vidovic-Zdrilic, I., **Vining, K.H.**, Vijaykumar, A., Kalajzic, I., Mooney, D.J., Mina, M. (2018) FGF2 Enhances Odontoblasts Differentiation by α -SMA⁺ Progenitors in vivo. *Journal of Dental Research*. PMID: 29649366

Complete List of Published Work in MyBibliography and ORCID:

<https://www.ncbi.nlm.nih.gov/myncbi/kyle.vining.1/bibliography/public/>

BIOGRAPHICAL SKETCH

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

NAME: Wells, Rebecca

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

POSITION TITLE: Professor (with tenure) of Medicine

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)	END DATE MM/YYYY	FIELD OF STUDY
Yale University, New Haven, CT	BS	05/1983	Molecular Biophysics and Biochemistry
Johns Hopkins University School of Medicine, Baltimore, MD	MD	05/1987	Medicine
Brigham and Women's Hospital, Boston, MA	Resident	06/1990	Internal Medicine
Brigham and Women's Hospital/Harvard Medical School, Boston, MA	Fellow	06/1998	Nephrology/Gastroenterology, including research

A. Personal Statement

Dr. Wells is a co-mentor for Dr. Makkar. She is Professor of Medicine (with tenure) in the Division of Gastroenterology and Hepatology of the University of Pennsylvania, with a secondary appointment in Bioengineering. Her expertise is in the interactions between cells, matrix proteins, and mechanical factors as regulators of fibrosis and tissue behavior, particularly of the liver and biliary tree. She has been a leader in defining the role of mechanical factors in the progression of liver fibrosis and in myofibroblastic activation of fibrogenic precursor cells in the liver. She has defined the mechanical impact (including DNA damage) of lipid droplets in liver steatosis, working with theory colleagues to produce a combined experimental and theoretical analysis; more recent work includes the identification of cholesterol crystals in hepatocytes and the finding that these crystals stiffen the liver. Dr. Wells collaborates extensively with multiple investigators, both bench scientists and theoreticians, at the School of Engineering and Applied Sciences, combining her studies of liver/biliary fibrosis with an understanding of matrix biology and cell and tissue mechanics. She is well published and is nationally respected as a leader in her field, formerly serving as an Associate Editor of Gastroenterology, Chair of the American Association for the Study of Liver Diseases (AASLD) Special Interest Group in Fibrosis, Chair of the AASLD Annual Meeting Education Committee and a member of the AASLD Basic Research Committee. She was one of two founding Associate Editors of the American Gastroenterological Association journal Cellular and Molecular Gastroenterology and Hepatology (first impact factor 7.076). She has organized multiple scientific meetings including the 2018 AASLD Basic Science Symposium on "Matrix Biology and the Liver". Dr. Wells is a Co-Director and Director of Education of the multi-site NSF Science and Technology Center, the Center for Engineering MechanoBiology (CEMB). She is also a Co-Associate Director for the Penn NIDDK-funded (DDRCC P30) Center for Molecular Studies in Digestive and Liver Diseases. Dr. Wells is former Vice Chief for Research in the Division of Gastroenterology and Hepatology and a former Interim Chief of the Division (12/20-6/21) and was just chosen as the Chair-Elect of the Mechanobiology Subgroup of the Biophysical Society. She is a dedicated teacher, serving on multiple PhD thesis committees in 7 graduate groups in the Schools of Medicine and Engineering and teaching highly regarded and impactful courses co-listed in Cell Biology and Bioengineering. Dr. Wells has not published papers under any other name.

1. Chen D, Du Y, Llewellyn J, Bonna A, Zuo B, Janmey PA, Farndale RW, Wells RG. Versican binds collagen via its G3 domain and regulates the organization and mechanics of collagenous matrices.

J Biol Chem. 2024 Dec;300(12):107968. PubMed Central PMCID: PMC11626796.

2. Fan W, Adebowale K, Váncza L, Li Y, Rabbi MF, Kunimoto K, Chen D, Mozes G, Chiu DK, Li Y, Tao J, Wei Y, Adeniji N, Brunsing RL, Dhanasekaran R, Singhi A, Geller D, Lo SH, Hodgson L, Engleman EG, Charville GW, Charu V, Monga SP, Kim T, Wells RG, Chaudhuri O, Török NJ. Matrix viscoelasticity promotes liver cancer progression in the pre-cirrhotic liver. *Nature*. 2024 Feb;626(7999):635-642. PubMed Central PMCID: PMC10866704.
3. Loneker AE, Alisafaei F, Kant A, Li D, Janmey PA, Shenoy VB, Wells RG. Lipid droplets are intracellular mechanical stressors that impair hepatocyte function. *Proc Natl Acad Sci U S A*. 2023 Apr 18;120(16):e2216811120. PubMed Central PMCID: PMC10120019.
4. Georges PC, Hui JJ, Gombos Z, McCormick ME, Wang AY, Uemura M, Mick R, Janmey PA, Furth EE, Wells RG. Increased stiffness of the rat liver precedes matrix deposition: implications for fibrosis. *Am J Physiol Gastrointest Liver Physiol*. 2007 Dec;293(6):G1147-54. PubMed PMID: 17932231.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2025 - 2027	Chair-Elect and Chair, Mechanobiology Subgroup, The Biophysical Society
2023 -	Scientific Advisory Board Member, FOR5628, Multiscale MRE: In Vivo Physics of Cancer, DFG funded, Berlin
2022 -	Scientific Advisory Board Member, LiSyM-Cancer (Liver Systems Medicine Cancer) Center, BMBF funded
2022 -	External Advisory Board Member, UCSF Liver Center (DDRCC P30), San Francisco, CA
2022 - 2022	Visiting Scientist (on sabbatical), Charite Universitätsmedizin, Berlin
2021 -	Co-Director, NSF Center for Engineering MechanoBiology, Philadelphia, PA
2020 - 2021	Interim Co-Chief, Division of Gastroenterology and Hepatology, Department of Medicine, University of Pennsylvania, Philadelphia, PA
2019 -	Associate Director, UPenn NIDDK Center for Molecular Studies in Digestive and Liver Diseases (P30; DDRCC), Philadelphia, PA
2019 -	Scientific Advisory Board Member, Southern California Research Center for Alcohol and Liver and Pancreatic Diseases and Cirrhosis, Los Angeles, CA
2019 - 2023	Vice-Chief for Research, Division of Gastroenterology and Hepatology, University of Pennsylvania, Philadelphia, PA
2017 -	Professor (with tenure) of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA
2017 -	Director of Education and Member of the Executive Committee, NSF Science and Technology Center for Engineering Mechanobiology, Philadelphia, PA
2017 - 2019	External Advisory Board Member, University of Cincinnati NIDDK Silvio O. Conte Digestive Disease Center, Cincinnati, OH
2016 -	Secondary Appointment, Department of Bioengineering, School of Engineering and Applied Sciences, University of Pennsylvania, Philadelphia, PA
2016 - 2017	Visiting Scholar (on sabbatical), Boston University Department of Biomedical Engineering, Boston, MA
2013 - 2019	Founding Associate Editor, Cellular and Molecular Gastroenterology and Hepatology
2011 - 2017	Associate Professor (with tenure) of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA
2009 - 2011	Chair, American Association for the Study of Liver Diseases, Fibrosis Special Interest Group
2006 -	Member, Graduate Group in Bioengineering, University of Pennsylvania, Philadelphia, PA

2006 - 2011	Associate Editor, Gastroenterology
2005 - 2009	Standing Member, Hepatobiliary Pathophysiology Study Section, NIH, Bethesda, MD
2002 -	Member, Cell and Molecular Biology Graduate Group, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA
2002 - 2011	Assistant Professor of Medicine, University of Pennsylvania, Philadelphia, PA
1998 - 2002	Assistant Professor of Medicine (Digestive Diseases) and Pathology, Yale University School of Medicine, New Haven, CT
1992 - 1998	Visiting Scientist, The Whitehead Institute for Biomedical Research, Cambridge, MA

Honors

2018	Neufeld Award winner (with Orith Waisbourd-Zinman), US-Israel Binational Science Foundation
2016	Elected Fellow, American Gastroenterological Association
2009	Sir William Osler Young Investigator Award, Interurban Clinical Club
2008	Elected member, American Society for Clinical Investigation
2003	AGA/Miles and Shirley Fiterman Basic Research Award, American Gastroenterological Association
1991 - 1994	Physician Scientist Award, Howard Hughes Medical Institute
1987	Elected member, Alpha Omega Alpha Honor Society
1983	Graduated magna cum laude with Distinction in Molecular Biophysics and Biochemistry, Yale University

C. Contribution to Science

1. The mechanics of liver fibrosis: I have made significant relevant contributions to understanding the role of mechanics in liver injury and fibrosis. Liver fibrosis was classically viewed as the result of the injury-induced secretion of soluble factors, particularly TGF-beta. We demonstrated that the fibrogenic cells in liver fibrosis (hepatic stellate cells and portal fibroblasts) require a mechanically-stiff environment (Olsen et al) in order to differentiate to fibrogenic myofibroblasts, and showed that the liver becomes stiff before it becomes fibrotic. This early increase in stiffness is largely due to lysyl oxidase-mediated collagen cross-linking. This lysyl oxidase is from the same cells that produce matrix. We have also characterized changes in tissue mechanics of the normal and fibrotic liver. Braun et al. resulted from a sabbatical in Berlin where I carried out magnetic resonance elastography on liver tissues with varying degrees of fibrosis. It is the first of several papers resulting from this work, and is notable in demonstrating that water diffusion may predict tissue organization, including inflammation vs. fibrosis. Papers listed after the personal statement all also relate to this topic, and demonstrate a role for mechanics in steatotic liver disease and liver cancer.
 - a. Braun J, Bernarding J, Snellings J, Meyer T, Dantas de Moraes PA, Safrdou Y, Wells RG, Guo J, Tzschätzsch H, Zappe A, Pagel K, Sauer IM, Hillebrandt KH, Sack I. On the relationship between viscoelasticity and water diffusion in soft biological tissues. *Acta Biomater.* 2024 Jul 1;182:42-53. PubMed PMID: 38729549.
 - b. Perepelyuk M, Chin L, Cao X, van Oosten A, Shenoy VB, Janmey PA, Wells RG. Normal and Fibrotic Rat Livers Demonstrate Shear Strain Softening and Compression Stiffening: A Model for Soft Tissue Mechanics. *PLoS One.* 2016;11(1):e0146588. PubMed Central PMCID: PMC4703410.
 - c. Perepelyuk M, Terajima M, Wang AY, Georges PC, Janmey PA, Yamauchi M, Wells RG. Hepatic stellate cells and portal fibroblasts are the major cellular sources of collagens and lysyl oxidases in normal liver and early after injury. *Am J Physiol Gastrointest Liver Physiol.* 2013 Mar 15;304(6):G605-14. PubMed Central PMCID: PMC3602686.
 - d. Olsen AL, Bloomer SA, Chan EP, Gaça MD, Georges PC, Sackey B, Uemura M, Janmey PA,

Wells RG. Hepatic stellate cells require a stiff environment for myofibroblastic differentiation. *Am J Physiol Gastrointest Liver Physiol*. 2011 Jul;301(1):G110-8. PubMed Central PMCID: PMC3129929.

2. The interrelated roles of matrix and mechanics on myofibroblast biology and hepatic stellate cell behavior: As part of our studies of liver fibrosis, we have made important contributions to understanding the biology of hepatic stellate cells and portal fibroblasts and the factors that influence their behavior and differentiation to myofibroblasts. This includes a demonstration of the synergistic impact of stiffness and TGF-beta on liver myofibroblast behavior, and the development of novel cell culture systems to study the interrelated roles of stiffness and variations in matrix proteins on myofibroblast differentiation. I was the senior author for Saums et al, where I mentored an undergraduate (the first author) in developing a new matrix- and mechanically-tunable cell culture system for fibrogenic cells of the liver. Charrier et al. was part of a collaborative study examining collagen cross-linking and viscosity in a whole organ context. All 3 papers demonstrated a significant impact of mechanical factors on hepatic stellate cell and liver tissue behavior, highly relevant to liver fibrosis.
 - a. Charrier EE, Pogoda K, Wells RG, Janmey PA. Control of cell morphology and differentiation by substrates with independently tunable elasticity and viscous dissipation. *Nat Commun*. 2018 Jan 31;9(1):449. PubMed Central PMCID: PMC5792430.
 - b. Caliri SR, Perepelyuk M, Cosgrove BD, Tsai SJ, Lee GY, Mauck RL, Wells RG, Burdick JA. Stiffening hydrogels for investigating the dynamics of hepatic stellate cell mechanotransduction during myofibroblast activation. *Sci Rep*. 2016 Feb 24;6:21387. PubMed Central PMCID: PMC4764908.
 - c. Saums MK, Wang W, Han B, Madhavan L, Han L, Lee D, Wells RG. Mechanically and chemically tunable cell culture system for studying the myofibroblast phenotype. *Langmuir*. 2014 May 20;30(19):5481-7. PubMed Central PMCID: PMC4030828.
3. The nature and mechanism of matrix proteins regulating cell behavior: As part of our studies of the relationship between matrix and mechanical factors in determining cell behavior, particularly in wound healing and cancer, I have directed and been involved in collaborative work demonstrating that these factors are critical in a variety of specific settings including liver and breast cancer and, more generally, in long-range cell-cell interactions. Olsen et al. (and others not listed), on which I was senior author, debunked long-standing dogmas about the functions of specific matrix proteins. Wang et al., on which I was a collaborator, was a theoretical study demonstrating the importance of a cross-linked fibrous network on cell-cell communication. This is highly relevant to liver fibrosis and provides a partial theoretical explanation for our previous finding on the importance of matrix and tissue stiffness to the progression of fibrosis. Chen X. et al. is a theory paper for which my lab did the experimental work; it showed that the presence of glycosaminoglycans such as hyaluronic acid alters the fibrous network-based long-range cell-cell communication described in Wang et al.. Chen D. et al., on which I was senior author, demonstrated that different matrix proteoglycans have different effects on collagen fibrillogenesis and organization.
 - a. Chen X, Chen D, Ban E, Toussaint KC, Janmey PA, Wells RG, Shenoy VB. Glycosaminoglycans modulate long-range mechanical communication between cells in collagen networks. *Proc Natl Acad Sci U S A*. 2022 Apr 12;119(15):e2116718119. PubMed Central PMCID: PMC9169665.
 - b. Chen D, Smith LR, Khandekar G, Patel P, Yu CK, Zhang K, Chen CS, Han L, Wells RG. Distinct effects of different matrix proteoglycans on collagen fibrillogenesis and cell-mediated collagen reorganization. *Sci Rep*. 2020 Nov 4;10(1):19065. PubMed Central PMCID: PMC7642422.
 - c. Wang H, Abhilash AS, Chen CS, Wells RG, Shenoy VB. Long-range force transmission in fibrous matrices enabled by tension-driven alignment of fibers. *Biophys J*. 2014 Dec 2;107(11):2592-603. PubMed Central PMCID: PMC4255175.
 - d. Olsen AL, Sackey BK, Marcinkiewicz C, Boettiger D, Wells RG. Fibronectin extra domain-A promotes hepatic stellate cell motility but not differentiation into myofibroblasts. *Gastroenterology*. 2012 Apr;142(4):928-937.e3. PubMed Central PMCID: PMC3321084.

4. New understanding of mechanisms of biliary damage, repair and fibrosis: Biliary atresia is the most common cause of liver fibrosis and the major indication for liver transplant in the pediatric population. It is characterized by fibrosis of the extrahepatic biliary tree and rapidly leads to cirrhosis of the liver. The etiology is unknown but likely involves an environmental insult. We have produced a body of novel work addressing the mechanism of this disease. We isolated (Lorent et al.) and synthesized a novel plant toxin (biliatresone) that causes biliary atresia in zebrafish and mammals, a proof of concept that biliary atresia could have a toxic cause, and in work not listed identified other toxins with similar motifs that specifically damage the neonatal extrahepatic bile duct. We identified the mechanism of biliatresone (waisbourd-Zinman et al.) and developed a microfluidic device mimicking the extrahepatic bile duct that demonstrates physiologic-levels of impermeability and shows differential effects of biliatresone and bile acids on the apical and basal surfaces of cholangiocytes (Du et al.). We have demonstrated significant differences between adult and neonatal extrahepatic bile ducts and suggest that the neonatal duct is particularly susceptible to injury, and have shown that fetal bile duct healing involves a program of fetal wound healing, with significant implications related to the role of hyaluronic acid (de Jong et al.). I was the senior or co-senior author for all of these papers.
 - a. de Jong IEM, Hunt ML, Chen D, Du Y, Llewellyn J, Gupta K, Li D, Erxleben D, Rivas F, Hall AR, Furth EE, Naji A, Liu C, Dhand A, Burdick JA, Davey MG, Flake AW, Porte RJ, Russo PA, Gaynor JW, Wells RG. A fetal wound healing program after intrauterine bile duct injury may contribute to biliary atresia. *J Hepatol.* 2023 Dec;79(6):1396-1407. PubMed Central PMCID: PMC10841314.
 - b. Du Y, Khandekar G, Llewellyn J, Polacheck W, Chen CS, Wells RG. A Bile Duct-on-a-Chip With Organ-Level Functions. *Hepatology.* 2020 Apr;71(4):1350-1363. PubMed Central PMCID: PMC7048662.
 - c. Waisbourd-Zinman O, Koh H, Tsai S, Lavrut PM, Dang C, Zhao X, Pack M, Cave J, Hawes M, Koo KA, Porter JR, Wells RG. The toxin biliatresone causes mouse extrahepatic cholangiocyte damage and fibrosis through decreased glutathione and SOX17. *Hepatology.* 2016 Sep;64(3):880-93. PubMed Central PMCID: PMC4992464.
 - d. Lorent K, Gong W, Koo KA, Waisbourd-Zinman O, Karjoo S, Zhao X, Sealy I, Kettleborough RN, Stemple DL, Windsor PA, Whittaker SJ, Porter JR, Wells RG, Pack M. Identification of a plant isoflavonoid that causes biliary atresia. *Sci Transl Med.* 2015 May 6;7(286):286ra67. PubMed Central PMCID: PMC4784984.
5. The anatomy of interstitial spaces: My group and collaborators have defined previously unappreciated features of the submucosal/dermal spaces, namely that they support fluid flow and are widely interconnected. This work expanded the concept of interstitial spaces to include large interstitial spaces and has significant implications for body-wide fluid management, signaling, and cancer metastasis. I was co-first author on Benias et al., co-senior author on Cenaj et al., and senior author on de Jong et al.
 - a. de Jong IEM, Theise ND, Wells RG. The space of Mall confirmed in humans: A response to "Portal venous branches as an anatomic railroad for a gut-bile duct axis". *J Hepatol.* 2024 Mar;80(3):e126-e127. PubMed PMID: 37821022.
 - b. Cenaj O, Allison DHR, Imam R, Zeck B, Drohan LM, Chiriboga L, Llewellyn J, Liu CZ, Park YN, Wells RG, Theise ND. Evidence for continuity of interstitial spaces across tissue and organ boundaries in humans. *Commun Biol.* 2021 Mar 31;4(1):436. PubMed Central PMCID: PMC8012658.
 - c. Benias PC, Wells RG, Sackey-Aboagye B, Klavan H, Reidy J, Buonocore D, Miranda M, Kornacki S, Wayne M, Carr-Locke DL, Theise ND. Structure and Distribution of an Unrecognized Interstitium in Human Tissues. *Sci Rep.* 2018 Mar 27;8(1):4947. PubMed Central PMCID: PMC5869738.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Hajishengallis, Georgios (George)

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

POSITION TITLE: Thomas W. Evans Centennial 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)	END DATE MM/YYYY	FIELD OF STUDY
University of Athens, School of Dentistry, Athens, Greece	D.D.S.	09/1989	Dentistry
University of Alabama at Birmingham, Birmingham, AL, USA	Ph.D.	12/1994	Cellular and Molecular Biology
University of Alabama at Birmingham, Birmingham AL, USA	Postdoctoral Fellow	09/1997	Microbiology and Immunology

A. Personal Statement

My laboratory has a long-standing interest in the local and systemic regulation of the host inflammatory response and its resolution in the context of bone loss disorders and comorbidities (see related references for Reviews below; original papers in Section C). My laboratory's work is highly cited (Google Scholar citations 47,163; H-Index=101) and combines basic immunological and translational interdisciplinary research (including rodent and non-human primate preclinical models), leading to innovative approaches to treatment, as exemplified by a clinical intervention trial for the treatment of human periodontal inflammation (see section C2). The impact of our research extends across different disciplines and our publication record includes papers in *Cell*, *Nature Immunology*, *Cell Host & Microbe*, *J. Clin. Invest.*, *Science Transl. Med.*, *N. Engl. J. Med.*, *Nature Communications*, *Molecular Psychiatry*, *PNAS*, as well as in *Nature Reviews* (Immunology, Microbiology). Concepts that we first discovered in the context of periodontitis have found application in other fields; for instance, our demonstration that the secreted protein DEL-1 homeostatically regulates inflammation in the periodontium was subsequently shown to be relevant in neuroinflammation/multiple sclerosis and rheumatoid arthritis. I have successfully directed several NIH-supported projects (see selected projects below) that have led to the dissection of the role of important regulatory and effector mechanisms of innate and adaptive immunity (e.g., DEL-1, complement, neutrophils, Th17 cells, Treg cells, IL-17, IL-22) in host defense and inflammation in different disease models, including periodontitis, which we have also explored in the context of aging. In recent years, we made significant contributions in establishing the bone marrow hematopoietic stem and progenitor cells (HSPCs) as the central hub in the generation and maintenance of innate/inflammatory immune memory that underlies trained immunity. We have also shown that maladaptive trained innate immunity constitutes a mechanistic basis for the comorbid connection between periodontitis and inflammatory arthritis (section C5). Alterations to HSPCs leading to the generation of progeny with enhanced proinflammatory capacity may result also from clonal hematopoiesis of indeterminate potential (CHIP), which arises from aging-related accumulation of somatic mutations in epigenetic regulators. In this context, we have shown that mutant *DNMT3A*-driven CHIP exacerbates the severity of periodontal disease and inflammatory arthritis. I have not published or created research products under any other name. Based on my expertise in inflammation and periodontal disease pathogenesis, I am well suited to advise Dr. Hardik Makkar on his proposed project to study the mechanical regulation of inflammation in periodontal disease.

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

- R01- DE033643, NIH/NIDCR, Hajishengallis, G (PI), 04/01/2024 – 03/31/2029
Clonal hematopoiesis and periodontal disease
- R01-DE031206, NIH/NIDCR, Hajishengallis, G (PI), 02/01/2022 – 01/31/2027

- Trained innate immunity and periodontitis-associated comorbidities
- R01-DE021104, NIH/NIDCR, Hajishengallis, G (MPI), 09/01/2023 – 05/31/2027
New upstream targets for HIF-1a-mediated regeneration in young and aged animals
- MERIT AWARD / R37-DE026152, NIH/NIDCR, Hajishengallis, G (PI), 08/01/2021 – 07/31/2026
Local endogenous regulators of functional immune plasticity in the periodontium
- R01-DE029436-01, NIH/NIDCR, Hajishengallis, G. (PI), 04/01/2020 – 03/31/2026 (NCE)
IL-22, immune plasticity, and autotherapy in the periodontium
- R01-DE028561 NIH/NIDCR, Hajishengallis, G. (Role: PI), 01/01/2020 – 12/31/2025 (NCE)
Aging and dysfunction of progenitor niches: Role of DEL-1

Citations (Reviews; see section C for primary research papers)

1. Chavakis T, Mitroulis I, **Hajishengallis G**. Hematopoietic progenitor cells as integrative hubs for adaptation to and fine-tuning of inflammation. *Nat Immunol*. 20:802-811 (2019). PMID: PMC6709414.
2. **Hajishengallis G**, Chavakis T. Local and systemic mechanisms linking periodontal disease and inflammatory comorbidities. *Nat Rev Immunol*. 21:426–440 (2021). PMID: PMC7841384.
3. **Hajishengallis G**, Lamont RJ, Koo H. Oral polymicrobial communities: Assembly, function, and impact on diseases. *Cell Host & Microbe* 31:528-538 (2023). PMID: PMC10101935.
4. **Hajishengallis G**, Netea MG and Chavakis, T. Trained immunity in chronic inflammatory diseases and cancer. *Nat Rev Immunol* 25 (On-line ahead of print)

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2019 - present	Thomas W. Evans Centennial Professor, University of Pennsylvania, Penn Dental Medicine, Department of Basic and Translational Sciences, Philadelphia, PA
2015 - 2019	Thomas W. Evans Centennial Professor, University of Pennsylvania, Penn Dental Medicine, Department of Microbiology, Philadelphia, PA
2012 - 2014	Professor, University of Pennsylvania, Penn Dental Medicine, Department of Microbiology, Philadelphia, PA
2010 - 2011	Professor and Distinguished University Scholar, University of Louisville, Center for Oral Health and Systemic Disease, Louisville, KY
2008 - 2010	Professor, University of Louisville, Center for Oral Health and Systemic Disease, Louisville, KY
2005 - 2008	Associate Professor, University of Louisville, Center for Oral Health and Systemic Disease, Louisville, KY
2003 - 2005	Assistant Professor, Louisiana State University Health Sciences Center, Department of Microbiology, Immunology, and Parasitology, New Orleans, LA
2001 - 2003	Research Assistant Professor, University at Buffalo, Department of Oral Biology, Buffalo, NY
2000 - 2001	Postdoctoral Associate, University at Buffalo, Department of Oral Biology, Buffalo, NY
1997 - 1999	Research Assistant Professor, University of Alabama at Birmingham, Department of Oral Biology, Birmingham, AL

Honors

2024	Highly Cited Researcher (Cross-Field category), Clarivate Analytics/Web of Science
2023	Highly Cited Researcher (Cross-Field category), Clarivate Analytics/Web of Science
2022	Highly Cited Researcher (Cross-Field category), Clarivate Analytics/Web of Science
2021	Highly Cited Researcher (Cross-Field category), Clarivate Analytics/Web of Science
2021	Keynote Speaker, NIH workshop on Metabolic Regulation of Inflammation and its Resolution
2020	Highly Cited Researcher (Cross-Field category), Clarivate Analytics/Web of Science
2018	Dean's Distinguished Professorship lecture, Perelman School of Medicine, Univ. Pennsylvania
2018	Highly Cited Researcher (Cross-Field category), Clarivate Analytics/Web of Science
2016	MERIT Award, NIH/NIDCR
2016	Keynote lecturer, British Society for Oral & Dental Research
2015	Endowed Professorship (Thomas W. Evans Centennial Professor), University of Pennsylvania

2014	William J. Gies Award in the Biological Research Category, AADR/IADR
2012	Distinguished Scientist Award in Oral Biology, International Association for Dental Research
2010	Distinguished University Scholar Award, University of Louisville
2008	Fellow, Japanese Society for the Promotion of Science
2006	Faculty Excellence Honor, University of Louisville
2005	Research Excellence Award, Louisiana State University School of Dentistry
2004	Research Excellence Award, Louisiana State University School of Dentistry
2001	Travel Award, International Association for Dental Research
1995	Travel Award, International Association for Dental Research
1995	Hatton Award, American Association for Dental Research

C. Contributions to Science

1. **Keystone pathogen-induced dysbiosis:** My laboratory's work has challenged established paradigms of periodontal disease pathogenesis by proposing that periodontitis is not a bacterial infection in the classical sense (i.e., not caused by a single or a select few "periopathogens") but rather results from polymicrobial synergy and dysbiosis (PSD). According to the PSD model of periodontitis, the disease is caused by reciprocally reinforced interactions between physically and metabolically integrated polymicrobial communities and a dysregulated host inflammatory response. Through collaborative research, we introduced the keystone-pathogen concept, according to which certain low-abundance microbes manipulate the host response and remodel a normally benign microbiota into a dysbiotic one. This work led to a re-evaluation of the roles of *Porphyromonas gingivalis* and other red complex bacteria in periodontitis.
 - a. **Hajishengallis G**, Liang S, Payne MA, Hashim A, Jotwani R, Eskan MA, McIntosh ML, Alsam A, Kirkwood KL, Lambris JD, Darveau RP, Curtis MA. Low-abundance biofilm species orchestrates inflammatory periodontal disease through the commensal microbiota and complement. *Cell Host Microbe*. 10:497-506 (2011). PMID: PMC3221781.
 - b. Maekawa T, Krauss JL, Abe T, Jotwani R, Triantafilou M, Triantafilou K, Hashim A, Hoch S, Curtis MA, Nussbaum G, Lambris JD, **Hajishengallis G**. *Porphyromonas gingivalis* manipulates complement and TLR signaling to uncouple bacterial clearance from inflammation and promote dysbiosis. *Cell Host Microbe*. 15:768-78. (2014) PubMed Central PMID: PMC4071223.
 - c. Lamont RJ, **Hajishengallis G**. Polymicrobial synergy and dysbiotic inflammation. *Trends Transl. Med*. 21:172-183 (2015). PMID: PMC4352384.
 - d. Hoare A, Wang H, Meethil A, Abusleme L, Hong B-Y, Moutsopoulos NM, Marsh PD, **Hajishengallis G**, Diaz PI. A cross-species interaction with a symbiotic commensal enables cell-density-dependent growth and in vivo virulence of an oral pathogen. *ISME J* 15:1490-1504 (2021). PMID: PMC8115154.
2. **Complement-targeted host modulation in periodontitis.** I have led collaborative studies that established complement as a key player in periodontitis, being involved both in the dysbiotic transformation of the periodontal microbiome and the ensuing destructive inflammation. Importantly, we showed that complement inhibition by a C3-targeted drug (compstatin Cp40; aka AMY-101) protects against periodontal inflammation and bone loss in non-human primates. In 2019, AMY-101 received Investigational New Drug approval by the U.S. Food and Drug Administration for the conduct of the first clinical study to evaluate its efficacy in adults with periodontal inflammation (gingivitis) (Clinical Trials Identifier: NCT03694444). This Phase 2a study was successfully completed and showed that AMY-101 blocks gingival inflammation in patients with periodontal disease.
 - a. Maekawa T, Abe T, Hajishengallis E, Hosur KB, DeAngelis RA, Ricklin D, Lambris JD, **Hajishengallis G**. Genetic and intervention studies implicating complement C3 as a major target for the treatment of periodontitis. *J Immunol*. 2014 Jun 15;192(12):6020-7. PubMed Central PMID: PMC4078411.
 - b. Maekawa T, Briones RA, Resuello RR, Tuplano JV, Hajishengallis E, Kajikawa T, Koutsogiannaki S, Garcia CA, Ricklin D, Lambris JD, **Hajishengallis G**. Inhibition of pre-existing natural periodontitis in non-human primates by a locally administered peptide inhibitor of complement C3. *J Clin Periodontol*. 2016 Mar;43(3):238-49. PubMed Central PMID: PMC4803614.

- c. Hasturk H, **Hajishengallis G**, Lambris JD, Mastellos DC, Yancopoulou D. Phase 2a clinical trial of complement C3 inhibitor AMY-101 in adults with periodontal inflammation. *J Clin Invest* 131:e152973 (2021). PMID: PMC8631591
 - d. **Hajishengallis G**, Hasturk H, Lambris JD and contributing authors. C3-targeted therapy in periodontal disease: moving closer to the clinic. *Trends Immunol* 42:856-864 (2021). PMID: PMC8487962
3. **Regulation of immune system plasticity and homeostasis by DEL-1 (in the context of aging):** My laboratory has developed new models and attained important insights into the impact of aging on innate immunity and periodontitis, the most influential being the discovery of aging-related declined expression of DEL-1, a secreted homeostatic protein. We attained important insights into the regulation of tissue homeostasis and immune plasticity by DEL-1, which, among other functions, regulates the recruitment and apoptotic clearance (efferocytosis) of neutrophils, the stability and function of T regulatory cells, and the activation of T helper follicular cells. DEL-1 deficiency was causally linked to periodontitis and arthritis in preclinical models. We documented that the diverse functions of DEL-1 depend on the location and cell types that secrete it ('location principle'); for instance, endothelial cell-derived DEL-1 exerts anti-leukocyte recruitment function while macrophage-derived DEL-1 mediates efferocytosis. These mechanisms involve both the initiation and resolution of inflammation and thus have profound implications for the treatment of inflammatory and autoimmune conditions.
- a. Eskin MA, Jotwani R, Abe T, Chmelar J, Lim JH, Liang S, Ciero PA, Krauss JL, Li F, Rauner M, Hofbauer LC, Choi EY, Chung KJ, Hashim A, Curtis MA, Chavakis T*, **Hajishengallis G***. The leukocyte integrin antagonist Del-1 inhibits IL-17-mediated inflammatory bone loss. *Nat Immunol.* 13:465-73 (2012). PMID: PMC3330141. *Contributed equally as senior authors.
 - b. Kourtzelis I, Li X, Mitroulis I, Grosser D, Kajikawa T, Wang B, Grzybek M, von Renesse J, Czogalla A, Troullinaki M, Ferreira A, Doreth C, Ruppova K, Chen LS, Hosur K, Lim JH, Chung KJ, Grossklaus S, Tausche AK, Joosten LAB, Moutsopoulos NM, Wielockx B, Castrillo A, Korostoff JM, Coskun Ü, **Hajishengallis G***, Chavakis T*. DEL-1 promotes macrophage efferocytosis and clearance of inflammation. *Nat Immunol.* 20:40-49 (2019). PMID: PMC6291356. *Co-senior and co-corresponding authors.
 - c. Li X, Colamatteo A, Kalafati L, Kajikawa T, Wang H, Lim JH, Bdeir K, Chung KJ, Yu X, Fusco C, Porcellini A, De Simone S, Matarese G, Chavakis T, De Rosa V, **Hajishengallis G**. The DEL-1/ β 3 integrin axis promotes regulatory T cell responses during inflammation resolution. *J Clin Invest.* 130:6261-6277 (2020). PMID: PMC7685741.
 - d. Wang H, Li X, Kajikawa T, Shin J, Lim J-H, Kourtzelis I, Nagai K, Korostoff J, Grossklaus S, Naumann R, Chavakis T, **Hajishengallis G**. Stromal cell-derived DEL-1 inhibits Tfh cell activation and inflammatory arthritis. *J Clin Invest* 131:e150578 (2021). PMID: PMC8483759.
4. **Cellular and molecular inflammatory mechanisms in periodontal and other mucosal tissues:** Our collaborative work with NIH scientists has conclusively implicated the IL-23/IL-17 axis and Th17 cells in inflammatory tissue destruction of the periodontal tissue in mice and humans. Human relevance of mechanistic mouse studies was established by clinical findings in subjects with a genetic defect in Th17 cell differentiation, who have reduced susceptibility to periodontitis. The relevance of the IL-23/IL-17 axis in destructive periodontal inflammation was also confirmed by our interventional studies in both mice and humans with an aggressive form of periodontitis (owing to leukocyte adhesion deficiency Type 1; LAD1). LAD1, which is caused by deficiency in leukocyte β 2-integrins, is also associated with colitis. In this context, we showed that β 2-integrins are required for effective production of intestinal IL-22, which is essential for host immunity against colitogenic bacteria.
- a. Moutsopoulos NM, Konkel J, Sarmadi M, Eskin MA, Wild T, Dutzan N, Abusleme L, Zenobia C, Hosur KB, Abe T, Uzel G, Chen W, Chavakis T, Holland SM, **Hajishengallis G**. Defective neutrophil recruitment in leukocyte adhesion deficiency type I disease causes local IL-17-driven inflammatory bone loss. *Sci Transl Med.* 6:229ra40 (2014). PMID: PMC4090608.
 - b. Moutsopoulos NM, Zerbe CS, Wild T, Dutzan N, Brenchley L, DiPasquale G, Uzel G, Axelrod KC, Lisco A, Notarangelo LD, **Hajishengallis G**, Notarangelo LD, Holland SM. Interleukin-12 and Interleukin-23

Blockade in Leukocyte Adhesion Deficiency Type 1. *N Engl J Med.* 376:1141-1146 (2017). PMID: PMC5494261.

- c. Dutzan N, Kajikawa T, Abusleme L, Greenwell-Wild T, Zuazo CE, Ikeuchi T, Brenchley L, Abe T, Hurabielle C, Martin D, Morell RJ, Freeman AF, Lazarevic V, Trinchieri G, Diaz PI, Holland SM, Belkaid Y, **Hajishengallis G***, Moutsopoulos NM*. A dysbiotic microbiome triggers T_H17 cells to mediate oral mucosal immunopathology in mice and humans. *Sci Transl Med.* 10:eaat0797 (2018). PMID: PMC6330016. **Senior authors.*
- d. Wang B, Lim JH, Kajikawa T, Li X, Vallance BA, Moutsopoulos NM, Chavakis T, **Hajishengallis G**. Macrophage β 2-Integrins Regulate IL-22 by ILC3s and Protect from Lethal *Citrobacter rodentium*-Induced Colitis. *Cell Rep.* 26:1614-1626.e5 (2019). PMID: PMC6404229.

5. Hematopoietic stem and progenitor cell (HSPC) adaptations/alterations and impact on immunity and inflammatory disease.

We established for the first time that the hematopoietic stem and progenitor cells (HSPC) are an integral component of trained immunity (TRIM), a form of epigenetic memory in innate immune cells. Specifically, we demonstrated that trained immunity is initiated in the bone marrow (BM) through long-lasting adaptations in HSPC. These adaptations may promote the host response quantitatively, by enhanced production of myeloid cells, and qualitatively, by generation of myeloid cells with enhanced inflammatory responsiveness. Our breakthrough discovery that TRIM is initiated at the level of BM progenitors resolved the earlier paradox regarding the long-term effects of TII on myeloid cells despite their short lifespan in circulation. TRIM can be protective against infections and tumors; in the latter regard, we showed that fungal-derived β -glucan can induce sustained epigenetic rewiring of BM granulopoietic progenitors leading to the generation of neutrophils with enhanced ROS-dependent tumor-killing phenotype. The ability of the innate immune system to remember also has detrimental consequences, especially if that memory is sparked by chronic systemic stimulation (maladaptive TRIM). In this regard, we have recently shown that maladaptive innate immune training of myelopoiesis bidirectionally links distinct inflammatory comorbidities, as exemplified by the periodontitis-arthritis axis. Although different mechanisms have been proposed earlier, no one mechanism explained this bidirectionality as did the unifying principle we established. The generation of leukocytes with enhanced inflammatory capacity may also result from certain aging-associated acquired mutations in HSPC in the context of clonal hematopoiesis of indeterminate potential (CHIP). In this regard, we have shown that mutant *DNMT3A*-driven CHIP aggravates inflammatory bone loss in periodontitis and arthritis.

- a. Mitroulis I, Ruppova K, Wang B, Chen LS, Grzybek M, Grinenko T, Eugster A, Troullinaki M, Palladini A, Kourtzelis I, Chatzigeorgiou A, Schlitzer A, Beyer M, Joosten LAB, Isermann B, Lesche M, Petzold A, Simons K, Henry I, Dahl A, Schultze JL, Wielockx B, Zamboni N, Mirtschink P, Coskun Ü, **Hajishengallis G***, Netea MG*, Chavakis T*. Modulation of myelopoiesis progenitors is an integral component of trained immunity. *Cell.* 172:147-161.e12 (2018). PMID: PMC5766828. **Contributed equally as senior authors.*
- b. Kalafati L, Kourtzelis I, Schulte-Schrepping J, Li X, Hatziioannou A, Grinenko T, Hagag E, Sinha A, Has C, Dietz S, de Jesus Domingues AM, Nati M, Sormendi S, Neuwirth A, Chatzigeorgiou A, Ziogas A, Lesche M, Dahl A, Henry I, Subramanian P, Wielockx B, Murray P, Mirtschink P, Chung KJ, Schultze JL, Netea MG, **Hajishengallis G***, Verginis P*, Mitroulis I*, Chavakis T*. Innate immune training of granulopoiesis promotes anti-tumor activity. *Cell.* 183:771-785.e12 (2020). PMID: PMC7599076. **Contributed equally as senior authors.*
- c. Li X, Wang H, Yu X, Saha G, Kalafati L, Ioannidis C, Mitroulis I, Netea MG, Chavakis T*, **Hajishengallis G***. Maladaptive innate immune training of myelopoiesis links inflammatory comorbidities. *Cell* 185:1709-1727.e18 (2022). PMID: PMC9106933 **Co-corresponding authors.*
- d. Wang H, Divaris K, Pan B, Li X, Lim J-H, Saha G, Barovic M, Giannakou D, Korostoff JM, Bing Y, Sen S, Moss K, Wu D, Beck JD, Ballantyne CM, Natarajan P, North KE, Netea MG, Chavakis T & **Hajishengallis G**, Clonal hematopoiesis driven by mutated DNMT3A promotes inflammatory bone loss. *Cell* 87:3690-3711.e19 (2024). PMID: PMC11246233

Complete List of Published Work in PubMed (250 publications)

<https://pubmed.ncbi.nlm.nih.gov/?term=Hajishengallis+G+%5BAuthor%5D&sort=date>

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Ko, Kang I.

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

POSITION TITLE: Assistant 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
University of California, San Diego, CA	B.S.	12/2009	Physiology & Neuroscience
University of California, San Diego, CA	M.S.	08/2011	Neuroscience
University of Pennsylvania, Philadelphia, PA	D.M.D	05/2015	Dental Medicine
University of Pennsylvania, Philadelphia, PA	D.Sc.D	12/2020	Stem Cell Biology
University of Pennsylvania, Philadelphia, PA	Residency	12/2020	Periodontology

A. Personal Statement

I am a clinician-scientist with a training background in clinical periodontology and research expertise in stem cell biology, wound healing and inflammation. I received an NIH-Ko8 award during my postdoctoral residency in 2017, and under this grant I developed an independent research focus on studies of mesenchymal progenitors using *in vivo* and *in vitro* wound healing models. I was appointed as an Assistant Professor in 2021 and have been a principal investigator of my own laboratory, leading a research team that consists of a postdoctoral fellow, research specialists and graduate students. My current research is funded by R01 grants, investigating the role of oral and skin fibroblast subsets for expediting wound healing and promoting type 2 inflammatory immune disorders. These studies were published in high impact journals such as *Science Translational Medicine* and *Journal of Experimental Medicine*. My clinical background as a board-certified periodontist and research expertise in studying oral immune sentinels are ideal for providing scientific support and mentorship for Dr. Makkar's K99/R00 career development award.

I have not published or created research products under another name.

Ongoing and Completed Research Support:

R01-DE030415 Ko (role: PI) 04/01/2021-03/30/2026
NIH/NIDCR "Wound healing mechanisms by distinct oral fibroblast population."
R01-AR082951 Ko (role: multi-PI) 09/21/2023-08/30/2028
NIH/NIAAMS "Fibroblast dysregulation promotes dermal eosinophilic and Th2 inflammation."
Ko8-DE027129 Ko (role: PI) 07/01/2017-06/30/2023 (completed)
NIH/NIDCR "The role of NF-kB in mesenchymal stem cells during diabetic wound healing"

Publications most relevant to the current application:

- Guan P, Ruan Q, Li J, Xi M, Qi W, **Ko KI**, Ni J. Ferroptosis in periodontitis: mechanisms, impacts, and systemic connections. *Cell Death Discovery*, 11:283, (2025).
- Easter QT, Matuck BF, Stark GB, Worth CL, Predeus AV, Fremin B, Huynh K, Ranganathan V, Pereira D, Weaver T, Miller K, Perez P, Hasuik A, Chen Z, Bush M, Warner BM, Lee J, Wallet SM, Sequeira I, Tyc KM, Liu J, **Ko KI**, Teichmann SA, Byrd KM. Single-cell and spatially resolved interactomics of tooth-associated keratinocytes in periodontitis. *Nature Communications* 15, 5016, (2024).
- Kim WS., Prasongyuenyong K, Ko A, Debnath R, Chen Z, Zhou JX, Shaaf E, **Ko KI**. ICAM1⁺ gingival fibroblasts modulate periodontal inflammation to mitigate bone loss. *Frontiers in Immunology* 15:

1484483, (2024).

- d. Easter QT, Alvarado-Martinez Z, Kunz M, Matuck BF, Rupp BT, Weaver T, Ren Z, Tata A, Caballero-Perez J, Oscarson N, Hasuike A, Ghodke AN, Kimple AJ, Tata PR, Randell SH, Koo H, **Ko KI**, Byrd KM. Polybacterial intracellular macromolecules shape single-cell inflammatory profiles in upper airway epithelia. *npj Biofilms and Microbiomes* 11, (2025).

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2022-present Diplomat, American Board of Periodontology
 2022-present Member, Wound Healing Society
 2021-present Assistant Professor, Department of Periodontics, University of Pennsylvania
 2021-2021 Instructor, Department of Periodontics, University of Pennsylvania
 2015-present Member, American Academy of Periodontology
 2015-2020 Member, American Dental Association
 2015-present Dental license, Commonwealth of Pennsylvania
 2013-present Member, International Association for Dental Research

Honors

2023 Difference Maker Wound Healing Researcher Scholarship, Wound Healing Society/SAWC
 2021 William R. Laney Award, Academy of Osseointegration
 2020 Ricardo Teles Research Award, University of Pennsylvania School of Dental Medicine.
 2018 AAP Educator Scholarship, American Academy of Periodontology Foundation.
 2017 NIH Career Development Award (Ko8), NIDCR.
 2016 Henry M. Thornton fellowship, the Student Clinician Research Program of the ADA (SCADA).
 2016 University of Pennsylvania, School of Dental Medicine; Travel Grant Award IADR 2017, San Francisco.
 2015 Dr. Earle Banks Hoyt Teaching Award, University of Pennsylvania School of Dental Medicine.
 2015 Abram Cohen Award in Periodontics, University of Pennsylvania School of Dental Medicine.
 2015 American Academy of Periodontology Dental Student Award for outstanding achievement in periodontics, University of Pennsylvania School of Dental Medicine.
 2015 Oral Biology Award, University of Pennsylvania School of Dental Medicine.
 2015 American Association for Dental Research (AADR) Bloc Travel Grant Award, NIDCR, Boston.
 2013 Vernon Brightman Research Society/Oral Health Fair, University of Pennsylvania, School of Dental Medicine, awarded first place in poster presentation.
 2013 American Association for Dental Research (AADR) Bloc Travel Grant Award, NIDCR, Seattle
 2011 Dean's scholarship, University of Pennsylvania School of Dental Medicine.

C. Contributions to Science

1. Fibroblasts are ubiquitous stromal cells that are crucial for extracellular matrix formation. However, their immune-modulatory functions and implications in skin and oral mucosal disorders are relatively unknown. We have demonstrated that distinct skin fibroblasts maintain cutaneous immune homeostasis by NF- κ B activity, as perturbation of this results in type 2 immunity via eosinophilic pathology that mimic human atopic dermatitis. Furthermore, oral mucosa harbors unique fibroblast progenitors that prime innate immune response to facilitate rapid wound healing. Recent collaborative work also highlights an important immune sentinel function of junctional epithelial cells in human periodontitis. These studies highlight a previously unrecognized role of resident sentinels in oral and skin inflammatory diseases and oral wound healing mechanisms.
 - a. **Ko K.I.**, Merlet J.J., DerGarabedian B.P., Zhen H., Horiuchi Y, Hedberg M.L., Hu E., Nguyen A.T., Prouty S., Alawi F., Walsh M.C., Choi Y., Millar S.E., Cliff A., Romero J., Garvin M.R., Seykora J.T., Jacobson D., Graves D.T. NF- κ B Perturbation Reveals Unique Immunomodulatory Functions in Prx1⁺ Fibroblasts that Promote Atopic Dermatitis. *Science Translational Medicine*, 14, eabj0324 (2022). PMID: 35108061 PMCID: PMC8979241.
 - b. **Ko K.I.**, DerGarabedian B.P., Chen Z., Debnath R., Ko A., Link B.N., Korostoff J.M., Graves D.T. Distinct fibroblast progenitor subpopulation expedites regenerative mucosal healing by immunomodulation. *Journal of Experimental Medicine*, 220 (3), e20221350 (2023). PMID: 36584405 PMCID: PMC9827523.
 - c. Easter Q.T., Matuck B.F., Stark G.B., Worth C.L., Predeus A.V., Fremin B., Huynh K., Ranganathan V., Pereira D., Weaver T., Miller K., Perez P., Hasuike A., Chen Z., Bush M., Warner B.M., Lee J., Wallet S.M., Sequeira I., Tyc K.M., Liu J., **Ko K.I.**, Teichmann S.A., Byrd K.M. Single-cell and spatially resolved interactomics of tooth-associated keratinocytes in periodontitis. *Nature Communications*, 15, 5016

- (2024). PMID: 38876998 PMCID: PMC11178863.
- d. Kim W.S., Prasongyuenyong K., Ko A., Debnath R., Chen Z., Zhou J.X., Shaaf E., **Ko K.I.** ICAM1⁺ gingival fibroblasts modulate periodontal inflammation to mitigate bone loss. *Frontiers in Immunology*, 15, 1484483 (2024). PMID: 39650645, PMCID: PMC11621011.
2. Diabetes has a detrimental effect on soft and hard tissue healing, thus posing a significant health issue. In long bones, this is due to a direct impact of diabetes on skeletal stem cell (SSC) viability and the loss of immune modulation by these progenitor cells to curtail inflammation. Diabetes causes aberrant activation of inflammatory transcription factor, nuclear factor kappa-B (NF- κ B) to induce SSC apoptosis and inhibit SSC proliferation during fracture repair, while downregulating TGF β 1 to prevent macrophage polarization from an inflammatory (M1) to pro-resolving phenotype (M2). We have also demonstrated that soft tissue healing and chronic inflammatory diseases such as periodontitis are negatively influenced by diabetes through epigenetic or dendritic cell-mediated mechanisms.
 - a. **Ko K.I.**, Coimbra L.S., Chen T., Alblowi J., Kayal R.A., Einhorn T.A., Gerstenfeld L.C., Pignolo R.J., & Graves D.T. Diabetes reduces mesenchymal stem cells in fracture healing through TNF α -mediated mechanism. *Diabetologia* 58(3), 633-42 (2015). PMID: 25563724 PMCID: PMC4346353.
 - b. **Ko K.I.**, Syverson A.L., Kralik R.M., Choi J., DerGarabedian B.P., Chen C., & Graves D.T. Diabetes-Induced NF- κ B Dysregulation in Skeletal Stem Cells Prevents Resolution of Inflammation. *Diabetes* 68(11):2095-2106 (2019). PMID: 31439641 PMCID: PMC6804629.
 - c. Yang B, Alimperti S, Gonzalez MV, Dentchev T, Kim M, Suh J, Titchenell PM, **Ko KI**, Seykora J, Benakanakere M, Graves DT. Re-epithelialization of Diabetic Skin and Mucosal Wounds is Rescued by Treatment with Epigenetic Inhibitors. *Diabetes* db230258, (2024).
 - d. Alghamdi B, Liu M, Huang X, Debnath R, Afzali H, Troka M, Hasuik A, Easter Q, Zhou M, Byrd KM, Gonzalez M, **Ko KI**, Graves DT. Single-cell RNA profiling identifies immune cell population shifts in diabetes associated mucosal inflammation. *Mucosal Immunology* (2025) Oct;18(5):1082-1097. PMID:40582570
 3. My early publications in the laboratory of Dr. Jing Wang investigated how internal physiologic status modulates sensory neural circuit and odor-driven behavior. Using an optimized behavioral assay and two-photon calcium imaging in *Drosophila*, we found that the modulation of starvation-dependent appetitive behavior required a presynaptic facilitation at the axon terminals of olfactory sensory neurons via the action of local neuropeptide, sNPF. In addition, starvation suppresses the neuronal activity in olfactory channels that are hardwired for aversion via an inhibitory neuropeptide drosophila tachykinin (DTK). We found that insulin acts as a metabolic cue to upregulate gene expression of sNPF1 and DTKR in the olfactory receptor neurons to generate concerted appetitive behaviors. The work demonstrated an important link between internal metabolic status and sensory modulation of neuronal activity that mediates olfactory behaviors.
 - a. Root C.M., **Ko K.I.**, Jafari A. & Wang J.W. Presynaptic facilitation by neuropeptide signaling mediates odor-driven food search. *Cell* 145, 133-144 (2011). PMID: 21458672 PMCID: PMC3073827
 - b. Zaninovich O.A., Kim S.M., Root C.M., Green D.S., **Ko K.I.**, & Wang J.W. A Single-Fly Assay for Foraging Behavior in *Drosophila*. *Journal of Visualized Experiments* 81, (2013). PMID: 24299900 PMCID: PMC3969902.
 - c. **Ko K.I.**, Root C.M., Lindsay S.A., Shepherd A.K., Wasserman S.A., & Wang J.W. Starvation promotes concerted modulation of appetitive olfactory behavior via parallel neuromodulatory circuits. *Elife*, 4 (2015). PMID: 26208339 PMCID: PMC4531282
 4. Dental implants are an essential tool that restores the form and function of missing dentition. Much is known about the success rate in healthy individuals, but potential complications associated with dental implants are less explored. I have collaborated with expert clinicians and published clinical and pre-clinical studies as a co-author and a senior author. These publications reflect my ongoing efforts to remain engaged in clinical research to bridge the bench science and clinical care.
 - a. Sarmiento H.L., Norton M., Korostoff J., **Ko K.I.**, & Fiorellini J.P. Surgical alternatives for treating peri-implantitis. *Int J Periodontics Restorative Dent* 38, 665-671 (2018). PMID: 30113606
 - b. Fiorellini J.P., Sourvanos D., Crohin C.C., Crohin M., Chang J.J., Mattos M., & **Ko K.I.** Diabetic serum inhibits osteoblast adhesion to titanium surface through AGEs: an *in vitro* study. *Int J Oral Maxillofac Implants*, DOI: 10.11607/jomi.8114 (2020). PMID: 32406653.

Complete List of Published Work in MyBibliography:

<https://www.ncbi.nlm.nih.gov/myncbi/kang.ko.1/bibliography/public/>

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Abt, Michael

eRA COMMONS USER NAME: mcabt13

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Loyola University of Maryland, Baltimore, MD	BS	05/2006	Biology
University of Pennsylvania, Philadelphia, PA	PHD	08/2012	Immunology
Memorial Sloan Kettering Cancer Center, New York, NY	Postdoctoral Fellow	12/2017	Immunology

A. Personal Statement

I have dedicated my scientific career to investigating mucosal immunity along the gastrointestinal tract. My independent research program focuses on immune-microbiome interactions in the context of infectious disease. I started my lab as a tenure-track, Assistant Professor in the Department of Microbiology at the University of Pennsylvania in 2018 investigating the immune response to the enteric bacterial pathogen *Clostridioides difficile*. I am currently the PI on a NIAID R01 grant investigating the immune factors that support fecal microbiota transplantation and a Project Leader on a NIAID U19 program awarded investigating vaccine induced immunity to *C. difficile* infection. My training experiences at all stages of my scientific career has uniquely positioned me to investigate the interplay between barrier immunity and the surrounding microbial communities.

My graduate studies at the University of Pennsylvania gain expertise in mucosal immunology from Dr. David Artis' lab and identified a role for commensal bacteria in calibrating the systemic and mucosal immune response to viral infection. Next, my post-doctoral training in the laboratory of Dr. Eric Pamer at the Memorial Sloan Kettering Cancer Center focused on understanding the Type-3 Innate Lymphoid Cell response to enteric bacterial infection. I was awarded an Irvington Fellowship from the Cancer Research Institute that supported my postdoctoral research examining the role of Innate Lymphoid Cells in host defense against *C. difficile* infection. These achievements demonstrate a strong scientific track record in the field mucosal immunology

I will advise Dr. Makkar on immune-microbiome interactions and mucosal immunology, providing analytical guidance on the animal workflow for Aim 2 through quarterly meetings and one-on-one discussions. I will support his efforts to investigate how gingival matrix stiffness influences microbiome changes in vivo and to develop future research directions in this area.

1. Abt MC, Osborne LC, Monticelli LA, Doering TA, Alenghat T, Sonnenberg GF, Paley MA, Antenus M, Williams KL, Erikson J, Wherry EJ, Artis D. Commensal bacteria calibrate the activation threshold of innate antiviral immunity. *Immunity*. 2012 Jul 27;37(1):158-70. PubMed PMID: 22705104
2. Abt MC, Lewis BB, Caballero S, Xiong H, Carter RA, Sušac B, Ling L, Leiner I, Pamer EG. Innate Immune Defenses Mediated by Two ILC Subsets Are Critical for Protection against Acute *Clostridium difficile* Infection. *Cell Host Microbe*. 2015 Jul 8;18(1):27-37. PubMed PMID: 26159718

I started my independent research program in 2018 and my lab's research program fuses the disciplines of mucosal immunology and microbial ecology to investigate contributing factors that shape *C. difficile* pathogenesis. We have demonstrated that the toxin-specific CD4⁺ T cell response following *C. difficile* infection is impaired by the glycosyltransferase activity of the *C. difficile* toxins, however, non-toxin specific CD4⁺ T regulatory cells are critical to support successful fecal microbiota transplantation engraftment for treatment of *C. difficile*.

1. . Maslanka JR, Londregan JA, Denny JE, Hult EN, Mdluli NV, Peritore-Galve FC, Alam MZ, Alameh MG, Lacy DB, Zackular JP, **Abt MC**. Clostridioides difficile toxin A and toxin B inhibit toxin-specific adaptive immune responses through glucosyltransferase-dependent activity. Mucosal Immunol. 2025 Aug 16: Epub ahead of print. PMID: 40825509.
2. Littmann ER, Lee JJ, Denny JE, Alam Z, Maslanka JR, Zarin I, Matsuda R, Carter RA, Susac B, Saffern MS, Fett B, Mattei LM, Bittinger K, Abt MC. Host immunity modulates the efficacy of microbiota transplantation for treatment of Clostridioides difficile infection. Nat Commun. 2021 Feb 2;12(1):755. PubMed PMID: 33531483; PMCID: PMC7854624.

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

R01 AI158830

Abt (PI)

02/01/21-01/31/26

Investigating Immune-Microbiota interactions in the treatment of *Clostridioides difficile*

U19 AI174998

Abt (PI)

02/28/23-03/01/28

mVACS – mRNA vaccines for C. difficile suppression

Dept of Army PR240366

Abt (Collaborator)

01/01/2025-12/31/2028

Next generation mRNA-LNP therapy to allow gluten tolerance in Celiac disease

I have not published or created research products under a different name.

Honors

2023	Pilot Award , Institute for RNA Innovation – University of Pennsylvania
2021	Pilot Award , ACD Spatial Genomics Validation Grant, Advanced Cell Diagnostics Bio-Techne – University of Pennsylvania
2020	Peggy Cotter Award for Early Career Branch Members – Eastern Pennsylvania American Society of Microbiology
2019	AAI Early Career Faculty Travel Grant , American Association of Immunologist – AAI 2019 San Diego, CA
2018	Pilot Award, Center for Molecular Studies in Digestive and Liver Diseases - University of Pennsylvania
2018	McCabe Fellow Award, The McCabe Fund – University of Pennsylvania
2018	Pilot Award , PennCHOP Microbiome Program – University of Pennsylvania
2016	Next Gen Immunology Travel Fellowship , Weizmann Institute
2016	K99/R00 NIH Pathway to Independence Award , NIH - National Institute of Allergy & Infectious Disease
2015	Distinguished Research Article , Weill Cornell Medical College & Graduate Studies - Immunology & Microbiology Department
2014 - 2016	Irvington Postdoctoral Fellowship , Cancer Research Institute
2014	Ruth L. Kirschstein NRSA Fellowship (F32) (declined) , National Institute of Health
2008 - 2012	Competitive Institutional T32 Training Grant , National Institute of Health
2005	Hauber Research Fellowship , Loyola University of Maryland
2002 – 2006	Presidential Scholarship , Loyola University of Maryland

C. Contribution to Science

1. **Defining the *C. difficile* toxin-mediated mechanism that impairs formation of a quality toxin-specific immunity.**

Following a primary episode of *C. difficile* infection patients often fail to generate antibody responses to Toxin A (TcdA) and Toxin B (TcdB) leaving them vulnerable to multiple rounds of recurrent infection. Why protective adaptive immunity against *C. difficile* infection often fails to form is poorly defined. My lab has generated TcdA and TcdB peptide libraries and MHC-II TcdB tetramers to track the mucosal toxin-specific CD4⁺ T cell response following infection. *C. difficile*-infected mice generated antibody responses to TcdA, but not TcdB, which corresponded with the presence of TcdA-responsive, but not TcdB-responsive, IL-17A-producing CD4⁺ T cells in the large intestine. To determine the mechanism of impaired anti-TcdB immunity, *C. difficile* mutant strains expressing glucosyltransferase inactive (GTx) TcdA, and/or glucosyltransferase inactive TcdB were employed and toxin-specific immune responses assessed. Infection with TcdB_{GTx} restored TcdB-specific antibody and TcdB-specific CD4⁺ T cell responses, but did not alter the formation of TcdA-specific immunity. Infection with TcdA_{GTx} or TcdA_{GTx} TcdB_{GTx} strains, however, led to a more robust TcdA-specific antibody and TcdA-specific CD4⁺ T cell response compared to infection with TcdA functional strains. These data demonstrate that the glucosyltransferase activity of TcdA and TcdB hinders the toxin-specific adaptive immune response to itself and may be a mechanism that enables multiple rounds of *C. difficile* recurrence in patients.

a. Maslanka JR, Londregan JA, Denny JE, Hult EN, Mdluli NV, Peritore-Galve FC, Alam MZ, Alameh MG, Lacy DB, Zackular JP, **Abt MC**. Clostridioides difficile toxin A and toxin B inhibit toxin-specific adaptive immune responses through glucosyltransferase-dependent activity. Mucosal Immunol. 2025 Aug 16: Epub ahead of print. PMID: 40825509.

2. The immune system supports successful fecal microbiota transplantation treatment of *Clostridioides difficile* infection.

Fecal microbiota transplantation (FMT) is a highly effective treatment for recurrent *C. difficile* infection. Despite remarkable efficacy, FMT mechanism of action and the host immune system's role in FMT engraftment is poorly understood. Work completed in my lab and supported by my R01 award provides evidence that the FMT recipient's immune system status contributes to success of FMT. We identified three lines of evidence to support this observation. First, FMT did not resolve *C. difficile* infection in T and B cell deficient *Rag1*^{-/-} mice that exhibited exacerbated intestinal inflammation at time of FMT in comparison to littermate, heterozygous mice. Second, targeted ablation of adaptive immune cell subsets revealed a necessary role for CD4⁺ Foxp3⁺ T-regulatory cells, but not B cells or CD8⁺ T cells, in FMT-mediated resolution of *C. difficile*. Third, the FMT bacterial consortium failed to engraft in FMT non-responsive mice while the microbiota of responsive mice shifted to resemble the FMT consortium. The disparate response to FMT treatment in mice due to their respective immune status highlights the need to conduct basic biological research to better understand the relationship between immune system and microbiota.

a. Littmann ER, Lee JJ, Denny JE, Alam Z, Maslanka JR, Zarin I, Matsuda R, Carter RA, Susac B, Saffern MS, Fett B, Mattei LM, Bittinger K, **Abt MC**. Host immunity modulates the efficacy of microbiota transplantation for treatment of Clostridioides difficile infection. Nat Commun. 2021 Feb 2;12(1):755. PubMed PMID: 33531483; PMCID: PMC7854624.

b. Maslanka JR, Gu CH, Zarin I, Denny JE, Broadaway S, Fett B, Mattei LM, Walk ST, **Abt MC**. Detection and elimination of a novel non-toxigenic *Clostridioides difficile* strain from the microbiota of a mouse colony. Gut Microbes. 2020 Nov 9;12(1):1-15. PubMed PMID: 33305657. PMCID: PMC7734020.

3. Innate immune defense mechanisms that limit *Clostridioides difficile* infection.

As an independent investigator I have continued my postdoctoral research focused on innate immunity to enteric bacterial infections. I have demonstrated that type-1 and type-3 Innate Lymphoid Cells (ILCs) are critical in limiting disease severity during the acute stage of *C. difficile* infection. Further, my lab at the University of Pennsylvania demonstrated that loss of the immunoregulatory cytokine IL-10 prior to infection promotes IL-22-mediated immune activation in the intestine that is protective against severe *C. difficile* infection. This protective IL-22-mediated innate immune activation can be replicated by administering a synthetic TLR-7 agonist without the deleterious effects of IL-10 deficiency. Transiently activating the IL-22 pathway with the TLR-7 agonist limits *C. difficile* toxin-mediated damage by promoting epithelial proliferation.

Both activation of ILCs and the TLR-7/IL-22 axis are potential therapeutic targets to support innate immunity against emerging antibiotic resistant pathogens.

- a. Mears KS, Denny JE, Maslanka JR, Mdluli NV, Hult EN, Matsuda R, Furth EE, Buffie CG, **Abt MC**. Therapeutic activation of IL-22-producing innate lymphoid cells enhances host defenses to *Clostridioides difficile* infection. *Cell Rep*. 2025 Apr 22;44(4):115438. PMID: 40138315.
- b. Denny JE, Alam MZ, Mdluli NV, Maslanka JR, Lieberman LA, **Abt MC**. Monoclonal antibody-mediated neutralization of *Clostridioides difficile* toxin does not diminish induction of the protective innate immune response to infection. *Anaerobe*. 2024;88. Pubmed PMID: 38701911. PMCID: PMC11347114
- c. Cribas, ES, Denny JE, Maslanka JR, **Abt MC**. Loss of IL-10 signaling promotes IL-22 dependent host defenses against acute *Clostridioides difficile* infection. *Infection and Immunity* 2021 Apr 16; 89(5). PubMed PMID: 33649048.
- d. **Abt MC**, Lewis BB, Caballero S, Xiong H, Carter RA, Sušac B, Ling L, Leiner I, Pamer EG. Innate Immune Defenses Mediated by Two ILC Subsets Are Critical for Protection against Acute *Clostridium difficile* Infection. *Cell Host Microbe*. 2015 Jul 8;18(1):27-37. PubMed PMID: 26159718. PMCID: PMC4537644.

4. The microbiota calibrates basal immune activation.

My publications during my thesis work focused on the role of commensal bacteria-derived signals in shaping immune cell development and function in peripheral tissue sites. I helped establish a murine model that uses broad-spectrum antibiotics to deplete intestinal commensal bacterial communities. This model, along with the use of gnotobiotic mice, lead to the discovery that commensal bacteria-derived signals influence basophil progenitor development in the bone marrow and, in turn, helped establish a mechanistic link between commensal bacteria and development of T_H-2 mediated allergies. My primary thesis work identified a role for commensal bacteria-derived signals in calibrating the activation threshold of innate antiviral defense genes in macrophages thereby poised these cells to rapidly response to viral infection. This research demonstrated a link between intestinal microbial communities and the systemic host immune response to a viral infection.

- a. **Abt MC**, Osborne LC, Monticelli LA, Doering TA, Alenghat T, Sonnenberg GF, Paley MA, Antenus M, Williams KL, Erikson J, Wherry EJ, Artis D. Commensal bacteria calibrate the activation threshold of innate antiviral immunity. *Immunity*. 2012 Jul 27;37(1):158-70. PubMed PMID: 22705104. PMCID: PMC3679670.
- b. Hill DA, Siracusa MC, **Abt MC**, Kim BS, Kobuley D, Kubo M, Kambayashi T, Larosa DF, Renner ED, Orange JS, Bushman FD, Artis D. Commensal bacteria-derived signals regulate basophil hematopoiesis and allergic inflammation. *Nat Med*. 2012 Mar 25;18(4):538-46. PubMed PMID: 22447074. PMCID: PMC3321082.
- c. Hill DA, Hoffmann C, **Abt MC**, Du Y, Kobuley D, Kirn TJ, Bushman FD, Artis D. Metagenomic analyses reveal antibiotic-induced temporal and spatial changes in intestinal microbiota with associated alterations in immune cell homeostasis. *Mucosal Immunol*. 2010 Mar;3(2):148-58. PubMed PMID: 19940845. PMCID: PMC2824244.

Complete List of Published Work in My Bibliography:

<http://www.ncbi.nlm.nih.gov/myncbi/michael.abt.1/bibliography/40455971/public/?sort=date&direction=ascending>

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

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: Koo, Hyun

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

POSITION TITLE: Professor and Director

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Sao Paulo State University, Sao Paulo, Brazil	D.D.S.	03/1993	Dentistry
The University of Campinas, Campinas, Brazil	M.Sc.	06/1996	Food Engineering
The University of Campinas, Brazil and University of Rochester Medical Center, New York, NY	Ph.D.	06/1999	Oral Biology & Pathology
University of Rochester Medical Center, New York, NY	Post-doc	11/2001	Microbiology
National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD	Sabbatical training	01/2012	Cell & Developmental Biology

A. Personal Statement

My research is focused on understanding the role of biofilms, interkingdom and host-microbe interactions in the pathogenesis of oral diseases while seeking novel therapeutic strategies against dental caries and periodontitis by fostering cross-disciplinary collaborations in engineering, materials sciences and robotics. We have adapted many of the principles governing cell & developmental biology to study oral biofilms and virulence mechanisms. I have extensive experience (25+ years) conducted animal- and human-based studies to help translate our discoveries. Our research program has been continuously funded by NIDCR, and we have published in top-tier dental, biomedical, and multidisciplinary journals. I am an AAAS fellow, a Clarivate Highly Cited Researcher, and the recipient of the IADR Distinguished Scientist and Innovation in Oral Care Awards. I have served in several scientific committees, including AADOCR/IADR Hatton and Distinguished Scientist Awards, and as standing member of the NIDCR/ODCS study section (R01/R21) and the NIH DSR study section (K/F awards).

I have a career-long mentoring commitment and experience. I have mentored >25 postdoctoral fellows, 12 of whom were recipients of the *AADOCR/IADR Hatton*, the *IADR Innovation in Oral Care*, the *AADOCR Joseph Lister*, and the *IADR Women in Science* awards. The majority of them have gone on to compete successfully for NIH funding (F, K, and R awards) and to obtain academic faculty positions in dental schools both in the US (e.g. University of Pennsylvania, University of Rochester, New York University, Indiana University) and abroad (e.g. University of Seville/Spain, Jeonbuk National University/Korea, UNICAMP/Brazil).

I have co-founded an inter-school center, the Penn's *Center for Innovation & Precision Dentistry* (CiPD), which bridges the faculty and resources from the Schools of Dental Medicine and Engineering. We were awarded an NIDCR T90/R90 postdoctoral training program "*Advanced Training at the Interface of Engineering and Oral-Craniofacial Sciences*", in which Dr. Makkar (PI of the proposed K99) was one of the selected trainees (R90 fellow). Dr. Makkar is an outstanding candidate for the K99 award. He is an exceptionally mature dentist-scientist and well-trained in biotechnology and bioengineering, who is deeply committed for an independent research career in oral and craniofacial research. He is one of the best postdoc fellows from our training program, and certainly in the top 1% based on my mentoring experience. As detailed in his K99 mentoring plan, I will be a member of Dr. Hardik Makkar's Advisory Committee. In this capacity, I will assist in his growth as an independent

researcher, facilitate access to resources within Penn Dental, while also introducing him to relevant collaborators. Additionally, I will guide Dr. Makkar in engaging with the workshops and seminars available at Penn Dental and across the University. As co-Director of CiPD, I will also offer numerous opportunities for him to present at seminars and symposia, apply for seed funds to foster collaboration (such as CiPD's annual IDEA prize), and provide dedicated spaces for collaboration, such as CiPD's Innovation Hub. I will enthusiastically support his career development and help him in any way I can to ensure a successful transition towards independence.

I have not published research or research products under any other name.

5 T90 DE030854 (NIH/NIDCR)

07/01/21-06/30/26

Advanced Training at the Interface of Engineering and Oral-Craniofacial Sciences

The program focusses on post-doctoral training at the interface of the disciplines to: (1) apply engineering and computational sciences to study disease mechanisms and to develop precision diagnostics, therapies and devices, and (2) develop a new cohort of cross-trained dentists and engineers for careers in advanced dental & craniofacial research and innovation in precision oral health care. Role: PD

2R01DE025848-05 (NIH/NIDCR)

12/09/2016-08/31/2026

Biofilm elimination and caries prevention using multifunctional nanocatalysts

This project employs nanocatalysts that integrates a multifunctional strategy to degrade the biofilm matrix and kill the embedded bacteria, while preventing demineralization under acidic pH. This approach uses low cost and biocompatible, FDA-approved nanoparticles facilitating clinical translation to promote oral health. Role: PI

Citations:

1. Tran HH, Watkins A, Oh MJ, Babeer A, Schaer TP, Steager E, Koo H. Targeting biofilm infections in humans using small scale robotics. **Trends in Biotechnology**, 42(4):479-495, 2024
2. Hajishengallis G, Lamont RJ, Koo H. Oral polymicrobial communities: Assembly, function, and impact on diseases. **Cell Host Microbe**, 31(4):528-538, 2023
3. Lamont RJ, Koo H, Hajishengallis G. The oral microbiota: dynamic communities and host interactions. **Nature Reviews Microbiology**, 16(12):745-759, 2018
4. Bowen WH, Burne RA, Wu H, Koo H. Oral Biofilms: Pathogens, matrix, and polymicrobial interactions in microenvironments. **Trends in Microbiology**, 26(3):229-242, 2018.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2019 - Present	Co-Founder/Director, Penn Center for Innovation and Precision Dentistry (CiPD), School of Dental Medicine and School of Engineering & Applied Sciences, University of Pennsylvania
2013 - Present	Professor (tenured) and Director of Research, Levy Center for Oral Health, Dept. of Orthodontics, School of Dental Medicine, University of Pennsylvania
2011 - 2012	NIH Adjunct Researcher, National Institute of Dental and Craniofacial Research (NIDCR)
2008 - 2013	Associate Professor (tenured) of Dentistry, Microbiology and Immunology in the Center for Oral Biology, University of Rochester
2006 - 2008	Assistant Professor, Dept. of Microbiology and Immunology, University of Rochester
2002 - 2008	Assistant Professor, Eastman Dept. of Dentistry, University of Rochester

Other Experience and Professional Memberships

2019 - Present	Chair, Faculty Senate, School of Dental Medicine, University of Pennsylvania
2018 - 2019	Acting Chair, ODCS Study section, NIH/NIDCR
2018 - 2018	Acting Chair, Special Emphasis Panel for DSR Study section members
2017 - Present	Standing member, ODCS Study section, NIH/NIDCR
2017 - Present	Member, Hatton Awards Committee of the AADR
2016 - 2019	Chair, Nominating Committee of the Faculty Senate, School of Dental Medicine, University of Pennsylvania
2015 - 2018	Chair, Committee on Faculty Appointments and Promotions, University of Pennsylvania
2015 - 2015	NIDCR/NIH Consultant Panel, Workshop on Remineralization: Current State of Science and

	Future Directions
2013 - 2019	Chair, Penn Dental Medicine Research Day
2012 - 2015	Member, Hatton Awards Committee of the AADR
2012 - 2013	Associate Director, NIDCR T90/R90 Training Program in Oral Science
2010 - 2014	Standing member, Special Grants Review, DSR Study section, NIH/NIDCR
2009 - 2012	Associate Director, NIDCR T32 Training Program in Oral Science
2008 - Present	Editorial board of several journals including <i>Journal of Dental Research</i> , <i>Frontiers Cellular & Infection Microbiology</i> , <i>International Journal of Oral Sciences</i>
2008 - Present	<i>Ad hoc</i> reviewer for NIH Study section, RCMI and COBRE review panels, NIDCR Dental Materials RFA Review, Challenge Grants in Health and Science Research review panel, RUMP Special Emphasis review panel, Dental-related SBIR/STTR review panel, and Drug Discovery and Mechanisms of Antimicrobial Resistance Study Section
2005 - 2006	President, Rochester Section of the AADR
2004 - 2005	Chair, Basil G. Bibby Fellowship Award Review Committee
2001 - 2013	External Advisory Committee, Ph.D. program, School of Dentistry, The University of Campinas (UNICAMP), Brazil

Honors

2022	Honorary Skou Professor of Microbiology and Immunology, Arhus University, Denmark
2020-2024	Clarivate Highly Cited Researcher
2020	Emerging Inventor of the Year, Penn Center for Innovation, University of Pennsylvania
2019	Fellow of the American Association for the Advancement of Science (AAAS)
2019	Penn Health-Tech Award for Small Scale Robotics, University of Pennsylvania
2018	IADR Distinguished Scientist Award – William H. Bowen Caries Research Award Selected
2016	as Penn Fellow, University of Pennsylvania
2013	IADR/GlaxoSmithKline Innovation in Oral Care Awards
2011	Honorary Professor, West China College of Stomatology, Sichuan University, China IADR
2007	Basil G. Bibby Young Investigator in Cariology Award
2006	Excellence in Research Incentives Program – University of Rochester Medical Center
2006	IADR/GlaxoSmithKline Innovation in Oral Care Awards
2006	IADR Distinguished Scientist Award – Young Investigator Award
2001	IADR/Colgate-Palmolive Research in Prevention Award
2001	The Basil G. Bibby Fellowship Award for Excellence in Oral Health Research

By graduate students and postdocs (as mentor)

2024	Federation of European Microbiological Societies Best ECR Award (Zhi Ren)
2024	IADR Microbiology & Immunology Arnold Bleiweis Travel Award (Zhenting Xiang)
2024	AADOCR Mini-Symposium for Young Investigators – 1 st Place Award (Zhenting Xiang)
2023	AADOCR Paula Five-Taylor Award Mini-Symposium for Young Investigators (Zhi Ren)
2023	AADOCR Robert Marquis Award Mini-Symposium for Young Investigators (Yilan Miao)
2023	National Student Research Group (NSRG) – 3 rd Place Award (Yilan Miao)
2022	American Society for Microbiology - Early Career Best Presentation Award (Zhi Ren)
2021	AADOCR Joseph Lister Award for New Investigators (Yuan Liu)
2021	IADR Hatton Award - Senior category (Zhi Ren)
2021	AADOCR Hatton Award – Senior category (Zhi Ren)
2020	IADR Women in Science Award for Promising Talent (Yuan Liu)
2019	IADR Women in Science Award for Distinguished Research (Aurea Simon Soro)
2019	IADR Colgate Research in Prevention Travel Award (Kenneth Sims)
2019	AADR Bloc Travel Grant (Kenneth Sims)
2019	Colgate-Palmolive Award for Excellence in Oral Health Research (Yuan Liu)
2018	IADR Colgate Research in Prevention Travel Award (Kassapa Ellepola)
2018	AADR/Unilever Hatton Award - Senior category (Yuan Liu)
2018	AADR Joseph Lister Award for Young Investigators (Dongyeop Kim)
2017	Colgate-Palmolive Fellowship – Penn Dental Medicine (Yuan Liu)
2017	AADR/Unilever Hatton Award - Senior category (Dongyeop Kim)
2017	IADR Lion Dental Research Award (Kassapa Ellepola)
2015	IADR/GlaxoSmithKline Innovation in Oral Care Awards (Lizeng Gao)

2012	AADR/Unilever Hatton Award - Senior category (Megan Falsetta)
2011	AADR/Unilever Hatton Award - Senior category (Jin Xiao)
2008	AADR/Unilever Hatton Award - Senior category (Marlise Klein)
2007	AADR/Unilever Hatton Award - Senior category (Simone Duarte)
2004	IADR/Unilever Hatton Award, Brazilian Division 1st place (Simone Duarte)

C. Contributions to Science

1. Polymicrobial communities in the oral cavity. We have focused on understanding the spatiotemporal development of interspecies interactions in saliva and in biofilms using super-resolution microscopy, genetics, biophysical, microfluidics and computational approaches. Our findings offer new insights on how pathogenic biofilms are assembled on a surface, which may help to design more precise therapeutic approaches to prevent or treat biofilm-associated diseases such as dental caries and periodontitis.
 - a. Kim D, Barraza JP, Arthur RA, Hara A, Lewis K, Liu Y, Scisci EL, Hajishengallis E, Whiteley M, Koo H. Spatial mapping of polymicrobial communities reveals a precise biogeography associated with human dental caries **Proc Natl Acad Sci U S A**, 117(22):12375-12386, 2020 PMID: 32424080 PMCID: PMC7275741.
 - b. Paula AJ, Hwang G, Koo H. Dynamics of bacterial population growth in biofilms resemble spatial and structural aspects of urbanization. **Nature Communications** 11(1):1354, 2020. PMID: 32170131 PMCID: PMC7070081.
 - c. Cho H, Ren Z, Divaris K, Roach J, Lin BM, Liu C, Azcarate-Peril MA, Simancas-Pallares MA, Shrestha P, Orlenko A, Ginnis J, North KE, Zandona AGF, Ribeiro AA, Wu D, Koo H. *Selenomonas sputigena* acts as a pathobiont mediating spatial structure and biofilm virulence in early childhood caries. **Nature Communications**, 14(1):2919, 2023.
 - d. Easter QT, Fernandes Matuck B, Beldorati Stark G, Worth CL, Predeus AV, Fremin B, Huynh K, Ranganathan V, Ren Z, Pereira D, Rupp BT, Weaver T, Miller K, Perez P, Hasuik A, Chen Z, Bush M, Qu X, Lee J, Randell SH, Wallet SM, Sequeira I, Koo H, Tyc KM, Liu J, Ko KI, Teichmann SA, Byrd KM. Single-cell and spatially resolved interactomics of tooth-associated keratinocytes in periodontitis. **Nature Communications**, 15(1):5016, 2024
2. Interkingdom (bacterial-fungal) interactions in saliva and biofilms. Using clinical data, biophysical methods, and animal models, we found a pathogenic role for *Candida albicans* in oral biofilms associated with severe childhood caries by developing a symbiotic partnership with *Streptococcus mutans*. *C. albicans* develops synergistic interactions with *S. mutans* that enhances biofilm accumulation and virulence, leading to rampant and extensive severe lesions in rodent caries models.
 - a. Ren Z, Jeckel H, Simon-Soro A, Xiang Z, Liu Y, Cavalcanti IM, Xiao J, Tin NN, Hara A, Drescher K, Koo H. Interkingdom assemblages in human saliva display group-level surface mobility and disease-promoting emergent functions. **Proc Natl Acad Sci U S A**, 119(41): e2209699119, 2022 PMID: 36191236
 - b. Hwang G, Liu Y, Kim D, Li Y, Krysan DJ, Koo H. *Candida albicans* mannans mediate *Streptococcus mutans* exoenzyme GtfB binding to modulate cross-kingdom biofilm development *in vivo*. **PLoS Pathogens**, 13(6):e1006407, 2017. Selected by Faculty of 1000 (2017). PMID: 28617874 PMCID: PMC547232.
 - c. Falsetta ML, Klein MI, Colonne P, Scott-Anne KK, Gregoire S, Pai CH, Gonzalez M, Krysan DJ, Bowen WH, Koo H. Symbiotic relationship between *Streptococcus mutans* and *Candida albicans* synergizes the virulence of plaque-biofilms *in vivo*. **Infection and Immunity**, 82(5):1968-81, 2014. Highlighted in ASM Microbe journal.
 - d. Kim D, Liu Y, Benhamou RI, Sanchez H, Simón-Soro Á, Li Y, Hwang G, Fridman M, Andes DR, Koo H. Bacterial-derived exopolysaccharides enhance antifungal drug tolerance in a cross-kingdom oral biofilm. **The ISME Journal**, 12(6):1427-1442, 2018. PMID: 29670217 PMCID: PMC5955968.
3. Novel antibiofilm and drug delivery approaches. Concomitantly, we have identified and developed new antibiofilm approaches using *in silico* approaches, nanotechnology and bioengineering. By targeting the vital structural traits of biofilms and drug tolerance mechanisms, novel and highly precise methods of preventing biofilm-associated diseases can be developed against dental caries and periodontitis.

- a. Babeer A, Liu Y, Ren Z, Xiang Z, Oh MJ, Pandey NK, Simon-Soro A, Huang R, Karabucak B, Cormode DP, Chen C, Koo H. Ferumoxylol nanozymes effectively target chronic biofilm infections in apical periodontitis. **The Journal of Clinical Investigation**, 135(3):e183576, 2024
 - b. Naha PC, Liu Y, Hwang G, Huang Y, Gubara S, Jonnakuti V, Simon-Soro A, Kim D, Gao L, Koo H, Cormode DP. Dextran coated iron oxide nanoparticles as biomimetic catalysts for localized and pH-activated biofilm disruption. **ACS Nano**, 13(5):4960-4971, 2019. PMID: 30642159 PMCID: PMC7059368.
 - c. Liu Y, Naha, PC, Hwang G, Kim D, Huang Y, Simon-Soro A, Jung HI, Ren Z, Li Y, Gubara S, Alawi F, Zero D, Hara AT, Cormode DP, Koo H. Topical ferumoxylol nanoparticles disrupt biofilms and prevent severe tooth decay in vivo via intrinsic catalytic activity. **Nature Communications** 9(1):2920, 2018. PMID:30065293 PMCID: PMC6068184. Selected for Editors' Highlights (2018).
 - d. Hajfathalian M, de Vries CR, Hsu JC, Amirshaghghi A, Dong YC, Ren Z, Liu Y, Huang Y, Li Y, Knight SA, Jonnalagadda P, Zlitni A, Grice EA, Bollyky PL, Koo H, Cormode DP. Theranostic gold-in-gold cage nanoparticles enable photothermal ablation and photoacoustic imaging in biofilm-associated infection models. **The Journal of Clinical Investigation**, 133(21), e168485, 2023. doi: 10.1172/JCI168485.
4. Developing new methodologies. We have developed novel methods to study and disrupt single cell binding dynamics, polymicrobial and matrix interactions as well as spatio-temporal analysis of biofilm microenvironments in real-time. These methodologies may have broad applicability because our concepts and approaches can be adapted in other systems as matrix and cell-matrix interactions are inherent in biofilms, organs and tissues.
- a. Petrie RJ, Koo H, Yamada KM. Generation of compartmentalized pressure by a nuclear piston governs cell motility in a 3D matrix. **Science**, 345(6200):1062-5, 2014. PMCID: PMC5248932. Selected for perspective/commentary articles in Nature Reviews Molecular Cell Biology (2014) and Science (2014).
 - b. Hwang G, Paula AJ, Hunter EE, Liu Y, Babeer A, Karabucak B, Stebe K, Kumar V, Steager E, Koo H. Catalytic antimicrobial robots for biofilm eradication. **Science Robotics**, 4 (29), eaaw2388, 2019. PMID: 31531409 PMCID: PMC6748647.
 - c. Huang Y, Liu Y, Pandey NK, Shah S, Simon-Soro A, Hsu JC, Ren Z, Xiang Z, Kim D, Ito T, Oh MJ, Buckley C, Alawi F, Li Y, Smeets PJM, Boyer S, Zhao X, Joester D, Zero DT, Cormode DP, Koo H. Iron oxide nanozymes stabilize stannous fluoride for targeted biofilm killing and synergistic oral disease prevention. **Nature Communications**, 14(1):6087, 2023
 - d. Oh MJ, Yoon S, Babeer A, Liu Y, Ren Z, Xiang Z, Miao Y, Cormode DP, Chen C, Steager E, Koo H. Nanozyme-based robotics approach for targeting fungal infection. **Advanced Materials**, 36(10):e2300320, 2024.

Complete List of Published Work (>170 peer reviewed publications) in MyBibliography:

<http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/40779761/?sort=date&direction=ascending>

BIOGRAPHICAL SKETCH

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

NAME: Stebe, Kathleen J.

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

POSITION TITLE: Goodwin 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
City College of New York, New York, NY	BA	05/1989	Economics
City College of New York, New York, NY	MSE	05/1989	Chemical Engineering
City University of New York, New York, NY	PhD	10/1980	Chemical Engineering
UTC, Compiegne, France	Post-doc	11/1990	Biomechanics

A. Personal Statement

I am a Chemical and Biomolecular Engineer with a record of leadership in post-graduate education and mentorship, and in the creation of new opportunities for researchers to interact at the interfaces between disciplines. With Dr. Hyun (Michel) Koo, I have co-founded an inter-school center, the Penn's *Center for Innovation and Precision Dentistry* (CiPD), which bridges the faculty and resources from the Schools of Dental Medicine and Engineering & Applied Sciences. Together, we were awarded a T90/R90 postdoctoral training program from NIDCR "Advanced Training at the Interface of Engineering and Oral-Craniofacial Sciences". A major aim of this center is to develop new cohorts of talented researchers who will bring innovative approaches in engineering into fields of clinical relevance. A significant aspect of our efforts is devoted to providing mentorship and support to trainees, and to seed new collaborations in the community of engineers and oral-health scientists for impact in the field. Trainee mentorship is formalized via regular meetings of Career Mentoring Committees chaired by us and comprised of senior faculties from both schools and at least one clinician as well as ad hoc trainee interactions as needed. I have also served as the Deputy Dean of Penn Engineering, with oversight of doctoral and postdoctoral programs, research, and innovation. In this role, I have developed and led workshops on the development of research programs, the faculty search process, and professional development. I have decades of experience in training of doctoral and postdoctoral researchers and in academic leadership. I have trained 34 current or former doctoral students and 28 postdoctoral researchers, with former trainees in positions of note in fundamental research, development, and technology translation in leading institutions, including Utrecht University, Delft University, and Yale, among others, and in leading research and development organizations around the globe. Representative alumni include Tagbo Niepa, Associate Professor of Chemical Engineering at Carnegie Mellon University, who was recently awarded NIH Director's New Innovator Award and Valeria Garbin, Professor of Chemical Engineering, TU Delft, who was recognized by the Royal Society's McBain medal (RSC/SCI) and the 2020 recipient of the Soft Matter Lectureship (RSC).

My research focuses on materials engineering, hydrodynamics, mechanics of assemblies, and the directed motion of colloids, microscale and millimeter scale objects in complex fluids. I am an expert in the design and fabrication of functional, complex shaped particles to promote interactions with boundaries and mechanics of complex fluids. I have authored 170 peer reviewed papers, presented >30 plenary or named lectures, and >300 invited talks at universities or conferences. I have led initiatives to develop interaction at the interface between engineering and biomedical focused disciplines, including to develop cross disciplinary interactions between the robotics, materials and biological focused research communities at the University of Pennsylvania.

Specifically for this proposal, I will serve on Dr. Hardik Makkar's Advisory committee, which will meet every 6 months or more as needed. In this role, I will support his development as an independent researcher, aid him in networking and access to resources at Penn Engineering, and introduce him to relevant collaborators. Furthermore, I will help guide or connect Dr. Makkar to the rich workshop and seminar environment within Penn Engineering and around the University. Furthermore, in my capacity as co-Director of CiPD, I will provide ample opportunities to present at seminars/symposia, to apply for seed funds that stimulate collaborations (e.g. CiPD's annual IDEA prize). Finally, within CiPD, we will provide a dedicated space for collaborations (e.g. CiPD's *Innovation Hub*)

Ongoing and recently completed projects:

T90/R90 DE030854 (NIH/NIDCR) 07/01/21-06/30/26

Advanced Training at the Interface of Engineering and Oral-Craniofacial Sciences

The program focusses on post-doctoral training at the interface of the disciplines to: (1) apply engineering and computational sciences to study disease mechanisms and to develop precision diagnostics, therapies and devices, and (2) develop a new cohort of cross-trained dentists and engineers for careers in advanced dental & craniofacial research and innovation in precision oral health care. Role: co-PD

DOE-BES DE-SC0022240 09/01/2021- 08/31/2025

Peptide Surfactants at Air-Aqueous Interfaces for Lanthanide Recovery

This project addresses fundamental science to develop peptide surfactants based on lanthanide binding tags to selectively bind to lanthanide cations and adsorb at aqueous-air interfaces to enable a new, green, foam-based selective separation technique. Principal Investigator: Kathleen Stebe, \$3M; 13 co-PIs at 4 institutions:UPENN, UIC, CCNY, Northwestern

DOE (BES) Award DE-SC0022892 7/01/2022 - 6/30/2025

Far-from-equilibrium topological defects on active colloids in nematic liquid crystals for bio-inspired materials assembly, Stebe, PI, We develop reconfigurable materials inspired by nature's complex, adaptive structures and emergent interactions. Ferromagnetic colloidal particles with rotational motion controlled by external magnetic fields generate companion topological defects that dramatically reconfigure propelling translation. An integrated experimental and numerical program will be pursued to understand topological flagella and their role in generating broken symmetries essential to swimming for rotating colloids with differing geometries, features, and genus.

NSF CBET 1945841 7/1/2020-6/30/2023

Process Intensification via Bijels for Simultaneous and Continuous Catalytic Reaction and Separation

Stebe, PI, Lee, co-PI This project develops bicontinuous interfacially jammed emulsion gels (bijels), a unique class of particle-stabilized fluid-bicontinuous structures, as a new class of membrane reactors for simultaneous reaction and separation of e.g. oily reactants that form aqueous-soluble products.

Dr. Stebe has not previously published or created research projects under another name.

Citations:

1. Deng, Jiayi, Mehdi Molaei, Nicholas G. Chisholm, Tianyi Yao, Alismari Read, and Kathleen J. Stebe. "Active colloids on fluid interfaces". *Current Opinion in Colloid & Interface Science* (2022): 101629; doi: 10.1016/j.cocis.2022.101629.
2. Geelsu Hwang, Amauri de Paula, Elizabeth Hunter, Yuan Liu, Alaa Babeer, Bekir Karabucak Kathleen J. Stebe, Vijay Kumar, Edward Steager, Hyun Koo. "Catalytic antimicrobial robots for biofilm eradication". *Science Robotics*, 4 (29), eaaw2388, 2019. PMID: 31531409 PMCID: PMC6748647.
3. Cavallaro Jr, Marcello, Lorenzo Botto, Eric P. Lewandowski, Marisa Wang, and Kathleen J. Stebe. "Curvature-driven capillary migration and assembly of rod-like particles. *Proceedings of the National Academy of Sciences* 108, no. 52 (2011): 20923-20928; doi: 10.1073/pnas.1116344108.

4. Garbin, Valeria, John C. Crocker, and Kathleen J. Stebe. "Nanoparticles at fluid interfaces: Exploiting capping ligands to control adsorption, stability and dynamics." *Journal of Colloid and Interface Science* 387, no. 1 (2012): 1-11; doi: 10.1016/j.jcis.2012.07.047.

B. Positions and Honors

Positions and Employment

2012-2020 Deputy Dean for Research, School of Engineering and Applied Science, PENN
 2008-present Goodwin Professor, Chemical and Biomolecular Engineering, PENN
 2008-2012 Chair, Chemical and Biomolecular Engineering, PENN
 2006-2008 Chair, Department of Chemical and Biomolecular Engineering, JHU
 2002 Fellow, Radcliffe Institute for Advanced Study, Harvard University
 2000-2008 Professor, Chemical and Biomolecular Engineering, JHU
 1996-2000 Associate Professor, Chemical Engineering, JHU
 1991-1996 Assistant Professor, Chemical Engineering, JHU
 1984-1985 Adjunct Faculty, Department of Mathematics, City College of New York

Honors

2024 Debye Visiting Professor Utrecht University
 2023 Schowalter Lecturer, AIChE
 2021 National Academy of Engineering
 2020 American Academy of Arts and Sciences
 2018 Langmuir Lecturer, American Chemical Society
 2015 Johns Hopkins Society of Scholars
 2010 Fellow, American Physical Society
 2002 Fellow, Radcliffe Institute, Harvard University
 1993 Robert S. Pond, Sr. Teaching Award, Johns Hopkins University
 1992 François N. Frenkiel Award, American Physical Society
 1989 Bourse Chateaubriand for Postdoctoral Research, Mission Scientifique, France
 1989 Stanley Katz Memorial Award, City College of New York
 1987-89 Patricia Robert Harris Fellowship for Graduate Research, City University of New York
 1982, 1984 Ketchum Award, Department of Economics, City College of New York

C. Contributions to Science

1. We are developing active colloids in nematics for micro-robotics and reconfigurable, bio-inspired materials. Colloid shape, surface chemistry and dynamic displacement are designed to introduce topological defects whose non-linear dynamics generate new modalities of motion and interaction. As the colloid moves, e.g., under the action of an external field, these defects undergo complex, non-linear rearrangements. Interactions emerge that differ strikingly in range and form from their static counterparts (a). These interactions provide a toolkit for hybrid top-down, bottom-up assembly schemes based on active nematic colloids for micro-robotic tasks including building and reconfiguring structures (b). We have developed related concepts for robots at fluid interfaces, harnessing long-ranged capillary interactions for directed assembly (c). In collaboration with Dr. Ed. Steager.

- a. Yao, Tianyi, Žiga Kos, Qi Xing Zhang, Yimin Luo, Edward B. Steager, Miha Ravnik, and Kathleen J. Stebe. "Topological defect-propelled swimming of nematic colloids." *Science Advances* 8, no. 34 (2022): eabn8176; doi:10.1126/sciadv.abn8176.
- b. Yao, Tianyi, Žiga Kos, Qi Xing Zhang, Yimin Luo, Francesca Serra, Edward B. Steager, Miha Ravnik, and Kathleen J. Stebe. "Nematic Colloidal Micro-Robots as Physically Intelligent Systems." *Advanced Functional Materials* 32, no. 44 (2022): 2205546; doi: 10.1002/adfm.202205546.
- c. Yao, Tianyi, Nicholas G. Chisholm, Edward B. Steager, and Kathleen J. Stebe. "Directed assembly and micro-manipulation of passive particles at fluid interfaces via capillarity using a magnetic micro-robot." *Applied Physics Letters* 116, no. 4 (2020): 043702; doi:10.1063/1.5130635.

2. We are developing swimming colloids trapped at fluid interfaces as *Active Surface Agents*, whose motion and trapping state can be designed to promote interaction to guide structure formation and to promote mixing. Fluid

interfaces are highly non-ideal, complex domains that impose constraints that fundamentally alter swimming behavior (a). We study the bacterium *Pseudomonas Aeruginosa* (PA01) as model micro-scale swimmers at aqueous-hexadecane interfaces and characterize several distinct swimming behaviors. We measure the flow generated by these swimmers in the pusher mode using a recently developed flow visualization method *correlated displacement velocimetry* (b). We find a flow field with unexpected asymmetries. Hydrodynamic theory allows us to understand this flow field fundamentally. We explore the implications of our results on mixing in the interface and in the design of biomimetic systems (c).

- a. Chisholm, Nicholas G., and Kathleen J. Stebe. "Driven and active colloids at fluid interfaces." *Journal of Fluid Mechanics* 914 (2021); doi: 10.1017/jfm.2020.708
- b. Deng, Jiayi, Mehdi Molaei, Nicholas G. Chisholm, and Kathleen J. Stebe. "Interfacial flow around a pusher bacterium." *Journal of Fluid Mechanics* 976 (2023): A18; doi: 10.1017/jfm.2023.905
- c. Deng, Jiayi, Mehdi Molaei, Nicholas G. Chisholm, Scarlett E. Clarke, and Kathleen J. Stebe. "Swimmers at interfaces enhance interfacial transport." *Soft Matter* 20, no. 26 (2024): 5245-5257; doi: 10.1039/D4SM00140K.

3. We are developing functional materials based on bicontinuous interfacially jammed emulsion gels (bijels), a unique class of particle-stabilized fluid-bicontinuous structures formed by arresting the spinodal decomposition process of two liquid phases via jamming of nanoparticles at interface. We have developed processes that allow these materials to be formed by scalable methods (a)-(c), strengthened to remain robust in the fluid or cross-linked states (b). When using monomer oil as one of the two phases, this phase can be polymerized to form a porous polymer network crusted by densely packed nanoparticles at all surfaces (a) and (c) and exploited as functional materials ranging from separation membranes to metasurfaces for management of thermal energy. In collaboration with Prof. Daeyeon Lee.

- a. Wang, Tiancheng, Robert A. Riggleman, Daeyeon Lee, and Kathleen J. Stebe. "Bicontinuous interfacially jammed emulsion gels with nearly uniform sub-micrometer domains via regulated co-solvent removal." *Materials Horizons* 10, no. 4 (2023): 1385-1391; doi: 10.1039/D2MH01479C
- b. Di Vitantonio, Giuseppe, Daeyeon Lee, and Kathleen J. Stebe. "Fabrication of solvent transfer-induced phase separation bijels with mixtures of hydrophilic and hydrophobic nanoparticles." *Soft Matter* 16, no. 25 (2020): 5848; doi: 10.1039/D0SM00071J.
- c. Wang, Tiancheng, Yuzhe Xiao, Jonathan L. King, Mikhail A. Kats, Kathleen J. Stebe, and Daeyeon Lee. "Bioinspired switchable passive daytime radiative cooling coatings." *ACS Applied Materials & Interfaces* 15, no. 41 (2023): 48716-48724; doi: 10.1021/acsami.3c11338

PHS OTHER SUPPORT

*Name of Individual: Kyle Vining

Commons ID: KVINING

Other Support – Project/Proposal

ACTIVE

*Title: Targeting mechanical regulation of monocyte fate in head and neck cancer

*Major Goals: This project aims to determine the role of NFKBIZ on monocyte fate in fibrotic oral cancers.

*Status of Support: Active

Project Number: R00-DE-030084-03

Name of PD/PI: Kyle Vining

*Source of Support: NATIONAL INSTITUTE OF DENTAL AND CRANIOFACIAL RESEARCH/NIH/DHHS

*Primary Place of Performance: University of Pennsylvania

Project/Proposal Start and End Date: (MM/YYYY) (if available): 07/2022 - 06/2026 NCE

*Total Award Amount (including Indirect Costs): \$858,217

*Title: Targeting mechanical dysregulation of stromal-myeloid crosstalk in Jak2-mediated myeloproliferative neoplasms

*Major Goals: This pending project focuses on the role of PI3K-gamma inhibition on monocytes in their regulation of mesenchymal cells in vivo.

*Status of Support: Active

Project Number: RSG-1152051

Name of PD/PI: Kyle Vining

*Source of Support: AMERICAN CANCER SOCIETY

*Primary Place of Performance: University of Pennsylvania

Project/Proposal Start and End Date: (MM/YYYY) (if available): 01/2024 - 12/2027

*Total Award Amount (including Indirect Costs): \$598,884

*Title: Overcoming Resistance to CAR T-cell Therapy in Acute Leukemia by Targeting Mechanical Regulation in Fibrosis

*Major Goals: This project investigates mechanical regulation of CAR T-cells in pediatric leukemia.

*Status of Support: Active

Project Number: N/A

Name of PD/PI: Kyle Vining

*Source of Support: HARTWELL FOUNDATION

*Primary Place of Performance: University of Pennsylvania

Project/Proposal Start and End Date: (MM/YYYY) (if available): 04/2024 - 03/2027

*Total Award Amount (including Indirect Costs): \$270,000

*Title: Immuno-mechanical regulation of monocytes in fibrotic niches

*Major Goals: The goal of this project is to investigate the mechanical regulation of cell fate of myeloid development from hematopoietic stem cells using hydrogel models of the bone marrow niche

*Status of Support: Active

Project Number: R35-GM-157079-01

Name of PD/PI: Kyle Vining

*Source of Support: NATIONAL INSTITUTE OF GENERAL MEDICAL SCIENCES/NIH/DHHS

*Primary Place of Performance: University of Pennsylvania

Project/Proposal Start and End Date: (MM/YYYY) (if available): 01/2025 - 12/2029

*Total Award Amount (including Indirect Costs): \$2,031,250

*Title: Adaptive strain-responsive biopolymer networks

*Major Goals: We will develop strain-dependent extracellular matrix hydrogels.

*Status of Support: Active

Project Number: MRSEC IRG1 Seed Grant, NSF DMR 2309043

Name of Individual: Kyle Vining
Commons ID: KVINING

Name of PD/PI: Kyle Vining
*Source of Support: NSF DMR
*Primary Place of Performance: University of Pennsylvania
Project/Proposal Start and End Date: (MM/YYYY) (if available): 06/2025 - 05/2027
*Total Award Amount (including Indirect Costs): \$100,000

*Title: Targeted Bone Regeneration with Piperazine Ring-based Bisphosphonate Nanomaterials
*Major Goals: Develop lipid nanoparticles for controlled local delivery of mRNA and siRNA for mineralized tissues.
*Status of Support: Active
Project Number: Collaborative Research Grant
Name of PD/PI: Kyle Vining
*Source of Support: Institute of Regeneration Medicine, University of Pennsylvania
*Primary Place of Performance: University of Pennsylvania
Project/Proposal Start and End Date: (MM/YYYY) (if available): 01/2025 - 12/2026
*Total Award Amount (including Indirect Costs): \$150,000

PENDING

*Title: Biomaterial models of matrix stiffness for developing dendritic cell immunotherapies
*Major Goals: The overall goal of this project is to develop new dendritic cellular immunotherapies to overcome the suppressive mechanical cues in oral cancer and promote anti-tumor immune responses.
*Status of Support: Pending
Project Number: R01-DE-035122
Name of PD/PI: Kyle Vining
*Source of Support: NATIONAL INSTITUTE OF DENTAL AND CRANIOFACIAL RESEARCH/NIH
*Primary Place of Performance: University of Pennsylvania
Project/Proposal Start and End Date: (MM/YYYY) (if available): 7/2025 - 06/2030
*Total Award Amount (including Indirect Costs): \$2,899,646

*Title: Tumor Mechanobiology and Immunotherapy for Improving Treatments for Oral SCC
*Major Goals: This project aims to develop novel dendritic cell therapies for oral cancer with EVs.
*Status of Support: Pending
Project Number: N/A
Name of PD/PI: Kyle Vining
*Source of Support: Department of Defense
*Primary Place of Performance: University of Pennsylvania
Project/Proposal Start and End Date: (MM/YYYY) (if available): 07/2026 - 06/2028
*Total Award Amount (including Indirect Costs): \$490,000

*Title: CAREER: Regulation of Immune Responses in Defined Mechanical Environments
*Major Goals: The project aims to lay the foundation for new immunotherapies.
*Status of Support: Pending
Project Number: N/A
Name of PD/PI: Kyle Vining
*Source of Support: NSF CMMI
*Primary Place of Performance: University of Pennsylvania
Project/Proposal Start and End Date: (MM/YYYY) (if available): 05/2026 - 04/2031
*Total Award Amount (including Indirect Costs): \$812,800

Name of Individual: Kyle Vining
Commons ID: KVINING

*Title: Biocompatible adhesive resins for improving the longevity of pulp capping materials
*Major Goals: Develop anti-caries materials for preventing secondary decay and for restoring pulpal health.
*Status of Support: Pending
Project Number: R01DE035826
Name of PD/PI: Kyle Vining
*Source of Support: NIH/NIDCR
*Primary Place of Performance: University of Pennsylvania
Project/Proposal Start and End Date: (MM/YYYY) (if available): 04/2026 - 03/2031
*Total Award Amount (including Indirect Costs): \$3,150,731

*Title: Engineering Cellular Neighborhood Models of Bone Marrow Malignancies
*Major Goals: Develop engineered models of cellular neighborhoods of AML.
*Status of Support: Pending
Project Number: R61CA30974
Name of PD/PI: Kyle Vining
*Source of Support: NIH/NCI
*Primary Place of Performance: University of Pennsylvania
Project/Proposal Start and End Date: (MM/YYYY) (if available): 12/2025 - 11/2028
*Total Award Amount (including Indirect Costs): \$722,062

Title: RET Site: Feel the Force: Bringing Mechanobiology to 6-12 Classrooms
*Major Goals: This proposal provides summer research experiences in mechanobiology to teachers from Philadelphia to help them integrate interdisciplinary STEM content in their classrooms. The program focuses on mechanobiology - the study of how mechanical forces influence biological processes and aims to address gaps in grades 6-12 STEM education through hands-on research training.
*Status of Support: Pending
Project Number: N/A
Name of PD/PI: Loebel and Wells
*Source of Support: National Science Foundation
*Primary Place of Performance: University of Pennsylvania
Project/Proposal Start and End Date: (MM/YYYY) (if available): 8/2026 - 07/2029
*Total Award Amount (including Indirect Costs): \$600,000

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.

 Kyle Vining
2025.11.06
10:06:29 -05'00'

PHS OTHER SUPPORT

*Name of the Individual : Wells, Rebecca Gray
Commons ID: RGWELLS

Other Support- Project/Proposal

ACTIVE

*Proposal/Active Project Title: Whole Person Reference Physiome Research and Coordination Center

*Status of Support: Current

Proposal/Award Number: 1U24AT013504-01

*Source of Support: NIH

*Primary Place of Performance: Stanford University

*Proposal/Active Project Start Date: (MM/YYYY): 08/2025

*Proposal/Active Project End Date: (MM/YYYY): 07/2026

*Total Anticipated Proposal/Project Amount: \$422,500

*Overall Objectives: The goal of this project is to develop a unified and integrated database of human physiology.

*Proposal/Active Project Title: Injury, progression, and fibrosis of the extrahepatic bile duct

*Status of Support: Current

Proposal/Award Number: R01DK119290

*Source of Support: NIDDK

*Primary Place of Performance: University of Pennsylvania

*Proposal/Active Project Start Date: (MM/YYYY): 07/2023

*Proposal/Active Project End Date: (MM/YYYY): 04/2027

*Total Anticipated Proposal/Project Amount: \$1,430,000

*Overall Objectives: Biliary atresia is a major liver disease in the pediatric population, affecting healthy newborns and progressing rapidly to cirrhosis of the liver. The cause is unknown, and treatment is palliative. We hypothesize that the unique features of the neonatal bile ducts predispose to injury; we propose to study the role of those features in bile duct injury, progression, and fibrosis in order to gain insights into the human disease.

*Proposal/Active Project Title: Pathological consequences of altered tissue mechanics in fibrosis

*Status of Support: Current

Proposal/Award Number: R01EB017753

*Source of Support: NIBIB

*Primary Place of Performance: University of Pennsylvania

*Proposal/Active Project Start Date: (MM/YYYY): 09/2022

*Proposal/Active Project End Date: (MM/YYYY): 06/2026

*Total Anticipated Proposal/Project Amount: \$3,264,085

*Overall Objectives: The goal of this proposal is to understand the structural determinants of tissue mechanics, especially the organization of cells and matrix within tissues.

*Proposal/Active Project Title: Center for Molecular Studies in Digestive and Liver Diseases

*Status of Support: Current

Proposal/Award Number: P30DK050306

*Source of Support: NIDDK

*Primary Place of Performance: University of Pennsylvania

*Proposal/Active Project Start Date: (MM/YYYY): 07/2022

*Proposal/Active Project End Date: (MM/YYYY): 06/2027

*Total Anticipated Proposal/Project Amount: \$6,093,750

Overall Objectives: The Center supports four scientific cores, conferences, and a pilot and feasibility program to advance research of center members and junior faculty as well as to attract new investigators to digestive and liver topics.

*Proposal/Active Project Title: Center for Engineering Mechanobiology

*Status of Support: Current

Proposal/Award Number: CMMI-1548571

*Source of Support: NSF

*Primary Place of Performance: University of Pennsylvania

*Proposal/Active Project Start Date: (MM/YYYY): 09/2021

*Proposal/Active Project End Date: (MM/YYYY): 09/2026

*Total Anticipated Proposal/Project Amount: \$22,470,000

*Overall Objectives: The goal of CEMB is to foster and support research and training in mechanobiology that is at the interface between the biological and the physical sciences and engineering.

PENDING

*Proposal/Active Project Title: RET Site: Feel the Force: Bringing Mechanobiology to 6-12 Classrooms

*Status of Support: Pending

Proposal/Award Number: CMMI-1548571

*Source of Support: N/A

*Primary Place of Performance: University of Pennsylvania

*Proposal/Active Project Start Date: (MM/YYYY): 08/2026

*Proposal/Active Project End Date: (MM/YYYY): 07/2029

*Total Anticipated Proposal/Project Amount: \$600,000

*Overall Objectives: This proposal provides summer research experiences in mechanobiology to teachers from Philadelphia to help them integrate interdisciplinary STEM content in their classrooms. The program focuses on mechanobiology – the study of how mechanical forces influence biological processes – and aims to address gaps in grades 6-12 STEM education through hands-on research training

*Proposal/Active Project Title: Solid stress as a driver of hepatocellular carcinoma development in cirrhotic nodules

*Status of Support: Pending

Proposal/Award Number: CA251447

*Source of Support: Department of Defense

*Primary Place of Performance: University of Pennsylvania

*Proposal/Active Project Start Date: (MM/YYYY): 07/2026

*Proposal/Active Project End Date: (MM/YYYY): 06/2028

*Total Anticipated Proposal/Project Amount: \$600,000

*Overall Objectives: The goal of this proposal is to investigate the causes and impacts of solid stress in nodules of the cirrhotic liver; in particular to determine the role of collagen organization and the impact on the development of hepatocellular carcinoma. Additionally, we will determine diagnostic tests for the presence of solid stress in cirrhosis.

*Proposal/Active Project Title: Tissue mechanics driving fibrosis in high cholesterol- associated steatitic liver disease

*Status of Support: Pending

Proposal/Award Number: 1R01DK144619

*Source of Support: NIDDK

*Primary Place of Performance: University of Pennsylvania

*Proposal/Active Project Start Date: (MM/YYYY): 01/2026

*Proposal/Active Project End Date: (MM/YYYY): 12/2030

*Total Anticipated Proposal/Project Amount: \$3,959,071

*Overall Objectives: The goal is to use both theoretical modeling and experimentation to understand the impact of anisotropic solid and liquid cholesterol-containing crystals on tissue, cell, and nuclear mechanics.

In-Kind Contributions

*Status of Support: Current

*Source of Support: German Research Foundation SA 901/26-1

*Primary Place of Performance: University of Pennsylvania

*Receipt (or Anticipated Receipt) Date of In-Kind Contribution: (MM/YYYY) : 01/2026

*Summary of In-Kind Contribution: Reimbursement of travel, housing, and per diem expenses for sabbatical support at Charite Universitätsmedizin, Berlin, Germany

*U.S. Dollar Value of In-Kind Contribution: \$11,056

*Overall Objectives: The goal is development of international cooperation on the topic "Untersuchung des Zusammenhangs zwischen Leberstoffwechsel und mechanischen Gewebeeigenschaften mittels neuartiger Tabletop-MR-Elastographie" (Investigation of the relationship between liver metabolism and mechanical tissue properties using novel tabletop MR elastography", at Charite Universitätsmedizin, Berlin, Germany

Certification:

I certify that the information provided is current, accurate, and complete. This includes but is not limited to current, pending, and other support (both foreign and domestic) as defined in 42 U.S.C. § 6605.

I also certify that, at the time of submission, I am not a party to a malign foreign talent recruitment program.

Misrepresentations and/or omissions may be subject to prosecution and liability pursuant to, but not limited to, 18 U.S.C. §§ 287, 1001, 1031 and 31 U.S.C. §§ 3729-3733 and 3802.

Certified by Wells, Rebecca in SciENcv on 2025-10-30 14:46:24

Rebecca Wells

Digitally
signed by
Rebecca Wells

Date:
2025.11.07
15:55:59 -05'00'

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

UEI*: GM1XX56LEP58

Budget Type*: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE

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.	HARDIK		MAKKAR		PD/PI		12			86,275.00	18,549.00	104,824.00
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons: File Name:											Total Senior/Key Person	104,824.00

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	Total Number Other Personnel					Total Other Personnel	
Total Salary, Wages and Fringe Benefits (A+B)							104,824.00

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

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

UEI*: GM1XX56LEP58

Budget Type*: ☒ Project ☐ Subaward/Consortium

Organization: TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE

Start Date*: 07-01-2026

End Date*: 06-30-2027

Budget Period: 1

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

D. Travel	Funds Requested (\$)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	3,000.00

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

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

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

UEI*: GM1XX56LEP58

Budget Type*: ☒ Project ☐ Subaward/Consortium

Organization: TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE

Start Date*: 07-01-2026

End Date*: 06-30-2027

Budget Period: 1

F. Other Direct Costs	Funds Requested (\$)*
1. Materials and Supplies	15,500.00
2. Publication Costs	1,500.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Other Costs	5,000.00
Total Other Direct Costs	22,000.00

G. Direct Costs	Funds Requested (\$)*
Total Direct Costs (A thru F)	129,824.00

H. Indirect Costs			
Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
1 . MTDC	8	129,824.00	10,386.00
Total Indirect Costs			10,386.00
Cognizant Federal Agency	DHHS, Stephen Hobday, (301) 492-4855		
(Agency Name, POC Name, and POC Phone Number)			

I. Total Direct and Indirect Costs	Funds Requested (\$)*
Total Direct and Indirect Institutional Costs (G + H)	140,210.00

J. Fee	Funds Requested (\$)*

K. Total Costs and Fee	Funds Requested (\$)*
	140,210.00

L. Budget Justification*	File Name: Budget Justification final.pdf
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RESEARCH & RELATED Budget (F-K) (Funds Requested)

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

UEI*: GM1XX56LEP58

Budget Type*: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE

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.	HARDIK		MAKKAR		PD/PI		12			86,275.00	18,549.00	104,824.00
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons: File Name:											Total Senior/Key Person	104,824.00

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	Total Number Other Personnel					Total Other Personnel	
Total Salary, Wages and Fringe Benefits (A+B)							104,824.00

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

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

UEI*: GM1XX56LEP58

Budget Type*: ☒ Project ☐ Subaward/Consortium

Organization: TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE

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)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	3,000.00

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

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

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

UEI*: GM1XX56LEP58

Budget Type*: ☒ Project ☐ Subaward/Consortium

Organization: TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE

Start Date*: 07-01-2027

End Date*: 06-30-2028

Budget Period: 2

F. Other Direct Costs	Funds Requested (\$)*
1. Materials and Supplies	19,500.00
2. Publication Costs	1,500.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Other Costs	1,000.00
Total Other Direct Costs	22,000.00

G. Direct Costs	Funds Requested (\$)*
Total Direct Costs (A thru F)	129,824.00

H. Indirect Costs			
Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
1 . MTDC	8	129,824.00	10,386.00
Total Indirect Costs			10,386.00
Cognizant Federal Agency	DHHS, Stephen Hobday, (301) 492-4855		
(Agency Name, POC Name, and POC Phone Number)			

I. Total Direct and Indirect Costs	Funds Requested (\$)*
Total Direct and Indirect Institutional Costs (G + H)	140,210.00

J. Fee	Funds Requested (\$)*

K. Total Costs and Fee	Funds Requested (\$)*
	140,210.00

L. Budget Justification*	File Name: Budget Justification final.pdf
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RESEARCH & RELATED Budget (F-K) (Funds Requested)

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

UEI*: GM1XX56LEP58

Budget Type*: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE

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.	HARDIK		MAKKAR		PD/PI		12			0.00	0.00	0.00
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons: File Name:											Total Senior/Key Person	0.00

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	Total Number Other Personnel					Total Other Personnel	
Total Salary, Wages and Fringe Benefits (A+B)							0.00

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

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

UEI*: GM1XX56LEP58

Budget Type*: ☒ Project ☐ Subaward/Consortium

Organization: TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE

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)	0.00
2. Foreign Travel Costs	
Total Travel Cost	0.00

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

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

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

UEI*: GM1XX56LEP58

Budget Type*: ☒ Project ☐ Subaward/Consortium

Organization: TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE

Start Date*: 07-01-2028

End Date*: 06-30-2029

Budget Period: 3

F. Other Direct Costs	Funds Requested (\$)*
1. Materials and Supplies	0.00
2. Publication Costs	0.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Other Costs	249,000.00
Total Other Direct Costs	249,000.00

G. Direct Costs	Funds Requested (\$)*
Total Direct Costs (A thru F)	249,000.00

H. Indirect Costs			
Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
		Total Indirect Costs	
Cognizant Federal Agency		DHHS, Stephen Hobday, (301) 492-4855	
(Agency Name, POC Name, and POC Phone Number)			

I. Total Direct and Indirect Costs	Funds Requested (\$)*
Total Direct and Indirect Institutional Costs (G + H)	249,000.00

J. Fee	Funds Requested (\$)*

K. Total Costs and Fee	Funds Requested (\$)*
	249,000.00

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

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

UEI*: GM1XX56LEP58

Budget Type*: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE

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.	HARDIK		MAKKAR		PD/PI		12			0.00	0.00	0.00
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons: File Name:											Total Senior/Key Person	0.00

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	Total Number Other Personnel					Total Other Personnel	
Total Salary, Wages and Fringe Benefits (A+B)							0.00

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

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

UEI*: GM1XX56LEP58

Budget Type*: ☒ Project ☐ Subaward/Consortium

Organization: TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE

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)	0.00
2. Foreign Travel Costs	
Total Travel Cost	0.00

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

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

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

UEI*: GM1XX56LEP58

Budget Type*: ☒ Project ☐ Subaward/Consortium

Organization: TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE

Start Date*: 07-01-2029

End Date*: 06-30-2030

Budget Period: 4

F. Other Direct Costs	Funds Requested (\$)*
1. Materials and Supplies	0.00
2. Publication Costs	0.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Other Costs	249,000.00
Total Other Direct Costs	249,000.00

G. Direct Costs	Funds Requested (\$)*
Total Direct Costs (A thru F)	249,000.00

H. Indirect Costs			
Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
		Total Indirect Costs	
Cognizant Federal Agency		DHHS, Stephen Hobday, (301) 492-4855	
(Agency Name, POC Name, and POC Phone Number)			

I. Total Direct and Indirect Costs	Funds Requested (\$)*
Total Direct and Indirect Institutional Costs (G + H)	249,000.00

J. Fee	Funds Requested (\$)*

K. Total Costs and Fee	Funds Requested (\$)*
	249,000.00

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

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

UEI*: GM1XX56LEP58

Budget Type*: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE

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.	HARDIK		MAKKAR		PD/PI		12			0.00	0.00	0.00
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons: File Name:											Total Senior/Key Person	0.00

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*	
	Post Doctoral Associates							
	Graduate Students							
	Undergraduate Students							
	Secretarial/Clerical							
0	Total Number Other Personnel					Total Other Personnel		
							Total Salary, Wages and Fringe Benefits (A+B)	0.00

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

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

UEI*: GM1XX56LEP58
Budget Type*: ☒ Project ☐ Subaward/Consortium
Organization: TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE

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)	0.00
2. Foreign Travel Costs	
Total Travel Cost	0.00

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

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

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

UEI*: GM1XX56LEP58

Budget Type*: ☒ Project ☐ Subaward/Consortium

Organization: TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, THE

Start Date*: 07-01-2030

End Date*: 06-30-2031

Budget Period: 5

F. Other Direct Costs	Funds Requested (\$)*
1. Materials and Supplies	0.00
2. Publication Costs	0.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Other Costs	249,000.00
Total Other Direct Costs	249,000.00

G. Direct Costs	Funds Requested (\$)*
Total Direct Costs (A thru F)	249,000.00

H. Indirect Costs			
Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
		Total Indirect Costs	
Cognizant Federal Agency		DHHS, Stephen Hobday, (301) 492-4855	
(Agency Name, POC Name, and POC Phone Number)			

I. Total Direct and Indirect Costs	Funds Requested (\$)*
Total Direct and Indirect Institutional Costs (G + H)	249,000.00

J. Fee	Funds Requested (\$)*

K. Total Costs and Fee	Funds Requested (\$)*
	249,000.00

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

Budget Justification

Hardik Makkar (PI, 12 calendar months) will direct all aspects of this project. He will devote >75% minimum effort to research activities, and the remainder towards career development the project in all years. \$86,275 is requested for PI's salary for Award Year 1 and Award Year 2.

Kyle H. Vining DDS, PhD, Mentor (Assistant Professor, Center for Innovation and Precision Dentistry) Scientific mentoring (training in in biomaterial characterization, focusing on mechanically tunable hydrogel models using advanced techniques like rheology, nanoindentation, AFM, and multi-photon microscopy while also pursuing relevant coursework), career development, and job search

Rebecca G. Wells MD, PhD, Co-Mentor (Professor, Co-Director, Center for Engineering Mechanobiology) Scientific mentoring (training in studying gingival tissue mechanics in mice with ligature-induced periodontitis, focusing on tissue characterization, ECM reorganization, and histological analysis, while also pursuing relevant coursework), career development, and job search

1) Scientific Advisory Committee

- **Kang I Ko**, DMD, DScD (Assistant Professor, Penn Dental Medicine)
Role - Access to gingival tissue will support my in vitro experiments, with his guidance as a board-certified periodontist and basic science researcher in fibroblast biology being essential to my work.
- **George Hajishengallis** DDS, PhD (Professor, Penn Dental Medicine)
Role - Advise on mouse model of ligature induced periodontitis and spatial transcriptomics, while also offering valuable career development guidance as a dentist-scientist.
- **Michael C. Abt**, PhD (Assistant Professor, Penn Medicine)
Role - Advice on immune-microbiome interactions and mucosal immunology, offering expertise in germ-free mouse models to study the impact of gingival matrix stiffness on host-microbiome interactions in vivo.
- **Michel (Hyun) Koo**, DDS, MS, PhD (Professor, Co-Director- Center for Innovation and Precision Dentistry, Penn Dental Medicine and Penn Engineering)
Role – Access to resources at Penn Dental and advice on career development, cross-disciplinary collaborations, and job search
- **Kathleen J. Stebe**, PhD and Professor, Co-Director- Center for Innovation and Precision Dentistry, Penn Dental Medicine and Penn Engineering)
Role - Access to resources at Penn Engineering and advice on career development, cross-disciplinary collaborations, and job search

Budget Details:

Other Direct Costs

Travel: \$3,000 per year is requested for the PI to attend the conferences to present research results. This travel supports the PI's professional development, as outlined in the Career Development Plan. Each trip includes round-trip airfare, lodging, per diem, ground transportation and registration fees.

Materials and Supplies: \$15,500 for Award Year 1 and \$19,500 for Award Year 2 per is requested for the purchase of project specific supplies. These materials and supplies will be used for achieving this proposal's Specific Aim 1 during mentored phase.

Publication costs: \$1,500 per year is requested for costs of anticipated publications, which are described in the Career Development Plan.

Intra and Extramural Training : \$5,000 is requested for Intra and extramural trainings for Award Year 1 and \$1,000 for Award Year 2 which are described in the Career Development Plan.

Data Management and Sharing Costs: No funds requested.

Indirect Costs

Indirect costs are calculated using the Trustees of the University of Pennsylvania's federally negotiated rate of 8% of modified total direct costs.

R00 Independent Phase

In Years 3-5, funds are requested in the amount of \$249,000 per year (total cost) for the R00 Independent Phase, with the understanding that the applicant institution for that phase will submit a detailed budget for each budget period that reflects their direct and indirect costs.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals (\$)	
Section A, Senior/Key Person		209,648.00
Section B, Other Personnel		0.00
Total Number Other Personnel	0	
Total Salary, Wages and Fringe Benefits (A+B)		209,648.00
Section C, Equipment		0.00
Section D, Travel		6,000.00
1. Domestic	6,000.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		791,000.00
1. Materials and Supplies	35,000.00	
2. Publication Costs	3,000.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	753,000.00	
9. Other 2	0.00	
10. Other 3	0.00	
11. Other 4	0.00	
12. Other 5	0.00	
13. Other 6	0.00	
14. Other 7	0.00	
15. Other 8	0.00	
16. Other 9	0.00	
17. Other 10	0.00	
Section G, Direct Costs (A thru F)		1,006,648.00
Section H, Indirect Costs		20,772.00
Section I, Total Direct and Indirect Costs (G + H)		1,027,420.00
Section J, Fee		0.00
Section K, Total Costs and Fee (I + J)		1,027,420.00

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)	Introduction_11102025.pdf
Candidate Section	
2. Candidate Information and Goals for Career Development	Candidate Background_11102025.pdf
Research Plan Section	
3. Specific Aims	Specific Aims_11102025.pdf
4. Research Strategy*	Research Strategy_Revised 11102025.pdf
5. Progress Report Publication List (for Renewal applications)	
6. Training in the Responsible Conduct of Research	TRAINING IN RESPONSIBLE CONDUCT OF RESEARCH.pdf
Other Candidate Information Section	
7. Candidate's Plan to Provide Mentoring	
Mentor, Co-Mentor, Consultant, Collaborators Section	
8. Plans and Statements of Mentor and Co-Mentor(s)	Plans and Statements of Mentor and Co-Mentor.pdf
9. Letters of Support from Collaborators, Contributors, and Consultants	Letters Advisor and Counsultants print.pdf
Environment and Institutional Commitment to Candidate Section	
10. Description of Institutional Environment	DESCRIPTION OF INSTITUTIONAL ENVIRONMENT.pdf
11. Institutional Commitment to Candidate's Research Career Development	Institutional Commitment to Candidate.pdf
12. Description of Candidate's Contribution to Program Goals	
Other Research Plan Section	
13. Vertebrate Animals	VERTEBRATE ANIMALS.pdf
14. Select Agent Research	
15. Consortium/Contractual Arrangements	
16. Resource Sharing	
17. Other Plan(s)	DMS PLAN.pdf
18. Authentication of Key Biological and/or Chemical Resources	AUTHENTICATION OF KEY BIOLOGICAL AND.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
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INTRODUCTION TO THE APPLICATION (No color, underline, markings, or bracketing were used to identify changes in the application. Color and bolding are used selectively to enhance readability but not to mark changes.)

I am grateful to the reviewers for their insightful and constructive feedback on my NIDCR K99/R00 application entitled, ***“Probing the Mechanical Regulation of Inflammation in Periodontal Health and Disease.”*** I have thoroughly considered all comments and have extensively revised the application. The result is a more focused, rigorous, and feasible research plan, a strengthened career development and mentoring plan, and a clearer path toward my transition to independence as a dentist-scientist. This revised application directly addresses all major critiques and substantially enhances the clarity, rigor, and feasibility of the proposed studies.

The most significant changes include:

- 1) **Refined and Focused Research Plan:** Reviewers noted that the original K99 phase combined multiple complex and high-risk techniques, which could exceed the scope of a trainee award. In response, **I have substantially simplified the K99 research plan by removing the bone marrow transplantation experiments and focusing on the ligature-induced periodontitis (LIP) model only.** This streamlining ensures feasibility and maintains scientific depth. I have also clearly delineated which experiments will be performed during the K99 versus R00 phases to match achievable milestones for each stage.
- 2) **Enhanced Experimental Rigor and Critical Controls:** Reviewers highlighted the need for additional experimental controls. **I have now incorporated no-matrix controls (plate-adhered and suspension cultures and enzyme-only controls (collagenase IV and alginate-lyase) to isolate enzyme-specific effects.** I have also clarified that the hydrogel digestion and flow cytometry techniques are well-established and optimized in our laboratory, with low enzyme concentrations minimizing impact on surface markers such as CD14, CD80, and PD-L1. These additions directly address concerns about technical artifacts and reproducibility.
- 3) **Strengthened In Vivo Rigor and Validation:** To address concerns regarding the feasibility and validation of the LIP mouse model, I now reference its **routine use in Dr. Hajishengallis’s lab, where reproducibility is well established.** The model’s application and success are now demonstrated through published data (Figure 4D). I have also included power analyses and detailed group size justifications under the Scientific Rigor section to ensure statistical validity, including considerations for potential sex-specific effects.
- 4) **Clarified Rationale for Transglutaminase Experiments:** Reviewer noted a conceptual inaccuracy in the previous description of transglutaminase. **This has been clarified with new preliminary data using human ex vivo gingival explants, demonstrating that transglutaminase treatment increases tissue stiffness (storage modulus) and attenuates TLR2-mediated IL-6 secretion.** These results directly support the revised Aim 2 hypothesis that matrix stiffening regulates local immune responses.
- 5) **Strengthened Mentoring and Career Development Plan:** I have provided a more detailed and structured mentoring plan, defining the specific roles of each mentor, frequency of meetings, and individualized training goals. The plan now includes dedicated **grantsmanship training, a revised Gantt chart with clear milestones, and enhanced documentation of mentor commitment and complementary expertise.**
- 6) **Addressed Administrative Note:** The hypertext issue has been rectified

Section-by-Section Summary of Revisions

Candidate: Expanded to highlight my dual expertise in periodontology and bioengineering and my long-term commitment to an independent academic research career.

Career Development Plan: Substantially revised with a detailed timeline, grantsmanship plan, and structured mentorship and advisory committee meetings.

Research Plan: Aim 1: Expanded prior research, limitation and rationale for studying epigenetic modifications, inclusion of additional controls, and new analyses such as **whole-genome bisulfite sequencing.** Aim 2: Focused on the LIP model, with power analysis and validated methodology; inclusion of new preliminary ex vivo data confirming feasibility of transglutaminase-induced matrix stiffening.

Mentoring and Environment: Roles and responsibilities of mentors are now clearly defined, with greater **emphasis on the lack of intellectual overlap** and the exceptional institutional resources available at the University of Pennsylvania. Further, the role of Bioinformatician and core expertise is highlighted and clarified.

I believe that these revisions have substantially strengthened my application. I have directly addressed all reviewer concerns, and I now present a clearer, more compelling, and achievable research and career development plan. I remain enthusiastic about the potential of this work to advance our mechanobiological understanding of periodontal disease and to enable the development of mechano-immune-targeted therapies.

CANDIDATE'S BACKGROUND – I am a dentist-scientist, NIDCR R90 postdoctoral fellow at the Center for Innovation & Precision Dentistry (CiPD) of the University of Pennsylvania. My research focuses on understanding the role of extracellular matrix (ECM) mechanical cues in periodontal health and disease¹. I integrate tools and techniques in Cell Biology, Bioengineering, Biomaterials, and Microbiology to investigate the impact of gingival ECM stiffness on stromal-myeloid crosstalk and immune responses.

My dental education at JSS Dental College and Hospital, India, provided the foundation for understanding oral and craniofacial health, followed by specialty training in Endodontics at KIIT University, India, where I also gained experience in the biotechnology innovation sector. I completed doctoral studies in Oral Sciences and Bioengineering at the National University of Singapore, shaping my commitment to advancing oral, dental, and craniofacial care at the intersection of bioengineering and life sciences. Through my D.D.S. training, I relied on evidence-based practice to guide treatment and realized how crucial research is in improving outcomes. I graduated with the **University Gold Medal**, the **Pierre Fauchard International Senior Student Merit Award**, and the **IDA-Colgate Merit Award** as the top student for two consecutive years. During my Endodontics residency thesis, I collaborated with the School of Biotechnology, blending clinical work with material sciences and biomedical engineering research, gaining skills in grant writing and intellectual property protection, which resulted in several first-author publications²⁻⁶. I received the **Biotechnology Ignition Grant** and led a project as Principal Investigator to develop a bio-polymeric root canal scaffold, resulting in patent applications (**IN201831014946; IN202031005561**). I also led a Social Innovation Project to create healthcare technologies for low-resource settings. As one of the top five innovators from my country for the **BIRAC-IGNITE fellowship, Judge Business School, University of Cambridge**, I received training on translating innovations into products. This experience reinforced my belief that mentored research is crucial for professional advancement.

These experiences led me to pursue a Ph.D. at the National University of Singapore under the **President's Graduate Fellowship**, co-mentored by Prof. Gopu Sriram and Prof. Chwee Teck Lim at the Faculty of Dentistry and Institute of Health Innovation and Technology. I developed core skills in developing micro-vascularized 3D gingival connective tissue models⁷ and integrated them in microfluidic organ-on-chip systems to study periodontal host-microbe interactions⁸. These systems aim to replicate native tissue properties, including tissue barrier functions and gingival crevicular fluid flow. I published several first-author articles, demonstrating that bioengineered models enabled studies on spatiotemporal colonization, vascular invasion, and immune cell polarization by biofilm colonizers⁷⁻¹². Using these models, I also investigated the role of tissue topography and fibroblast heterogeneity in immune responses to oral microbes⁹ and showed how gingival crevicular fluid protects the host by microbial clearance⁸. I was awarded the **IADR Hatton Award** and the **NUS Travel Award**. My PhD provided collaborative opportunities, which culminated in a co-authored review on biofabrication strategies for oral soft tissue regeneration¹¹ as well as original research on lipid nanoparticle-mediated oral drug delivery of antivirals¹³. Additionally, I interned with Umami Bioworks, focusing on developing animal-component-free media for cultivated seafood to gain industry experience.

During my Ph.D. at and while working at the NUS Mechanobiology Institute, I was introduced to the mechanobiology of wound healing. This sparked my interest in applying these principles to periodontal diseases, an area understudied in mechanobiological mechanisms. Building on my clinical background and doctoral training in bioengineering, I aimed to expand my research into the intersection of mechanobiology and periodontal health for postdoctoral training. A pivotal moment came when I met **Dr. Kyle Vining**, a dentist-scientist bridging materials science and mechanobiology, at the 2023 AADOCR conference. With shared research goals, I joined Dr. Vining's lab at the University of Pennsylvania, focusing on Immuno-Mechanobiology to understand chronic inflammatory diseases. Four months into training, I was selected for the prestigious **NIDCR T90/R90 program— "Advanced Training at the Interface of Engineering and Oral-Craniofacial Sciences"—at UPenn's Center for Innovation and Precision Dentistry (CiPD)**.

At UPenn, I leverage the vibrant research ecosystem to investigate periodontitis pathogenesis from a mechanobiological perspective, focusing on ECM mechanics and innate immunity. I have a strong track record of teaching and mentoring. During my Ph.D., I mentored three students—two now in the life science industry and one pursuing a Ph.D. at the University of Zurich. As a postdoc, I currently mentor three DMD students, two master's students (Bioengineering), and two Ph.D. students in Materials Science, resulting in co-author publications^{14,15}. I also mentor an undergraduate through the American Cancer Society's Cancer Research Internship, supporting students from marginalized backgrounds, and serve as a **regional postdoc representative of the AADOCR-National Student Research Group**, promoting collaborations, memberships,

and organizing workshops. My scholarly productivity as a postdoc reflects in my research outputs with [one first-author manuscript currently in preprint¹](#) and [two additional peer-reviewed publications^{14,15}](#). My goal is to build an academic career, fostering creative freedom, intellectual growth, and value creation for society. With my track record of productivity, creativity, and independent work, I am well-positioned to become an independent investigator. The mentoring plan proposed herein will provide me with the opportunity to achieve independence within the time frame of the K99 phase. The quality and output of my research in bioengineering, cell biology, microbiology and immunology ^{1,7-9,11-15} make me a strong contender for tenure track independent investigator.

CAREER GOALS AND OBJECTIVES – My career goal is to become a tenured professor at a leading research-intensive university, establishing a lab focused on immuno-mechanobiology and Tissue Engineering to study periodontal diseases and develop next-generation mechano-immune therapeutics. I also aim to develop non-animal technologies for studying chronic oral inflammatory diseases and lead a collaborative, egalitarian team that encourages creativity and innovation through decentralized work. To complement this, I plan to actively collaborate with clinicians in the Periodontology department. This continued engagement will not only ensure that my scientific questions remain relevant to important unmet clinical needs but will also provide a unique platform for training future clinician–scientists. By being actively engaged in clinical evidence-driven research, I hope to expose pre-dental and dental students to laboratory-based research early in their careers, thus developing an integrated clinical–research continuum that passionately promotes innovation, inter-disciplinary collaboration, and translational breakthroughs. I also believe this approach will foster a meaningful and autonomous environment where research is directly aimed at solving critical clinical problems, especially for vulnerable communities. Academia-industry partnerships will play a key role in translating my science into clinical solutions. The K99 career development plan will be crucial in advancing my training in mechano-immunobiology in periodontal health and disease. My research will build on my work as an NIDCR R90 fellow, where I investigated the mechanical regulation of gingival fibroblast-monocyte crosstalk in periodontal diseases using a tunable ECM hydrogel system. I aim to continue studying how ECM stiffness and viscoelastic properties affect innate immune responses and homeostasis.

Primary Mentoring Team, Transition to Independence, and Intellectual Overlap – My primary mentoring team includes **Dr. Kyle H. Vining** (Asst. Professor, School of Dental Medicine, UPenn) and **Dr. Rebecca G. Wells** (Professor, School of Medicine). Dr. Vining, a dentist–bioengineer scientist, is a leader in myeloid mechanobiology and biomaterial-based immunomodulation, focusing on the mechanical regulation of tissue inflammation in bone marrow myelofibrotic diseases and how mechanical cues affect cellular immunotherapies. His work has been supported by NIDCR K08 and K99/R00, the American Cancer Society Research Scholars Grant, the Hartwell Foundation Individual Biomedical Research Award, and NIGMS R35 MIRA. At Penn, he has mentored 6 PhD students, 1 DScD student, 4 postdoctoral fellows (including two T90/R90 fellows), and 2 visiting scholars. His strong mentoring record demonstrates his commitment and ability to support my development into an independent dentist-scientist. Dr. Wells, an internationally recognized expert in fibrosis and tissue mechanics, serves as Co-Director of the NSF Center for Engineering Mechanobiology and Co-Associate Director of Penn’s NIH-funded Digestive and Liver Center. Her research on tissue mechanics, matrix reorganization, and interstitial spaces provides a strong foundation to apply these concepts to gingival ECM biology in health and periodontal disease. [This co-mentorship structure ensures I receive both technical mentorship and seasoned career and scientific guidance.](#) The proposed training plan leverages their broad expertise while maintaining distinct aims: [gingival tissue mechanobiology at the host–microbe interface is novel and entirely independent of my mentors’ current and planned research](#)— While [Dr. Vining’s research focuses on bone marrow inflammation and head and neck cancer](#), and [Dr. Wells’ on liver and biliary fibrosis](#), my program is uniquely centered on the [role of ECM mechanics in periodontal disease](#). [Both mentors have agreed to transfer intellectual knowledge and proprietary reagents to support my independent research](#) and encourage my creative freedom. This project establishes my independent research niche and over the next five years, I aim to complete the proposed research and career development plans, obtain early career extramural funding (e.g., NIDCR R03, R21; NIGMS R35), and pursue follow-on NIH R01 support (*ref. Training Plan & Gantt Chart*).

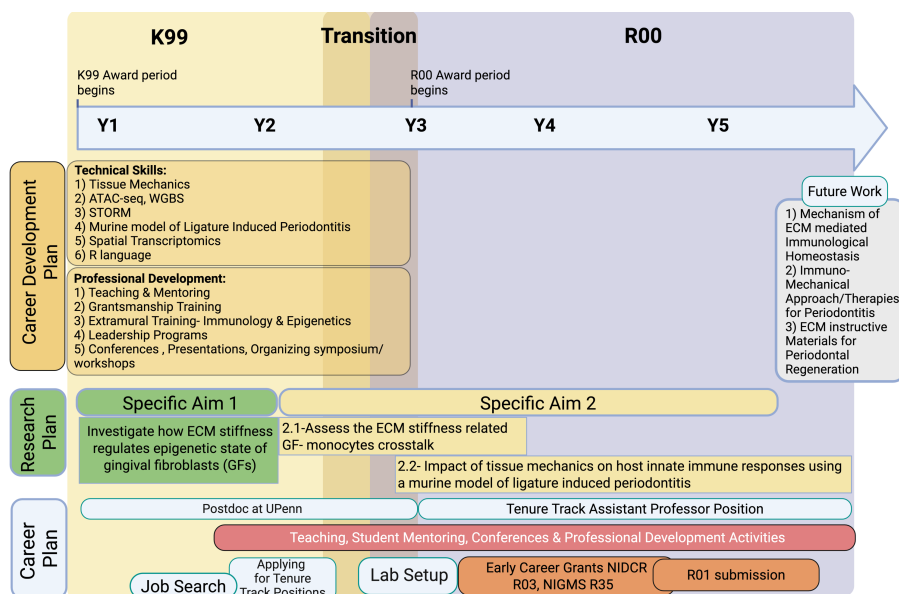
THE ADVISORY COMMITTEE [comprises members specializing in dentistry, immunology, bioengineering, and microbiology while leading NIH training programs at Penn](#). They will provide mentoring via advisory and one-on-one meetings, which will be critical for my career development during the K99 phase. **1) Dr. George Hajishengallis** (Dentist, Immunologist)- a world-renowned leader in immunology, has seminal contribution in studying immunoinflammatory mechanisms in periodontitis. During the K99 phase, I will

leverage his lab's expertise to learn workflow for a murine model of ligature-induced periodontitis^{16,17}, as well as develop technical proficiency in processing animal tissues for downstream molecular and protein-based assays, including spatial biology and whole-genome methylation sequencing workflow¹⁸. Overall, his expertise will be critical for the successful execution of in vitro workflow under Aim 2 (stromal-myeloid cross-talk using hydrogel model) and in vivo immunology workflow in the mouse model, including interpretations of spatial transcriptomics data. I will have biannual advisory meetings and one-on-one guidance as needed. As a dentist-scientist, he will also provide valuable guidance for my career development. (*refer to letter of support*). **2) Dr. Kang I. Ko, DMD, DScD (Periodontist)**. As a clinician scientist and board-certified periodontist, he will provide scientific advice and a clinical perspective on my work. Dr. Ko's expertise in studying the role of oral fibroblast heterogeneity in wound healing. His lab's gingival tissue biobank and archived clinical samples (IRB #844933, PI: Ko) will support my in vitro experiments (Specific Aims 1 and 2.1). Further, his lab's expertise in studying gingival fibroblast-driven periodontal tissue damage in a murine model of ligature-induced periodontitis¹⁹ using spatial transcriptomics will be key in obtaining a reductionist approach to understand fibroblast-driven regulation of periodontal inflammation. Dr. Ko and I will have quarterly meetings and will offer one-on-one guidance as needed. (*refer to letter of support*). **3) Dr. Michael Abt (Immunologist, Microbiologist)** – Dr. Abt will provide analytical guidance on the animal workflow for Aim 2, focusing on immune-microbiome interactions and mucosal immunology via quarterly meetings and one-on-one guidance as needed. I will seek his expertise to study how gingival matrix stiffness impacts microbiome changes in vivo, opening new future research directions. **4) Dr. Michel (Hyun) Koo & Dr. Kathleen Stebe** – Co-directors of the NIDCR R90/T90 program at UPenn, Dr. Koo and Dr. Stebe will support my career development and cross-disciplinary collaborations. **Dr. Koo, an oral microbiology/biofilm expert**, will provide guidance on how ECM mechanical properties influence immune modulation at the host-microbe interface, ensuring scientific rigor and innovative approaches via quarterly meetings and one-on-one guidance as needed (*refer to letter of support*).

PROPOSED TRAINING PLAN (Technical Skills) – Mentored Phase

1. [Training in Tissue Mechanics](#) – I will receive comprehensive training in tissue mechanics and biomaterial characterization through three integrated components. First, under the mentorship of **Dr. Kyle Vining** (primary mentor), I will develop expertise in biomaterial characterization using his lab's mechanically tunable hydrogel models. I will gain hands-on experience with techniques including micro-rheology, atomic force microscopy (AFM), and multi-photon microscopy to assess the mechanical properties and microscale features of gingival ECM hydrogels. Dr. Vining, an expert in myeloid mechanobiology, will also guide me in studying ECM stiffness-induced stromal-myeloid crosstalk. Second, under the mentorship of **Dr. Rebecca Wells** (co-mentor), I will be trained in tissue mechanics studying inflammatory softening of gingival tissues. This training will focus on: (1) mechanical characterization of healthy and diseased gingival tissues (human and mouse), (2) investigating ECM reorganization and collagen crosslinking in disease, and (3) applying advanced histological and staining methods to analyze ECM properties at the cellular level. Finally, I will supplement this training with formal coursework by enrolling in [MSE 5000: Experimental Methods in Materials Science](#) at Penn. This course will provide theoretical and practical knowledge of material characterization techniques. Together, these components will provide rigorous, multidisciplinary training in tissue mechanics and biomaterial characterization, establishing a strong foundation for my independent research career.

2. [Advanced training on ATAC-seq and bioinformatics at the center for single cell biology, Children's Hospital of Philadelphia \(CHOP\)](#) – To achieve specific aim 1 (K99 phase) I will leverage the state of art center for single cell biology at CHOP headed by **Prof. Kai Tan** ([Consultant Bioinformatician, Computational & Systems biologist](#))



Career Development Plan Timeline & Milestones

and his bioinformatics team to get trained in ATAC-seq and methylation sequencing data analysis (*ref. letter of support*). In parallel, I will be attending the single cell biology seminar series and will devote time in learning bioinformatics and data analysis using the R language. I will attend [BSTA 7870 Methods for Statistical Genetics and Genomics](#) in Complex Human Disease and [GCB 5770 Advanced Epigenetics Technology](#) at UPenn and learn the latest statistical methods for sequencing data analysis.

3. [Super resolution microscopy](#) – Under Specific Aim 1 (K99 phase), I will visualize the impact of ECM stiffness on global histone spatial (nanoscale) reorganization in gingival fibroblasts. This will be critical in understanding if stiff ECM influences the formation of nuclear memory in stromal cells. I will be trained in Stochastic Optical Reconstruction Microscopy (STORM) under the guidance of **Dr. Su Chin Heo** (*Consultant, ref. letter of support*), Perelman School of Medicine, UPenn. I will attend the [BE 5370 Biomedical Image Analysis](#) course to supplement my training in advanced quantitative image analysis methods and high-dimensional statistical analysis of images.

PROPOSED TRAINING PLAN (Professional Development) – Mentored Phase

1. [Teaching and Mentoring](#) – As part of my K99 plans, I will continue to seek mentoring and teaching assistance (TA) opportunities from **Dr. Kyle Vining**, who currently teaches in Materials Science ([MSE5180 Biological Material Sciences](#)). I will serve as a guest lecturer in his course to gain further teaching experience. Additionally, to enhance my writing and teaching, I will take short professional development programs at the Penn College of Liberal and Professional Studies- [PROW 3010 “Power of storytelling”](#) and [CLCH-3000 “Communicating Science”](#).

2. [Grantsmanship training](#)- Currently, my mentors provide opportunities to peer review their grants, offering a valuable learning experience. During my K99 phase, I will receive formal training through the Penn Institute for Translational Medicine and Therapeutics and Biomedical Postdoctoral Programs, including ‘[MTR 6060: Grantsmanship](#)’ and ‘[MTR 6230: Writing an NIH Grant \(R01\)](#)’ (Year1-K99). Institutional funding opportunities, such as the [CiPD IDEA Award](#), Seed Funds, the [Penn Health Tech Award](#), and internal pilot grants from Penn Dental and Penn Engineering, will further enhance my ability to write and submit innovative research proposals. Additionally, my mentoring committee has committed to critiquing my future grants and providing feedback, and structured CiPD peer-led sessions, functioning as mock study sections, will provide critical experience in reviewing and refining grant proposals.

3. [Extramural training courses](#)- I will take the “[Advanced course in Immunology-on demand](#)” from the American Association of Immunologists. I will attend two in-person [Cold Spring Harbor Laboratory courses – Chromatin, Epigenetics & Gene Expression](#) (includes hands-on training on ATAC seq)- Year 1; K99 and [Single Cell & Spatial Transcriptomic Analysis](#) (includes training on spatial biology of tissues using Visium HD)- Year 2; K99

4. [Leadership](#) – To hone my leadership skills, I will participate in the “[Leadership Essentials Program](#)” at the Perelman School of Medicine, UPenn, which is a didactic three-tier training program including topics such as “[Shaping a Motivational Workspace](#)”, “[Collaborations in Science](#)”, “[Think like a Leader](#)”, and “[Grant Management](#)”. Additionally, I will take certificate program on organizational dynamics and ethics in research at the Penn College of Liberal and Professional Studies.

5. [Scientific Presentations, Networking & Job Search](#) – I will present my work at UPenn internal symposiums, International and American Associations for Dental, Oral, and Craniofacial Research (IADR, AADOCR), Gordon Conferences in Bioengineering and Immunology. To further develop my skills as an educator, I am organizing a workshop on ‘[Mastering the NIH Specific Aims Page](#)’ at IADR 2026. Beyond presenting at conferences, I will actively seek faculty positions—allocating ~10% effort to networking, engaging with department chairs, and pursuing openings at conferences and through professional contacts. My mentoring committee will support this process by providing feedback on applications, presentations, and chalk talks, as well as facilitating introductions to search committee members at dental schools. In addition, CiPD will help connect me with its vast network of research and industry leaders across DOC and Engineering fields.

PROPOSED PLAN (Independent Phase) – I will transition to an independent research career to achieve my long-term career goals (*ref. Career Goals and Gantt chart*). I will maintain an active publication record (2 manuscripts in the first two years, followed by ~2-4 per year), apply for early investigator awards (NIDCR R03, NIGMS R35), followed by an R01 by the end of the R00 phase. I will continue professional growth through teaching, mentoring, grant writing workshops, symposium organization, and conference presentations, advancing mechanistic discovery in Periodontology, and training the next generation of dentists and scientists.

SPECIFIC AIMS

Periodontitis is a prevalent chronic inflammatory disease, with over one billion severe cases globally. In the U.S., it affects more than 40% of adults over 30, costing over \$40 billion. Periodontitis progressively damages the tissues supporting teeth, leading to tooth loss, and is associated with cardiovascular, metabolic, and autoimmune diseases. Despite advances in understanding its microbial and immunological etiopathogenesis, knowledge remains limited on the changing mechanobiology of the gingival extracellular matrix (ECM) in periodontitis. An unmet clinical need exists for improved outcomes and disease management. My long-term goal is to develop novel mechano-immune therapeutics for periodontitis, leveraging the interplay between tissue mechanics and immune modulation to enhance clinical outcomes.

Periodontal disease is linked to ECM disruption by host and microbial proteases, but the bio-mechanical changes in tissue resulting from this disruption have not been investigated. Healthy gingiva has a connective tissue predominantly composed of gingival fibroblasts (GFs) and a highly crosslinked ECM of collagen type-1 fibers that provide stiffness and non-compliance to stretching. However, a knowledge gap remains in our understanding of how GFs respond to these mechanical cues to support homeostasis and immune responses.

I propose to investigate the impact of the gingival tissue's ECM mechanical cues on the regulation of myeloid immune responses by gingival fibroblasts. I will use an ECM hydrogel model system that mimics the gingival ECM in healthy and diseased states. Interpenetrating networks of polysaccharide alginate and collagen type-1 provide tunable stiffness independent of ligand density, mesh size, and collagen fiber architecture. **My preliminary data** show physiologically relevant rheological properties of the ECM hydrogel system comparable to gingival tissue. GFs in stiffer hydrogels downregulate inflammatory cytokines and matrix metalloproteinases, with enrichment of matrix-related genes and genome/epigenome regulation pathways. Stiffness-dependent inflammatory responses of GFs are mediated through nuclear organization and DNA methylation. GFs in stiff matrices promote differentiation of myeloid progenitors into activated antigen-presenting cells in co-culture. Induced ECM crosslinking of human gingival explants enhanced their mechanical properties and reduced IL-6 secretion. Together, these data suggest that stiffer ECM promotes tissue homeostasis and fibroblast–myeloid cell crosstalk. **I hypothesize that reduced tissue stiffness in periodontal disease impairs fibroblast-mediated matrix and immune homeostasis via epigenetic regulation.** I will dissect this mechanism using *in vitro* and *in vivo* models to test the overall hypothesis that ECM stiffness maintains gingival immune homeostasis.

Aim 1 (K99) - Investigate how ECM stiffness regulates the epigenetic state of gingival fibroblasts. I hypothesize that ECM stiffness programs gingival fibroblasts to maintain immune homeostasis, whereas loss of stiffness disrupts chromatin organization and shifts them toward a pro-inflammatory state. Preliminary results show stiffness-mediated nuclear changes and epigenetic pathways, including chromatin remodeling and methylation, are significantly enriched in GFs cultured in stiff hydrogels. Using a tunable ECM hydrogel model, I will **(i)** determine how stiffness regulates fibroblast inflammatory responses, **(ii)** define stiffness-dependent chromatin and histone modifications, and **(iii)** test whether these changes are reversible when stiffness is restored. I will receive extensive training in tissue mechanics, ATAC sequencing, bioinformatics, and super-resolution microscopy to achieve this objective.

Aim 2 (K99 & R00) – Determine how ECM stiffness regulates stromal-myeloid crosstalk. I hypothesize that increased ECM stiffness in healthy gingiva epigenetically promotes GFs' positive regulation of myeloid cell fate compared to soft ECM in periodontitis. I will study stromal-myeloid crosstalk using a co-culture gingival ECM model, which will be exposed to pattern recognition receptor ligands. I will investigate the fibroblast-induced transcriptional regulation of monocyte differentiation and activation during the K99 phase. I will continue this work during R00 phase while also studying the role of tissue mechanics in health and periodontal disease by utilizing a mouse model of ligature-induced periodontitis, where ECM crosslinking can be modulated. I will receive training (K99) in mouse model of ligature-induced periodontitis to study tissue mechanics & spatial transcriptomics.

This application will provide key insights into the mechanobiological mechanisms underlying periodontal disease pathogenesis. This will inform the identification of mechano-immune targets and the development of novel therapeutics, with broad implications for oral/craniofacial chronic inflammatory diseases. I have assembled a multidisciplinary mentoring and advisory committee with complementary expertise in mechanobiology, immunology, microbiology and clinical periodontology, whose guidance will be key shaping my scientific development through training and supporting my transition to independence.

RESEARCH STRATEGY- Significance – Severe Periodontitis affects 19% of adults globally, causing tooth loss and increasing the risk of systemic diseases like heart disease, diabetes, respiratory infections, and adverse pregnancy outcomes^{20,21}. The World Health Organization has prioritized this condition due to its impact on health, quality of life, and healthcare costs, which exceed \$40 billion USD²¹. With rising risk factors such as smoking, poor diet, and aging populations, addressing periodontitis through preventive and integrated care is crucial.^{22,23} While the role of microbial dysbiosis and host innate immune responses is a well-recognized driver of periodontitis progression²⁴⁻²⁶, the concurrent inflammatory tissue weakening and altered mechanical properties of the gingiva secondary to microbial and host-induced matrix degradation, with its inflammatory and immunological impact, remains understudied. I propose that these mechanical changes create an altered extracellular microenvironment that disrupts immunological homeostasis, promoting a pro-inflammatory state that further increases susceptibility to microbial invasion²⁷⁻²⁹. Therefore, this application will investigate the mechanical regulation of inflammation in both periodontal health and disease.

Prior research rigor, Limitations & Proposed Research - Prior research has shown that accelerated extracellular matrix (ECM) breakdown in periodontitis arises from an imbalance between matrix metalloproteinases (MMPs) and their inhibitors (TIMPs)³⁰, driven by inflammatory cytokines in response to bacterial challenge^{28,31,32}. Excessive ECM degradation, particularly of collagen type I, compromises gingival tissue's integrity and contributes directly to disease progression, highlighting the central role of ECM remodeling in periodontitis³⁰. **However, these studies have primarily focused on biochemical and enzymatic pathways, with limited understanding of how the ECM's physical properties and cell-matrix interactions regulate inflammatory signaling within the gingival microenvironment. Critically, the mechanisms by which ECM structure and mechanics integrate with host immune responses remain poorly defined.**

The physiological stiffness of healthy gingival tissue plays a key role in shaping stromal cell behavior and innate immune responses, which are essential for maintaining tissue homeostasis^{30,31,33,34}. Despite the constant microbial load in the oral cavity, healthy gingiva maintains a state of immunological equilibrium³⁵⁻³⁷. In contrast, chronic periodontal disease is associated with collagenous ECM breakdown, which reduces tissue stiffness and disrupts homeostasis. Gingival fibroblasts (GFs), the primary cell type in gingival connective tissue, adjacent to the pocket epithelium, are central to both tissue homeostasis and disease pathogenesis^{29,38}. Beyond their role in ECM biosynthesis and remodeling, GFs act as sentinel cells in immune defense, largely through Toll-like receptor signaling^{29,39-43}. Under healthy conditions, GFs maintain tissue integrity by balancing TIMPs and MMPs^{44,45}, but during periodontitis, they can adopt pro-inflammatory phenotypes that exacerbate tissue damage³². Importantly, the function of GFs is closely influenced by interactions with the ECM. Mechanical cues from the ECM modulate GF activation and immune responses^{34,46}. **Based on previous evidence⁴⁷⁻⁵³ and my preliminary data¹, I propose that these mechanical cues are transmitted to the nucleus, altering the epigenome and genome, and regulating transcriptional programs that control immune responses.** Importantly, how ECM degradation during chronic inflammation impairs these nuclear and transcriptional mechanisms—and thereby disrupts GF regulation of immune cell activation and tissue homeostasis—necessitates mechanistic understanding. **Myeloid cells drive periodontal inflammation through cytokine- and MMP-mediated ECM degradation, antigen presentation, and sustained immune activation^{32,54-56}. Periodontitis further exhibits a bone marrow-derived myeloid bias, linking systemic and local inflammatory regulation⁵⁴. To address these gaps, I will investigate how gingival ECM stiffness modulates GF epigenetic states and their interactions with myeloid cells.** To gain mechanistic insight, I will employ a 3D gingival ECM-mimicking hydrogel system that enables precise control of mechanical cues in vitro, complemented by a mouse model of periodontitis to assess the in vivo relevance of ECM stiffness on inflammation. This integrated approach combines mechanistic precision with physiological relevance. Defining how ECM mechanics regulate GF behavior and immune modulation is critical, as the pathways driving GFs toward pro-inflammatory phenotypes remain poorly understood.

Innovation – 1) Mechanobiology of ECM in periodontal disease: The mechanical properties of gingival ECM and their role in disease are underexplored. This application uses a tunable 3D gingival ECM-mimicking hydrogel to isolate the effects of mechanical changes from inflammation, providing a novel platform to study tissue mechanics in periodontal pathogenesis. **2) Linking ECM mechanics to innate immune memory:** Using CD34⁺ hematopoietic stem-cell-derived myeloid precursors, which provides a more representative model of immune cell fates, this proposal investigates how ECM stiffness and composition direct immune cell differentiation and epigenetically imprinted inflammatory memory, a previously unrecognized driver of chronic inflammation. This will enable a deeper exploration of how the ECMs' mechanical properties shape immune

memory— where "innate immune memory" refers to the epigenetically imprinted and recallable memory of inflammation, and "trained innate immunity" describes the immune system's heightened response to future challenges. Integration of chromatin accessibility profiling, super-resolution imaging, and spatial biology enables a comprehensive mechanistic understanding. 3) **ECM based therapies:** Insights from these studies will guide the development of mechano-immune therapies representing an innovative mechanobiology-informed strategy to manage periodontal diseases.

APPROACH – Central Hypothesis – I hypothesize that the stiffness of the gingival ECM plays a critical role in maintaining immunological homeostasis, which is disrupted in periodontitis due to ECM degradation and the resulting changes in tissue mechanics. Further, restoring ECM crosslinking and the mechanical properties of the gingival connective tissue will restore immunological homeostasis.

OVERALL RATIONALE - I propose to investigate how localized changes in tissue mechanics, driven by ECM degradation, contribute to the epigenetic alterations observed in gingival fibroblasts (GFs) during periodontitis. GFs are the predominant cells of the connective tissue and are essential for maintaining tissue structure and homeostasis^{32,35}. In addition to ECM synthesis and remodeling, they interact with innate immune cells to modulate local responses via TLR2 signaling, whose broad recognition is particularly relevant in the polymicrobial environment of periodontitis^{26,29,39,40,43,57}. Previous studies on GFs in monolayer culture have highlighted their mechanosensitivity in ECM formation and immune regulation³³. However, the coordinated response of GFs to mechanical cues in a 3D tissue environment, particularly in interaction with neighboring cells to elicit a tissue-level response, has not been explored. While GF activation plays a role in resolving inflammation, its dysregulated activation leads to proinflammatory phenotypes and tissue destruction in periodontitis^{41,58}. The recognition that microbial and lifestyle factors alone cannot fully explain an individual's susceptibility to disease has led to a hypothesis of the role of epigenetic regulation⁴⁹. Studies have identified abnormal DNA methylation patterns in genes related to inflammation in gingival tissue from periodontitis patients^{53,59-61}. Additionally, the expression of enzymes that control histone acetylation, particularly histone deacetylases (HDACs), is disrupted in affected gingival tissue⁴⁸ and drives excessive production of inflammatory mediators and tissue-degrading enzymes. However, how these changes are influenced via changing ECM mechanics in disease has never been investigated. We hypothesize that ECM degradation-induced mechanical changes drive epigenetic reprogramming in GFs, which we will investigate under the following experimental plan.

SCIENTIFIC RIGOR

- 1. Reagent Authentication & Quality** – All compounds/reagents will be authenticated for identity and purity, based on certificates of analysis by manufacturer. Ultra-pure alginates and bovine telocollagen type I with endotoxin <0.1 EU/mL will be used. Donor HSPCs and GFs will be authenticated by flow cytometry/expansion assays, routinely tested for contamination, and maintained at <5 passages.
- 2. Statistical Analysis** – Conducted in GraphPad Prism. Significance set at $p < 0.05$ or FDR <0.05 based on normality/variance. Two-group comparisons: unpaired t-test or Mann–Whitney U; multiple groups: one-/two-way ANOVA or Kruskal–Wallis with post hoc tests. Each animal is treated as the unit of measurement.
- 3. Animal Work (Power & Sample Size Justification)** – Studies performed in a pathogen-free, AAALAC-accredited facility with IACUC approval. Experiments will be initiated on 12 weeks old mice with equal numbers of male and female mice; randomization applied. Power analysis ($\alpha=0.05$, $\beta=0.2$, effect size ~1.2) supports $n=10$ /group to detect significance at $p < 0.05$ ¹⁷⁻¹⁹. (for details, ref. document- Vertebrate Animals)
- 4. Experimental Techniques & Scientific Controls** – Live cells will be enriched using a dead cell removal kit for sequencing. Hydrogel experiments will include no-matrix controls (plate-adhered and suspension cultures). Enzyme-only controls (alginate lyase, collagenase IV) will be applied to suspension cells to isolate enzyme effects. Flow cytometry includes bead compensation, isotype, and fluorescence-minus-one (FMO) controls, which ensure accurate gating and reliable interpretation of the cell population. Appropriate negative/positive controls included in all assays (e.g., untreated vs treated hydrogels, vehicle controls for compounds).
- 5. Biological replicates and rigor**- All gingival fibroblast (GF) experiments will use 4 male and 4 female tissue donors (sex-balanced and age less than 35 years old), with donor age recorded; age-dependent trends will be noted for potential follow-up studies (total $n=8$). For CD34⁺ hematopoietic cells, sex and age cannot be strictly controlled, but three donors will be included to capture variability. Sample sizes (n) are based on preliminary data and will be increased as feasible to ensure statistical power and reproducibility.

EXPERIMENTAL DESIGN: SPECIFIC AIM1

Investigate how ECM stiffness regulates the epigenetic state of gingival fibroblasts (GFs). I hypothesize that the mechanical properties of stiff (healthy) gingival ECM support immunomodulatory functions of GFs through nuclear changes in chromatin structure via accessibility and methylation.

Rationale of Aim 1 & Rigor of Prior Research-

Past work has shown periodontal microbe-induced histone modification and DNA methylation variations in the gingiva via increased histone H3 acetylation and reduced DNA methyltransferase-1 (DNMT1) gene expression, hypomethylation of the MMP-2 promoter, indicating extracellular matrix degradation⁵⁰. Gingival biopsies from humans and animal models have also shown altered methylation patterns of TLR2 and TLR4 promoters in periodontitis⁶²⁻⁶⁴. Further, epigenetic patterns of key cytokines and chemokines⁴⁷ have also been studied in periodontitis, where decreased methylation was found in the promoter regions of CCL25, IL17, and IL6^{51,52}. Interestingly, some of these epigenetic changes were reversed after treatment and inflammation resolution⁵⁰.

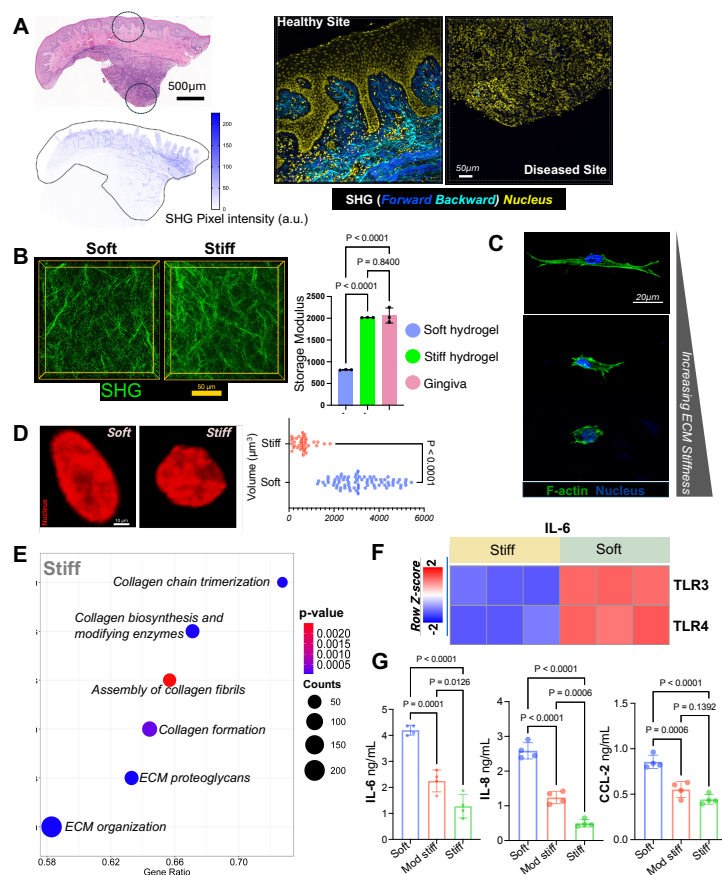
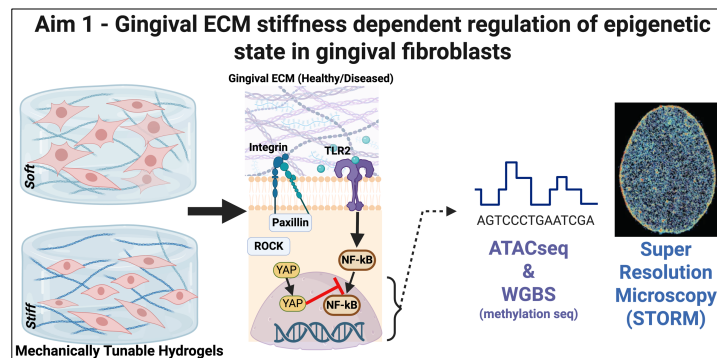


Fig.1. ECM changes in periodontal disease and influence of matrix stiffness on GFs morphology and inflammatory cytokine secretion

(A) Human gingival tissue section across different stages of periodontal disease. H&E staining (top) and pixel intensity of SHG signal (bottom). Exploded view of SHG imaging of healthy and diseased gingiva combined with nuclear staining reveals a progressive loss of collagen and an increase in immune cell infiltration from healthy to diseased sites.

(B) SHG images (z-projection) of soft and stiff hydrogels. Storage Modulus of Gingival ECM hydrogels and healthy human Gingival tissue.

(C) Cell morphology of GFs in soft and stiff ECM hydrogels. **(D)** High magnification micrograph of GF's nucleus and volume in soft and stiff hydrogels. **(E)** Pathway analysis of RNA-seq data from GFs cultured in stiff hydrogels. **(F)** IL-6 secretion (z-scored) by TLR3,4 activated GFs in soft and stiff hydrogels (Z-scored, n=3, p<0.01). **(G)** IL-6, IL-8, and CCL-2 secreted by TLR2 activated GFs cultured in soft, moderately stiff (Mod Stiff) and stiff ECM hydrogels.



Notably, GFs isolated from periodontitis patients exhibit sustained inflammation and reactive oxygen species production, distinguishing them from GFs from healthy counterparts^{41,58,65}. Although innate immune responses in GFs are well-studied, the mechanisms underlying their persistent inflammatory state in periodontitis remain unclear. While literature suggests site-specific epigenetic reprogramming that controls innate immunity and inflammation^{48-50,61}, the influence of extracellular matrix stiffness and matrix mechanics on epigenetic changes in periodontitis has not been investigated previously. I propose that periodontitis causes changes in tissue structure and mechanics secondary to ECM degradation. These changes can be seen in connective tissue closer to the disease front, creating small areas with altered mechanical properties that influence epigenetic modifications in GFs. Importantly, how these mechanical changes affect the genomic and epigenomic landscape of GFs in the context of inflammation is poorly understood. I hypothesize that a reduction in the mechanical properties of the gingival connective tissue ECM drives spatial reorganization of chromatin in the nucleus of GFs, thereby altering accessibility and transcriptional activity and modulating their response to TLR-mediated inflammatory signaling.

Preliminary Data for Aim 1- To strengthen my overall hypothesis, I first studied the impact of periodontal disease on ECM degradation. I used bio-archived tissue sections of healthy and diseased human gingival tissues and employed second harmonic generation (SHG) imaging of collagen. I observed extensive collagen degradation in areas with high inflammatory cell infiltrates within the gingival connective tissue (**Fig. 1A**) compared to adjacent unaffected sites¹. To mimic the stiff (healthy)

and soft (diseased) ECM microenvironments of gingival connective tissue^{46,66-68}, I used tunable viscoelastic hydrogels composed of interpenetrating networks (IPN) of alginate and collagen type I fibers. Bulk stiffness, quantified by oscillatory shear rheology as the storage modulus (G'), was controlled by the extent of ionic crosslinking of alginate. A higher cooperative binding of divalent cations (Ca^{2+}) produced a higher G' ^{66,68,69}, while viscoelasticity was defined by the ratio $\tan(\delta) = \text{loss modulus } (G'')/G'$. After casting, gels were equilibrated in buffer to remove unbound calcium, leaving minimal free ions. This system allows the independent examination of stiffness (G') and viscoelasticity ($\tan \delta$) on human cells, as shown previously in studies on wound healing, mesenchymal biology, and fibrosis^{70,71}. To assess physiological relevance, I compared the rheological properties of healthy donor gingival tissues with gingival ECM hydrogels, which showed a similar range of G' (**Fig. 1B**), indicating comparable mechanical properties between the engineered model and human tissue¹. For in-vitro studies, gingival fibroblasts (GFs) were isolated from excised healthy human gingival sulcular tissue (IRB-approved protocol #844933), characterized for mesenchymal markers (CD90, CD105, CD73)¹, and encapsulated in ECM hydrogels of varying stiffness. In soft hydrogels, 3D-cultured GFs exhibited active spreading and probing protrusions, typical of migratory behavior in compliant environments¹. In contrast, in stiff hydrogels, their compact morphology reflected adaptation to mechanically stable, load-bearing conditions requiring strong cell–matrix anchorage and internal tension (**Fig. 1C**). Nuclear imaging revealed stiffness-dependent reductions in nuclear volume and sphericity¹ (**Fig. 1D**), suggesting a potential role for actomyosin-mediated cytoskeletal tension in nuclear deformation⁷². GFs in stiff hydrogels upregulated a broad cluster of genes reflective of a strong, homeostatic, and matrix-maintaining phenotype typical of healthy gingival connective tissue (**Fig. 1E**). We mimicked microbial challenge by treating hydrogels with TLR2 (Pam3CSK4), TLR3 (poly(I:C) HMW), and TLR4 (ultra-pure LPS) agonists. GFs cultured in soft hydrogels exhibited significantly higher secretion of IL-6, IL-8, and CCL-2 (**Fig. 1F,G**), suggesting that stiffer matrices (reminiscent of the healthy state) suppress pro-inflammatory cytokine and chemokine secretion¹. No changes were observed after TLR9 stimulation. Given the polymicrobial etiology of periodontitis and the broad recognition profile of TLR2, we focused subsequent studies on TLR2 agonists²⁹. Bulk RNA-seq data showed that ECM markers (Col4a1, Col4a2, Col5a3) and TGF- β 2/ β 3 (Tgfb2, Tgfb3) were upregulated in GFs cultured in stiff matrices, whereas Mmp3, Mmp4, Il11, Ccl5, and Cxcl6 were enriched in soft matrices, and Timp3 was increased in stiff gels¹ (**Fig. 2A**). GO term analysis revealed significant upregulation of epigenetic regulation in GFs cultured in stiff hydrogels (**Fig. 2B**). Consistently, fibroblasts in stiff hydrogels showed higher DNMT1 expression and reduced cell spreading, while DNMT1 inhibition (1 μM) enhanced spreading and increased TLR-mediated IL-6 secretion¹ (**Fig. 2C**). Together, these preliminary findings support a mechano-regulatory role of ECM stiffness on gingival fibroblast phenotype, where stiff ECM promotes a homeostatic and matrix-maintaining state, and ECM softening during disease drives pro-inflammatory and degradative responses. We will study this under the following experimental plan.

Aim 1 Experiment 1 – The impact of ECM stiffness and actomyosin contractility on non-canonical inflammatory signaling in gingival fibroblasts (GFs) - We will assess how ECM stiffness and actomyosin contractility influence non-canonical inflammatory signaling in gingival fibroblasts (GFs). GFs will be encapsulated in stiff (2000 Pa) and soft (800 Pa) IPN hydrogels and cultured for 5 days ($n=8$). Immediately after equilibration (Day 0), cells will be treated with ROCK inhibitor (10 μM Y-27632) or myosin II inhibitor (10 μM blebbistatin)^{66,67}, with media and inhibitor replenished at 48 h. During the final 24 h (Day 4–5), hydrogels will be co-incubated with TLR2 agonist Pam3CSK4 (1 $\mu\text{g}/\text{mL}$); untreated hydrogels will serve as controls. We hypothesize that higher ECM stiffness promotes ROCK and

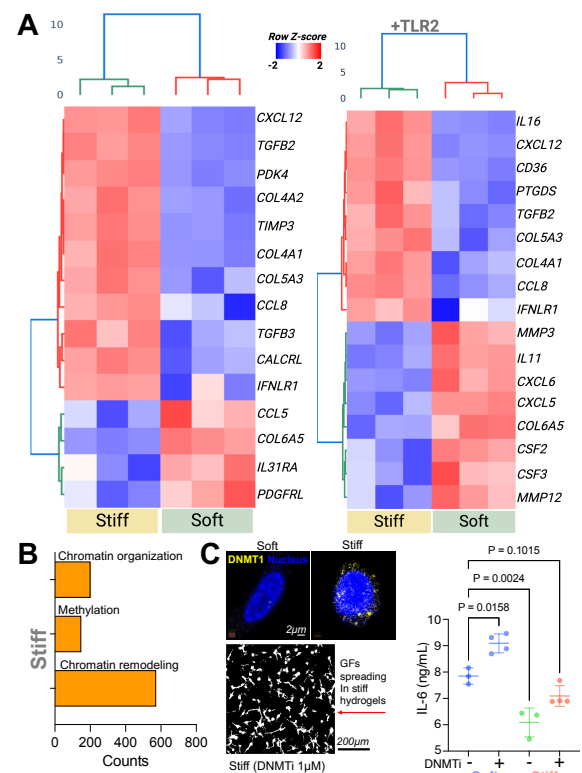


Fig.2. ECM stiffness-mediated regulation of matrisome, inflammation, and epigenome in GFs. (A) Heatmap of RNA-Seq z-scores for genes differentially expressed ($\text{padj} < 0.05$) in GFs cultured in stiff vs. soft hydrogels without (left) or with (right) TLR2 activation. (B) Top enriched GO terms associated with genome and epigenome regulation in GFs in stiff hydrogels. (C) DNMT1 expression (yellow), enhanced spreading, and elevated IL-6 following DNMT inhibition (DNMTi).

Myosin II-dependent YAP nuclear translocation, inhibiting TLR2-mediated NF-kappa-B (NFkB) phosphorylation. Hydrogels will be fixed, permeabilized, blocked, and subjected to whole-mount immunostaining for YAP1, NFkBp65, and paxillin. F-actin and DAPI staining will be included for cytoskeletal and nuclear visualization. Paxillin per cell and cell morphology will be quantified using ImageJ. Cytokine and chemokine secretion (IL-6, IL-8, CCL-2, CCL-5, IL-1 β , IL-17, TNF- α) will be measured by ELISA. All outcomes will be compared pairwise across groups using ANOVA with post-hoc multiple comparisons to directly assess the effects of ECM stiffness, actomyosin inhibition, and TLR2 activation.

Aim 1 Experiment 2 – Role of matrix stiffness in chromatin accessibility and mechanical memory in gingival fibroblasts (GFs). We hypothesize that GFs cultured in stiff ECM exhibit reduced global chromatin accessibility. We will test this by examining chromatin accessibility and mRNA transcription in GFs cultured in stiff (2000Pa) and soft (800Pa) hydrogels which models healthy and diseased ECM respectively. This will allow us to identify regions responsive to ECM mechanics and study the development of stiffness mediated nuclear memory.

Experiment 2.1 – To study genome-wide chromatin accessibility, GFs will be cultured in stiff and soft hydrogels for 5 days, with or without TLR2 activation during the final 24 h (n=8). Hydrogels will be digested, live cells collected, and nuclei prepared for ATAC-sequencing. ATAC-seq will profile genome-wide chromatin accessibility, identifying active regulatory elements and providing insights into gene regulation and chromatin dynamics. Libraries will be sequenced on a NovaSeq 6000 (50 bp paired-end reads). Sequencing reads will be processed using the ENCODE ATAC-seq pipeline: reads will be aligned, duplicates and mitochondrial reads removed, and a normalized count matrix generated. Motif analysis will rank regulatory elements by adjusted P value. Chromatin accessibility will be compared across stiffness (stiff vs. soft) and TLR2 activation using two-way ANOVA followed by Tukey's post-hoc tests. *I will be trained by Dr. Kai Tan on bioinformatics analysis and interpretation of integrated multi-omics high-dimensional data (Bioinformatician, Consultant Computational Biologist, Director – Single cell biology core, CHOP- ref. support letter).*

Experiment 2.2- Induction of mechanical and inflammatory memory in GFs. To model the transition from healthy to diseased ECM, GFs cultured in stiff hydrogels (healthy state) for 5 days will be retrieved and re-encapsulated in soft hydrogels (diseased state) for an additional 5 days, with or without TLR2 activation during the final 24 h (n=8). GFs maintained in stiff hydrogels for 10 days will serve as controls. Genome-wide chromatin accessibility will be assessed to identify stiffness- and TLR2-dependent changes. Supernatants will be collected, and multiplex ELISA will quantify proinflammatory cytokines and chemokines (IL-6, IL-8, CCL-2, CCL-5, IL-1 β , IL-17, TNF- α) to evaluate memory effects on inflammatory responses. Chromatin accessibility, cytokine secretion will be compared across re-encapsulated and control groups, using two-way ANOVA with post-hoc tests.

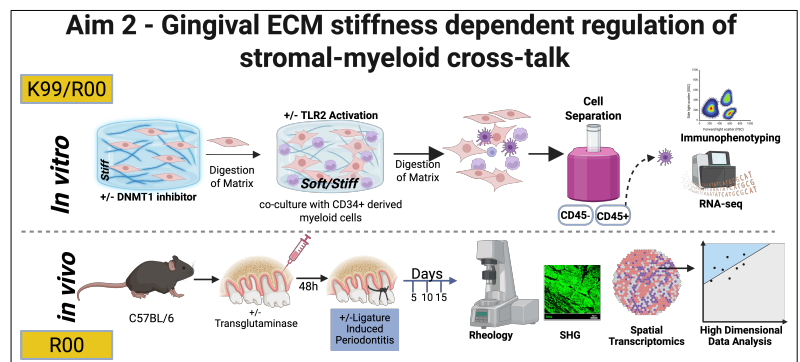
Aim 1 Experiment 3 – Whole-genome methylation and chromatin reorganization in GFs encapsulated in stiff and soft ECM hydrogels. We hypothesize that ECM degradation in periodontitis induces persistent epigenomic reorganization, which may underlie prolonged disease features until homeostasis is restored. To test this, GFs will be encapsulated in stiff (2000 Pa) and soft (800 Pa) IPN hydrogels and cultured for 5 days (n=8). Immediately after equilibration (Day 0), cells will be treated with ROCK inhibitor (10 μ M Y-27632) or myosin II inhibitor (10 μ M blebbistatin), with media and inhibitor replenished at 48 h. During the final 24 h (Day 4–5), hydrogels will be co-incubated with TLR2 agonist Pam3CSK4 (1 μ g/mL). Untreated stiff and soft hydrogels will serve as controls. After treatment, hydrogels will be digested for genomic DNA isolation and whole-genome bisulfite sequencing (Novaseq X Plus, 20x coverage) to assess DNA methylation. For imaging, hydrogels will be fixed, flash-frozen, sectioned, and immunostained for H3K4me3 (active pro-inflammatory gene promoters) and H3K27me3 (associated with transcriptional repression). STORM imaging, in collaboration with the **Heo lab** (ref. letter of support), will visualize chromatin organization⁷³. Image analysis will use custom MATLAB code applying Voronoi tessellation, plotting nuclear Voronoi densities to distinguish euchromatin from heterochromatin⁷³. Data from this experiment will be integrated with Experiments 1 and 2. DNA methylation, chromatin organization, and related imaging metrics will be compared across stiffness (stiff vs. soft), \pm ROCK/myosin II inhibition, and \pm TLR2 stimulation using two-way ANOVA with post-hoc tests. Relationships among ECM stiffness, TLR2 stimulation, non-canonical inflammation, chromatin accessibility, and organization will be assessed using multiple regression and principal component analysis.

Anticipated results, pitfalls, and alternatives - We expect soft viscoelastic hydrogels to suppress mechanotransduction-related transcription factors and enhance pro-inflammatory signaling, while stiff hydrogels will promote ROCK/Myosin II-dependent YAP nuclear translocation, reduced non-canonical NF- κ B activation, chromatin compaction, and increased DNA methylation. Potential pitfalls include low cell yield or sequencing

quality in ATAC-seq or methylation studies. These will be mitigated by optimizing hydrogel digestion, confirming nuclei integrity, and assessing library quality using ENCODE metrics (e.g., FRiP >0.3, bisulfite conversion >99%). If data quality is suboptimal, replicates will be pooled. Bioinformatic analyses will be supported by [Dr. Kai Tan's group](#) to ensure robust alignment, normalization, and integration. GF heterogeneity will be monitored by flow cytometry. If inhibitor effects are weak, concentration or exposure duration will be optimized. Although TLR2 signaling is central in GFs^{29,39,42,43}, alternative stimuli (heat-killed *F. nucleatum*, *P. gingivalis*, or LPS) will be tested to ensure reproducibility⁷. Future work will employ full-thickness 3D organotypic cultures integrated with periodontal biofilms on hydrogels to better model epithelial–stromal and host–microbiome interactions under altered ECM stiffness.

SPECIFIC AIM 2- Determine how ECM stiffness regulates Gingival fibroblast-myeloid crosstalk. I hypothesize that ECM stiffness plays a critical role in how GFs regulate monocyte fate in periodontal health and disease. I will study this in vitro using the mechanically tunable gingival ECM hydrogel system and in vivo using the mouse model of ligature-induced periodontitis (mLIP).

Rationale of Aim 2 and rigor of prior research - Despite continuous exposure to antigens, severe inflammatory responses in the gingiva are relatively rare⁵⁶. This is attributed to the tolerogenic properties of the oral mucosa, which are critical for maintaining immunological homeostasis^{35,55,56}. Gingival fibroblasts (GFs) influence the host's immune responses not only by triggering the release of inflammatory mediators in response to pathogens but also through direct interactions with immune cells or by secreting factors that modulate immune cell activity^{29,38}. Periodontitis has been shown to induce adaptations in hematopoietic stem cells, resulting in a long-term myeloid bias^{31,54}. GFs are site-specific and maintain positional identity, whereas myeloid cells from the bone marrow are constantly replenished in the gingival connective tissue. [Monocytes, being in a naïve state in the bone marrow environment, experience mechanically different extracellular environments as they migrate to the gingival extracellular matrix \(ECM\), coming in close contact with the GFs¹⁴. This plasticity requires mechanosensing and the gingival tissue's ECM architecture, mechanical properties, and fibroblasts themselves can influence their differentiation and activation.](#) Prior work shows GFs can both activate and suppress myeloid cells via IL-6 or TNF modulation^{74,75}, but how ECM mechanics affect this crosstalk remains unknown. Further, monocyte-derived dendritic cells (DCs), which are predominantly involved in activating and polarizing naïve T cells, play a crucial role in initiating immunity and tolerance by differentially regulating the expression of TLRs, co-inhibitory molecules, and co-stimulatory molecules^{55,76-78}. Although GFs' interaction with infiltrating myeloid cells in both healthy and periodontitis-affected gingival tissues highlights their significance in the regulation of immunological homeostasis, much less is known about the impact of ECM mechanics on this crosstalk. [Changing tissue mechanics secondary to ECM degradation in periodontitis can influence GF-myeloid cross-talk, which necessitates a mechanistic understanding. Further, I propose that ECM softening in periodontitis due to its degradation negatively impacts the GF-monocyte crosstalk, which disrupts the tissue's immunological homeostasis.](#) I will study this 1) by co-culturing GFs and Monocytes in mechanically tunable gingival ECM hydrogels (K99 and R00 phase) and 2) in vivo using a mouse ligature-induced periodontitis model (R00 phase), where ECM is perturbed with an ECM crosslinking agent -Transglutaminase (Tg). TG's crosslinking ability provides matrix stability, strength, and is naturally implicated in wound healing⁷⁹⁻⁸². [I will train in mucosal immunology and the ligature-induced periodontitis \(LIP\) model¹⁶⁻¹⁸ with Dr. George Hajishengallis and receive guidance from Dr. Michael Abt during the K99 phase. The routinely used LIP model in Dr. Hajishengallis's lab ensures reproducibility, and experiments will be divided between the K99 and R00 phases.](#)



Further, monocyte-derived dendritic cells (DCs), which are predominantly involved in activating and polarizing naïve T cells, play a crucial role in initiating immunity and tolerance by differentially regulating the expression of TLRs, co-inhibitory molecules, and co-stimulatory molecules^{55,76-78}. Although GFs' interaction with infiltrating myeloid cells in both healthy and periodontitis-affected gingival tissues highlights their significance in the regulation of immunological homeostasis, much less is known about the impact of ECM mechanics on this crosstalk. [Changing tissue mechanics secondary to ECM degradation in periodontitis can influence GF-myeloid cross-talk, which necessitates a mechanistic understanding. Further, I propose that ECM softening in periodontitis due to its degradation negatively impacts the GF-monocyte crosstalk, which disrupts the tissue's immunological homeostasis.](#) I will study this 1) by co-culturing GFs and Monocytes in mechanically tunable gingival ECM hydrogels (K99 and R00 phase) and 2) in vivo using a mouse ligature-induced periodontitis model (R00 phase), where ECM is perturbed with an ECM crosslinking agent -Transglutaminase (Tg). TG's crosslinking ability provides matrix stability, strength, and is naturally implicated in wound healing⁷⁹⁻⁸². [I will train in mucosal immunology and the ligature-induced periodontitis \(LIP\) model¹⁶⁻¹⁸ with Dr. George Hajishengallis and receive guidance from Dr. Michael Abt during the K99 phase. The routinely used LIP model in Dr. Hajishengallis's lab ensures reproducibility, and experiments will be divided between the K99 and R00 phases.](#)

Preliminary Data for Aim 2 - I generated myeloid cells from donor G-CSF mobilized CD34+ hematopoietic stem and progenitor cells (HSPCs- Fred Hutch Cancer Center) by culturing them for 7 days in IMDM supplemented with 50 ng/mL GM-CSF and 50 ng/mL FLT3L. Myeloid cell differentiation was assessed using flow cytometry, with both unstained and isotype controls included. CD34+ HSPCs differentiated monocytes were co-encapsulated with gingival fibroblasts (1:1 ratio) in hydrogels with shear moduli (G') of 2000 Pa (stiff; healthy state) and 800 Pa (soft; diseased state). On day 5, cultures were exposed to a TLR2 agonist for 24 hours, while

unexposed cultures served as controls. Cells were retrieved from the hydrogels for flow cytometry, allowing us to assess the impact of matrix stiffness and TLR2 activation on GF-monocyte crosstalk. Stiff matrix and GFs promoted dendritic cell differentiation of monocytes and enhanced the expression of MHC class II (HLA-DR), which was downregulated in softer hydrogels¹ (**Fig. 3A**). Notably, TLR2 activation in stiffer GF–monocyte co-cultures further increased expression of the co-inhibitory molecule PD-L1 (**Fig. 3B**). These phenotypic changes were also coupled to enhanced immunological function of phagocytosis as measured by the internalization of pH-sensitive fluorescent *E. coli* particles¹ (**Fig. 3C**). Notably, the same hydrogel system has been used to model the bone marrow microenvironment, where it provided mechanistic insights into how ECM stiffening alters myeloid cell fate in myeloproliferative neoplasms⁶⁷. In parallel, I worked towards developing a human relevant ex vivo gingival explant model which was perturbed with transglutaminase (10U/mL) to enhance ECM protein crosslinking (**Fig.4A**). My preliminary data shows that enhancing ECM crosslinking further enhances the mechanical properties of the explants and rescued the impact of TLR2 mediated IL6 secretion¹ (**Fig.4B**). Overall, these preliminary results suggest that GFs in stiffer ECM promotes a more favorable environment for immune cell activation, antigen presentation and immunomodulation. [I will probe stromal-myeloid crosstalk under the following experimental plan. All experiments described below will include no-matrix controls \(plate-adhered and suspension cultures\) and enzyme-only controls.](#)

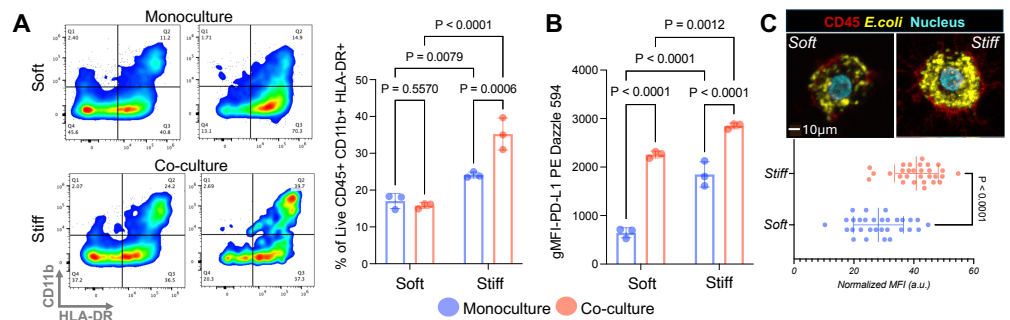


Fig.3. Gingival Fibroblast-Monocyte cross-talk. Flow cytometry of monocultures (monocyte only) and co-cultures (monocytes and GFs) in soft and stiff hydrogels showing expression of (A) HLA-DR CD11b (B) PD-L1 in monocytes. (C) phagocytosis by differentiated dendritic cells in soft and stiff hydrogels via uptake of labeled *E. coli* (yellow)

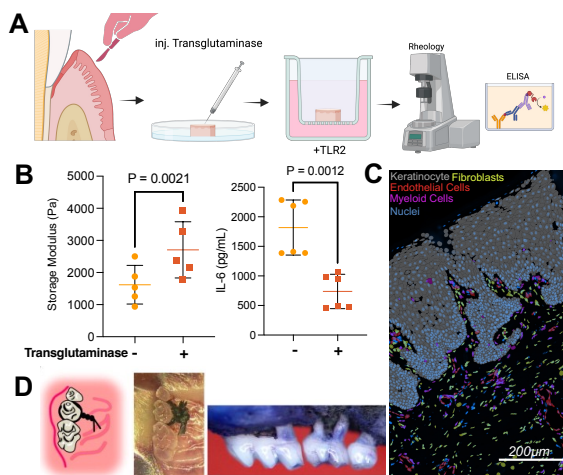


Fig.4. Gingival tissue stiffening and inflammation (A) Schematic showing perturbation of human gingival explants by transglutaminase (Tg). (B) Graphs showing storage modulus and IL6 secretion by TLR2 - activated human gingival explants treated with and without Transglutaminase. (C) Spatial transcriptomic map of healthy human gingiva using 10x Genomics Xenium, showing annotated cells (D) Murine Ligature - induced periodontitis model – adapted from ¹⁶

Aim 2 Experiment 1 (K99) – Determine how ECM stiffness and fibroblast mechanical memory regulate GF-monocyte crosstalk - I will use the gingival ECM hydrogel system to dissect how matrix mechanics and fibroblast mechanical memory shape monocyte fate and inflammation. Our preliminary data show that stiff ECM and GF co-culture enhance dendritic cell (DC) differentiation and phagocytic function, suggesting that fibroblast mechanosensing regulates immune activation. To probe mechanical memory, GFs will be cultured in stiff hydrogels (2000 Pa) with or without a DNMT1 inhibitor (1 μ M) for 5 days, followed by matrix digestion and cell retrieval. Consistent with preliminary data, stiff ECM increases DNMT1 expression and reduces fibroblast spreading, whereas DNMT1 inhibition enhances matrix spreading and TLR-mediated IL-6 secretion, mimicking a disease-like state. I hypothesize that DNMT1 inhibition reprograms fibroblasts toward a “soft-matrix” phenotype that alters monocyte differentiation and immune responses. Retrieved fibroblasts (\pm DNMT1 inhibition) will be co-cultured with HSC-derived monocytes in soft (800 Pa) or stiff (2000 Pa) hydrogels for 5 days, followed by 24 h TLR2 activation. After digestion, CD45⁺ (immune) and CD45⁻ (fibroblast) fractions will be isolated via immunomagnetic separation. RNA (RIN > 8) will be extracted, rRNA-depleted, and sequenced (Illumina NextSeq 500). Differentially expressed genes will undergo pathway enrichment, GO, and transcription factor motif analyses to define ECM-dependent GF–myeloid signaling. Key genes will be validated by qPCR. In parallel, flow cytometry (CD11b, CD11c, CD1c, HLA-DR, PD-L1, CD163, CD206, CD80, CD68) will assess myeloid phenotypes^{66,67,83}. Comparisons across conditions (stiff vs. soft ECM, \pm DNMT1 inhibition, \pm TLR2 activation; n = 8) will be analyzed using two-way ANOVA with post-hoc tests.

Aim 2 Experiment 2 (K99 & R00) – Investigate the impact of tissue mechanics on host innate immune responses using a mouse model of ligature-induced periodontitis (mLIP). This will be studied under the following experimental subsections.

Experiment 2.1 (K99 & R00) - Study the role of periodontitis-induced ECM degradation on tissue mechanics *in vivo*. First, I will study ligature time-dependent tissue softening in mice. Periodontitis will be induced in a C57Bl/6J (2-3 months of age)¹⁶. Briefly, a 5-0 silk ligature will be tied around the left maxillary second molar tooth for 5, 10, 15 days while keeping the contralateral tooth (right maxillary second molar) un-ligated to serve as a baseline control^{16,19}. Diseased gingival tissue will be retrieved using a 1mm punch biopsy and subjected to rheology and SHG imaging. For rheology, tissue samples will be tested at 37°C in HBSS/HEPES under 0.01N preload, measuring storage modulus and $\tan(\delta)$ via frequency sweep (0.01–25 Hz, 0.5% strain), followed by stress relaxation at 10% strain. Further, axial compression test until 80% strain will be performed with a strain rate of 1%/s to measure stiffness in the axial direction^{69,84}. Next, I will perturb the ECM via gingival transglutaminase injection (10-25U/mL), which would aid in the formation of covalent ϵ -(γ -glutamyl)lysine bonds between glutamine and lysine residues on ECM proteins⁷⁹⁻⁸². LIP will be induced after 48 hours. Micro-CT scans¹⁹ will be performed to assess bone loss, and the mouse will be sacrificed at the aforementioned time points. The gingival tissue (healthy and diseased sites) will be harvested aseptically and subjected to rheology to obtain complex shear modulus, storage G' , and loss G'' moduli. Additionally, SHG of collagen signal^{85,86} will be acquired from these tissues on a Leica SP8 confocal with multi-photon laser excitation (910nm). Collagen fiber number, alignment, width, and length will be quantified with CT-FIRE software⁸⁷. Part of the tissue will be fixed, processed, sectioned, and stained for Sirius red staining (stains collagen type 1 and type 3), Alcian blue (stains glycosaminoglycans). *I will be getting trained in the mLIP model from Dr. Hajishengallis (Advisor) and techniques to study tissue mechanics^{84,88,89} from Dr. Wells (co-mentor).*

Experiment 2.2 (R00)- Investigate matrix stiffness-dependent stromal-immune crosstalk *in vivo*. This experiment will determine how gingival tissue mechanics shape immune landscape remodeling during periodontitis. Wild-type C57BL/6 mice will receive gingival injections of transglutaminase (10–25 U/mL) to enhance ECM crosslinking, followed by ligature-induced periodontitis (LIP) after 48 h. Mice will be sacrificed on days 5, 10, and 15. Paraffin-embedded sections will undergo 10x Xenium spatial transcriptomics using the mouse pan-tissue and pathway panels. Data will be processed with *Seurat* for spatial co-occurrence and neighborhood analysis and integrated with regression models to correlate tissue stiffness with immune–stromal architecture. Fibroblasts (*Col1a1*, *Dcn*, *Fap*, *Pdgfra*), monocytes/macrophages (*Cd68*, *Cd14*, *Itgam*, *Fcgr1*), dendritic cells (*Itgax*, *Cd209a*), and keratinocytes (*Krt14*, *Krt5*, *Loricrin*) will be identified and quantified across regions. Differential gene expression and pathway enrichment will compare ECM-stabilized (transglutaminase + LIP) versus control (LIP) tissues. Cytokine and signaling transcripts (*Tnf*, *Il1b*, *Il6*, *Il10*, *Rankl/Rank*, *Il17a*) and epithelial remodeling markers (*Krt6*, *Cxcl1*, *Ccl20*) will be assessed to define mechanical regulation of inflammation and barrier integrity. Spatial data will be integrated with histologic and mechanical measurements to construct a stiffness–immune–epithelial network model. **Dr. Hajishengallis** and **Dr. Kang Ko** (Advisors) will guide the *in vivo* workflow and spatial data interpretation (Fig. 4C,D).

Anticipated results, pitfalls, and alternatives – I propose that ECM mechanics modulate fibroblast behavior by altering epigenetic memory into an immunoregulatory phenotype that promotes monocyte differentiation into tolerogenic myeloid subsets. *In vivo*, gingival tissues are predicted to progressively soften during ligature-induced periodontitis, reflected by reduced storage modulus (G') and diminished SHG signal. The optimal time window for matrix softening will be established experimentally; if tissue remodeling is insufficient, collagenase I injection will serve as an alternative to recapitulate the diseased phenotype. Potential challenges, including variability in ECM remodeling, incomplete transglutaminase crosslinking, or spatial-omics data complexity, will be mitigated through optimized ligature duration, pilot validation of workflows, and bioinformatic support from Dr. Kai Tan's team. If transglutaminase doesn't effectively restore ECM homeostasis, lysyl oxidase treatment will be tested as an alternative crosslinking strategy. This spatial-omics approach will also profile epithelial and other stromal cell changes, which can be explored as secondary outcomes in future mucosal organotypic model studies.

Future Directions – Future studies, supported by R03 (NIDCR), R35 (NIGMS), and R21/R01 mechanisms, will validate gingival ECM stiffness–driven epigenetic mechanisms in periodontal inflammation using immune competent organ-on-chip models, complementing animal models and biopsies of human gingival tissues to link ECM stiffness with clinical outcomes. I will expand the scope of ECM mechanics on the keratinocyte epigenome and function. Addressing a critical unmet need, I aim to develop ECM instructive biomaterials that complement mechanical debridement, restore tissue homeostasis, and improve periodontitis treatment and quality of life.

TRAINING IN RESPONSIBLE CONDUCT OF RESEARCH (RCR)

Training in responsible conduct of research will be increasingly important as I progress in my career development. Throughout my dental and research career I received extensive training in Responsible Conduct of Research (RCR) including face-to-face trainings with faculty in a discussion format and additional online instruction. As a dental student and Endodontics Resident at JSS University and KIIT University, India, respectively, my curriculum included studying ethics in clinical dental research, data privacy, confidentiality, and research integrity

During my doctoral research at the National University of Singapore, I completed a comprehensive online course and received Certificate on RCR developed by Collaborative Institutional Training Initiative (CITI). These online sessions covered topics including *Introduction to RCR, Authorship, Collaborative Research, Conflicts of Interest, Data Management, Mentoring, Peer Review, Plagiarism, Reproducibility of Research Results, Research Involving Human/Animal Subjects*, and *Research Misconduct*. I was also required to complete yearly online refresher for the RCR training.

Since I have joined as a postdoctoral researcher, I have attended the “Responsible Conduct of Research Symposium” organized by the Biomedical Postdoctoral Programs (BPP) at the University of Pennsylvania. This was special in person event focusing on the recently updated NIH policy and guidelines for training in Responsible Conduct of Research, providing in-depth lecture with discussions led by faculty mentors on a variety of topics including *Scientific Rigor and Reproducibility, Research misconduct and policies for handling it, Responsible authorship and publication, Safe research environment: mitigating biases and Microaggression*, and *Mentor/Mentee Responsibilities and Relationships*.

As a current NIDCR R90 Postdoctoral Fellow at CiPD, in addition to the RCR modules by the CITI, I am required to attend at least six in-person RCR workshops (1-1.5 hours each) per year organized by the Penn BPP office. Each of these workshops is led by faculty mentors and consists of lectures, discussion, and case studies. The course is designed to meet the new NIH requirements.

During the K99 award period, I will continue to attend these trainings during my mentored and independent phases of K99 through the office of the Vice provost for research and the Biomedical Postdoctoral programs at the University of Pennsylvania.

Topics of workshops I will attend include

- Conflict of interest – personal, professional, and financial
- Mentor/mentee responsibilities and relationships
- Collaborative research including collaborations with industry
- Peer review
- Data acquisition and laboratory tools; management, sharing and ownership
- Research misconduct and policies for handling misconduct
- Responsible authorship and publication
- The scientist as a responsible member of society, contemporary ethical issues in biomedical research, and the environmental and societal impacts of scientific research

My mentors also have significant training and experience in the responsible conduct of research. Dr. Vining’s and Dr. Well’s lab have at least one meeting per year (1-2 hours) dedicated to research rigor and RCR. I will lead these RCR sessions/group discussion and will discuss critical evaluation of research, such as rigorous experimental design, consideration of sex and other relevant biological variables, authentication of key biological and/or chemical resources, and data transparency. These sessions will also focus on other relevant topics such as data management, case studies on ethical issues in publication, truthfulness in reporting results, and financial conflicts of interest. In addition to the formal RCR training, I will look forward to opportunities to provide guidance on RCR for junior members (both dental students, undergraduates) and junior postdocs), which aligns with my career path to become a faculty member.

Center for Innovation & Precision Dentistry



Kyle Vining, DDS, PhD

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Dear Committee Members,

October 14, 2025

I am writing to enthusiastically support the application of Dr. Hardik Makkar for the Pathway to Independence K99/R00 Award. **Dr. Makkar is exceptionally well qualified for this award**, with advanced training in Bioengineering (PhD), dental surgery (DDS), and a demonstrated record of research excellence and drive to become a leading dentist-scientist positioned for a successful career in academia. **Dr. Makkar is a highly productive and skilled post-doctoral fellow in my lab.** I am excited to support his career development and research plan to investigate the Mechanical regulation of inflammation in periodontal health and disease. Co-mentor Dr. Rebecca Wells and I will provide guidance, expertise, resources, and mentorship. Dr. Wells and I have worked together for over 3 years since I joined Penn through the **Center for Engineering MechanoBiology (CEMB)** in 2022. Dr. Wells and I regularly interact through CEMB events, conferences, and regular meetings. I confirm that Dr. Makkar will commit **12 person-months (100% effort)** to the mentored research and career development activities.

1) Information on my research qualifications and previous experience as a research supervisor

I am committed to supporting Hardik's research with my lab's expertise and resources. My laboratory is focused on investigating the mechanical regulation of inflammation in cancer and regeneration with the goal of developing immunomodulatory strategies to improve patient outcomes. We are a multi-disciplinary team bridging the gap between materials science and biology. Relevant to Dr Makkar's proposal, a major focus of the lab is investigating immuno-mechanical regulation of chronic inflammation. During the chronic inflammatory process, tissues undergo changes in their mechanical properties, and we have found that these changes, particularly in viscoelasticity, modulate immune cells. **Dr. Makkar has proposed a new line of investigation on the role of mechanobiology in gingival tissue health and disease.**

As a dual-trained dentist-scientist, I have over 11 years of experience as a research mentor. I am jointly appointed as an Assistant Professor at the University of Pennsylvania in both the School of Dental Medicine in Preventive and Restorative Dentistry and the School of Engineering and Applied Science in Materials Science and Engineering. My lab's research is at the intersection of dental medicine, bioengineering, and materials science. I am the first faculty member recruited by the **Center for Innovation and Precision Dentistry (CiPD)** of Penn Dental and Penn Engineering. I started at Penn in 2022 after completing my D.D.S. at the University of Minnesota, my Ph.D. in bioengineering at Harvard University, and my NIH K99 Post-doctoral Fellowship at Dana-Farber Cancer Institute, as well as a pre-doctoral fellowship in the NIH Medical Research Scholars Program. My research has a strong track record of **continuous NIH funding since 2016** with an NIDCR K08, an NIDCR K99/R00, and a subsequent NIGMS MIRA R35 award. As an independent scientist, I have also been awarded several prestigious awards, including the 2024 IDEA Prize from the CiPD and Penn Health-Tech, the 2024 Center for Undergraduate Research Foundation Faculty Award, the 2024 Joseph and Josephine Rabinowitz Award for Excellence in Research from Penn Dental, and the 2025 CiPD RESTORE Prize. My work has also received outside private foundation support from the **American Cancer Society Research Scholar Award** (the R01 equivalent) and the **Hartwell Foundation Individual Biomedical Research Award**. I am a rising leader in research in IADR and was recently elected **Vice President of the Oral and Maxillofacial Surgery Research Group**. I contribute as an **expert in biomaterials and mechanobiology as a peer reviewer** in top-tier journals, including Nature Nanotechnology, Science Advances, Proceedings of the National Academy of Sciences, ACS Nano, Advanced Healthcare Materials, and the Journal of Dental Research. I serve as a standing member of the American Cancer Society

Postdoctoral Fellowship Cell Biology & Immunology study section and an ad-hoc reviewer for the NIH CSR Special Emphasis Panel MSOS Review Branch. I have also been an invited speaker at **prestigious international seminars**, including the International Conference on Innate Immunity, IADR, and the MechanoBiology Institute at the National University of Singapore. I have published **26 peer-reviewed scientific articles with over 3250 citations since 2013**.

I have a strong mentoring track record in bioengineering, materials science, mechanical engineering, and dental medicine. So far at Penn, I have served as **a research mentor** for 9 summer undergraduate students, 11 Master's of Science students, 7 dental students, **6 PhD students, 1 DScD student, 4 post-doctoral fellows (including 2 CiPD T90/R90 trainees)**, and 2 visiting scholars. 2 of my mentored dental students received an AADOCR research award. My first PhD student successfully defended her dissertation in July in Mechanical Engineering at Penn. 1 of my PhD students was awarded a prestigious NSF Graduate Research Fellowship, and 1 other PhD student was recognized as Honorable Mention. I prioritize mentoring my students through biweekly one-on-one meetings, weekly lab meetings, and weekly platform meetings. I foster a collaborative work environment by enabling students' independence and growth. I regularly communicate with the group informally in the lab, as well as through Slack messaging and channels, to make sure I am up to date on all group matters. **My success as a mentor thus far highlights my expertise and ability to mentor and support Dr. Makkar's career development.** I am confident that Dr. Makkar will be well-positioned for a successful career as an independent dentist-scientist.

2) A plan that describes nature of the supervision and mentoring that will occur during the proposed award period

Dr. Makkar has developed a rigorous training and research plan that draws on his extensive expertise in periodontal disease research. By incorporating cutting-edge approaches of mechanobiology and tissue mechanics, he seeks to advance the objectives of his work and further our understanding of the underlying mechanisms involved. **Dr. Makkar will have both informal and formal interactions with my lab members through experiments and lab meetings.** He will be co-advised by me with monthly one-on-one meetings. **Dr. Makkar currently leads the bio-subgroup platform meetings** in my group and will continue to do so during the award period. He will participate in my weekly lab meetings, bi-weekly materials subgroup meetings, journal clubs, and social events. Dr. Makkar will also participate in seminars through my member centers, including the opportunity to host a monthly Discovery Series of the Center for Innovation and Precision Dentistry and present at symposia, including the annual Symposium of the Center for Engineering MechanoBiology (CEMB), annual Symposium of the Penn Center for Musculoskeletal Disease (PCMD), monthly Penn Institute of Regenerative Medicine (IRM) Stem Cell Club, Rising Leaders Seminar of the CEMB, and the annual IRM Symposium. Dr. Makkar will also give research updates at the weekly Penn/CHOP Blood Club, and the Mucosal Immunology and Inflammation Research in Progress Group (MiiRIP) at the Institute for Immunology and Immune Health. Dr. Makkar will expand his expertise in biomaterials and tissue mechanics to answer his research questions studying the mechano-regulation of gingival fibroblast-myeloid crosstalk. **He will work closely with my students and has offered to continue mentoring junior lab members.**

3) Career Development Plan

Dr. Makkar's career development plan will support his transition into an **independent dentist-scientist investigator** focusing on immuno-mechanobiology and biomaterials-based therapies in periodontal diseases. His career transition is supported by his rigorous research plan for the K99 mentored phase and the R00 independent phase. **During the mentored K99 phase**, he will be co-advised by me and Dr. Wells as his primary scientific mentors. He will gain scientific skills in **tissue mechanics and biomaterials** (with formal coursework, such as Experimental Methods in Materials Science), **advanced sequencing and bioinformatics** (with courses such

as Statistics and Advanced Epigenetics), **super-resolution microscopy** (with courses such as Biomedical Image Analysis), **in vivo models of ligature-induced periodontitis** (with Dr. Hajishengallis's lab) and **spatial transcriptomics** (with the 10x Xenium platform). Specifically regarding teaching activities, I have already invited Dr. Makkar as a guest lecturer for engineering graduate students on functional biomaterials and engineered models of periodontal tissues in my course (MSE 5180). **In the R00 phase**, Dr. Makkar's lab will study ECM stiffness-dependent fibroblast–myeloid crosstalk in vitro and in vivo. Dr. Makkar has assembled an advisory committee composed of experts in the fields of periodontology, immunology, computational biology (with bioinformatics), and mechanobiology to support Dr. Makkar's transition to independence throughout the award period. His committee members will continue in the R00 phase to provide feedback on his grants and manuscripts and overall development as an independent investigator. Additional topics for career development activities throughout the award period will include **grantsmanship training, teaching and mentoring, leadership and networking, scientific communication skills, technology transfer, and navigating the job search**. Dr. Makkar's progress during the award will be measured by specific milestones, including conference presentations (1-2 per year), submitted manuscripts (~2 per year throughout the award period, resulting in ~10 total published articles over 5 years), and obtaining follow-on funding from extramural sources (NIH early career awards, including NIDCR R03 and NIGMS MIRA R35), as well as private foundations (IADR) and internal faculty/post-doc awards (e.g., CiPD IDEA Prize).

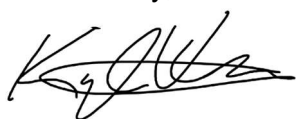
4) A plan for monitoring the candidate's research, publications, and progress toward independence.

Together with Dr. Rebecca Wells, I will meet with Dr. Makkar biweekly to discuss research and writing, provide evaluations of progress, and support a successful transition to a faculty position. As outlined in his career development plan, we expect ~2 submitted manuscripts per year as a co-author or first-author. Since joining my lab in 2024, Hardik has already been a **co-author on 2 published articles** from my lab (in ACS Materials Letters and APL Bioengineering). **He submitted 1 paper** and posted it as a preprint and is preparing an **additional 2-4 manuscripts for his K99 term**. I am fully confident in Dr. Makkar's ability to establish an independent research program. In the final phase of the mentored period, the Advisory Committee will provide candid feedback on Hardik's faculty application package and participate in practice interview seminars and chalk talks. As Hardik approaches the independent phase, I will review faculty application materials, provide candid discussions and feedback on research/chalk talks and career trajectory, and offer input on what search committees are looking for in faculty applications, including his strengths and weaknesses as a candidate. Additionally, I will continue to be a resource for Hardik, review future grants and manuscripts, share reagents and best practices for laboratory management, and provide feedback as he advances his career goals.

5) Components and resources of research that the applicant can take with them

Dr. Makkar's proposal is distinct from my active and pending projects in the lab. He will take the components related to his proposal with him in his independent research career. He will be able to take with him materials that he synthesizes in the lab and samples to his new academic setting. **I will also provide Dr. Makkar with any data he generates related to this project as preliminary data for his independent grants to support his lab in the future.**

Sincerely,



Kyle Vining, DDS, PhD,
Assistant Professor, Departments of Preventive and Restorative Sciences &
Materials Science and Engineering, University of Pennsylvania



Rebecca G. Wells, MD
Professor of Medicine, Bioengineering, and
Pathology and Laboratory Medicine
Co-Director, NSF Center for Engineering MechanoBiology
Associate Director, NIDDK Center for Molecular
Studies in Digestive and Liver Diseases

Department of Medicine
Division of Gastroenterology and Hepatology

October 30, 2025

Dear Committee Members,

I am pleased to provide my enthusiastic endorsement of Dr. Hardik Makkar's application for the NIDCR Pathway to Independence Award (K99/R00). I have had the opportunity to work closely with Dr. Makkar for the past two years, during which I have closely observed his exceptional growth and development as a researcher. As a current NIDCR R90 postdoctoral fellow, Dr. Makkar has demonstrated a rare combination of scientific rigor, intellectual curiosity, and technical expertise.

Dr. Makkar's interdisciplinary background is unique, as he holds both a dental degree in Oral Sciences and a doctoral degree in Bioengineering. This diverse training has enabled him to carry out an integrated program, applying engineering principles to solve complex biological problems, particularly in the field of periodontology. His past research in new approaches and methodologies to study periodontal host-microbe interactions and his record of publications attests to the quality and significance of his work. His most recent research identifies the role of changing tissue mechanics in periodontal disease progression and its effects on innate immune responses and host homeostasis – this is fascinating work, as mechanical pathology usually involves tissue stiffening, not softening as occurs in periodontitis. Dr. Makkar's focus on mechanobiology, particularly the role of extracellular matrix (ECM) mechanics, offers an innovative and novel approach to studying the pathogenesis of periodontitis. The research he proposes for the K99 award will make an important contribution to understanding the mechanobiological factors underlying periodontal disease and will almost certainly have a significant impact on the field. His preliminary data are robust, and the proposed project addresses a critical gap in current knowledge.

Mentorship Goals and Objectives

The primary goals of the mentorship plan are to:

- Provide guidance on the development, design, and interpretation of research projects that will contribute to Dr. Makkar's long-term research goals.
- Guide Dr. Makkar through his transition to independence by ensuring that he develops essential skills in grant writing, project management, mentoring junior trainees, and networking within the scientific community.
- Support Dr. Makkar in preparing for a successful transition to an independent academic or research position at the conclusion of the K99 phase.

Research Qualifications and Mentorship Plan

I am a highly experienced and successful mentor who has worked in mechanobiology for more than 20 years and am excited to serve as Dr. Makkar's co-mentor for the duration of his K99 award. Throughout this period, he will receive both formal and informal supervision in my laboratory. We will meet monthly to discuss his progress, refine experimental strategies, and ensure that he stays on track to achieve his scientific and

career goals. Dr. Makkar will have full access to the state-of-the-art equipment in my lab, which will be crucial for advancing his research.

As co-director of the Center for Engineering MechanoBiology (CEMB), a National Science Foundation-funded multi-institutional Science and Technology Center (STC), I am well positioned to provide Dr. Makkar with exceptional mentorship and a broad range of opportunities. The resources available through CEMB, including collaborations with top-tier researchers and access to advanced technologies, will be invaluable as he advances his work. Although CEMB will be formally sunsetting in one year, the educational and support activities of the center, as well as faculty and trainee interactions, will continue (as typically happens for STCs). As an example, Hardik will have the opportunity to present his research at the "Future Leaders in Mechanobiology Seminar Series," where he will engage with leading experts in the field and receive constructive feedback. He will also benefit from the extensive network of researchers and laboratories within the CEMB consortium, which will provide crucial support for his research and career development.

In my lab, Dr. Makkar will continue to build on his expertise in tissue mechanics and the role of mechanical factors in large-scale tissue rearrangements during chronic inflammatory diseases. His proposed project will be significantly enhanced by our lab's experience with relevant animal models and cutting-edge techniques linking mechanics to disease pathology.

During the mentored phase of the K99 award, Dr. Makkar will acquire expertise in advanced techniques, including tissue mechanics (macro, meso, and nano scale), ATAC sequencing, superresolution nuclear imaging, and spatial transcriptomics. Training in these areas will occur in his mentor's lab (Dr. Kyle Vining), in my lab, and in collaboration with experts in the fields of clinical periodontology (Dr. Kang I. Ko – Advisor) and immunology (Dr. George Hajishengallis and Dr. Michael Abt – Advisors). These collaborations will ensure that Dr. Makkar has access to the most current methodologies and cutting-edge technologies for his work.

Dr. Makkar will devote the majority of his time during the mentored phase to completing his proposed research project, for which I will provide full access to my lab's resources and equipment. To enhance his development as a well-rounded researcher, he will also participate in training in Responsible Conduct of Research (RCR) through Penn's Biomedical Postdoctoral Program (BPP), as well as in faculty-led discussion groups. For leadership development, Dr. Makkar will continue to lead sessions for the American Association for Dental, Oral, and Craniofacial Research (AADOCR) and the International Association for Dental Research (IADR), as well as participate in leadership workshops at Penn.

I will hold monthly meetings with Dr. Makkar to track his progress, and quarterly meetings with him, his primary mentor, and the Advisory Committee to assess his research, coursework, publications, and grant applications. These meetings began and have occurred as stated for the last year. Additionally, I am committed to helping him expand his academic and professional network, and to assisting in his job search during the transition to his independent investigator phase.

Career Development and Professional Skills

- **Grant Writing:** I will mentor Dr. Makkar through the process of writing competitive research grants. Along with his primary mentor, I will guide him in preparing applications for NIGMS R35, NIH R01 grants, foundation grants, and other relevant funding mechanisms. We will discuss specific aims, hypotheses, experimental approaches, and timelines. I will review their drafts and provide detailed feedback on scientific content, clarity, and overall structure.
- **Publications:** I will encourage Dr. Makkar to publish their findings in high-impact journals. Along with the mentor, we will work together on manuscript drafts, from the initial concept through submission and revision. I will assist with strategies for selecting appropriate journals, responding to reviewer comments, and navigating the publication process.
- **Presentation Skills:** I will work with Dr. Makkar to develop his oral presentation skills, both in formal academic settings and in informal group meetings. This will include mock presentations, constructive feedback on presentation style, and guidance on communicating their research effectively to both specialist and general audiences.

Long-Term Career Development

The goal of this mentorship is to ensure that Dr. Makkar successfully transitions to the independent investigator phase of his career. I am confident that he will establish an impactful multi-disciplinary research program that combines periodontology, mechanobiology, and bioengineering to address significant scientific and clinical challenges. I will support him in every step of this journey, from expanding his skillset to establishing a robust academic network and securing future funding. In summary, I am confident in Dr. Makkar's potential to succeed in his K99 career development award and to establish himself as a leader in the field of periodontology and mechanobiology. He is an exceptional scientist with the intellectual curiosity, technical skills, and dedication required to make lasting contributions to his field. I look forward to continuing to mentor Dr. Makkar and supporting his growth as an independent investigator.

Metrics for Success and Evaluation

We will regularly evaluate progress towards the following milestones:

- Successful completion of the career development plan and research project
- He has assembled an advisory committee with expertise in periodontology, immunology, computational biology, and mechanobiology to guide his transition to independence, providing ongoing feedback on research, grants, and manuscripts.
- Career development activities will focus on grantsmanship, teaching, leadership, communication, and job search preparation.
- Progress will be evaluated through defined milestones, including 1–2 conference presentations and ~2 manuscripts per year (targeting ~10 publications over 5 years), along with pursuit of follow-on funding from NIH (e.g., NIDCR R03, NIGMS R35), private foundations (e.g., IADR), and institutional awards (e.g., CiPD IDEA Prize) with the long-term goal of obtaining independent R01 funding.

I will conduct quarterly evaluations of Dr. Makkar's progress. These evaluations will be based on:

- **Research Milestones:** Completion of research objectives outlined in the K99 proposal, including manuscript submissions and successful presentations.
- **Career Development Goals:** Achievement of career milestones such as successful grant applications, networking, and the ability to establish a collaborative research agenda.
- **Professional Skills:** Progress in leadership, mentoring, and independent research project management and job search preparations.

Transferability of Research and Resources

Dr. Makkar's research interests are aligned with, but do not overlap with, mine. He will be able to take all techniques and data generated during the mentored phase of the award with him as he transitions to his independent research role.

Sincerely,



Rebecca G. Wells, MD

905 Basic Research Building • 421 Curie Blvd. • Philadelphia, PA 19104-6160
Phone: 215-573-1860 • Fax: 215-573-2024



October 30th, 2025

Re: Letter of support for Dr. Hardik Makkar

To whom it may concern,

I am writing to offer my enthusiastic support for Dr. Hardik Makkar's application for the NIDCR K99/R00 award titled "Probing the Mechanical Regulation of Inflammation in Periodontal Health and Disease". I have had the pleasure of advising Dr. Makkar throughout his current NIDCR R90 fellowship. I am committed to continuing this during both the mentored and independent phases of his proposed project. Dr. Makkar is an exceptionally talented clinician-scientist, and I am confident that this award will support his eventual transition to an independent investigator. His proposed project focuses on the mechanobiology of extracellular matrix (ECM) and its impact on immunopathology during periodontitis pathogenesis, a research area that remains underexplored. Dr. Makkar has a research background that combines oral biology and bioengineering approaches; thus, he is in a unique position to unveil key disease mechanisms driven by biomechanical cues, which may lead to development of new therapeutic targets.

I am a board-certified periodontist and an Assistant Professor in the Department of Periodontics at Penn Dental Medicine. I have been funded by the K08 award for my own career transition to independence and now lead two R01-funded projects that study fibroblast heterogeneity and its functional significance in health and disease at the oral and cutaneous barrier. The work from our lab unveiled remarkable heterogeneity of the oral fibroblasts in murine and human gingiva and identified a special subtype of fibroblasts that accelerates wound healing by facilitating early macrophage trafficking post-injury. Our lab has also published on the role of gingival fibroblasts for mitigating periodontal damage in a murine model of ligature-induced periodontitis. Dr. Makkar's proposed studies that focus on the effect of biomechanical cues on gingival fibroblasts to explain periodontal pathogenesis are novel and timely, and I am excited for his significant contribution in the field of periodontal pathogenesis.

I am pleased to serve on Dr. Makkar's Advisory Committee, meeting with him quarterly and providing one-on-one guidance as needed. My laboratory will support his research by providing archived primary human gingival fibroblasts and histologic tissue sections, as outlined in his proposal. Through the Center for Clinical and Translational Research (CCTR) at Penn Dental Medicine, I will also facilitate access to discarded fresh gingival tissues obtained during routine dental care procedures from patients who provide informed consent, in accordance with IRB-approved protocols (IRB #844933, PI: Ko).

As a clinician-scientist and board-certified periodontist, I will offer scientific consultation and clinical insight into Dr. Makkar's studies of fibroblast-immune interactions in periodontal inflammation. My laboratory's expertise in gingival fibroblast heterogeneity, wound healing, and fibroblast-driven periodontal tissue inflammation—using murine ligature-induced periodontitis models and spatial transcriptomic approaches—will complement and strengthen his mechanobiology-focused research.

I am fully committed to supporting Dr. Makkar's scientific and career development by providing both technical expertise and mentorship to facilitate his transition to independence. His creativity, intellectual rigor, and commitment to translational research make him an outstanding candidate for the K99/R00 Pathway to Independence Award. Please do not hesitate to contact me for any additional information.

Sincerely yours,

A handwritten signature in black ink, appearing to read "Kang I. Ko".

Kang I. Ko, DMD, DScD
Assistant Professor, Department of Periodontics
School of Dental Medicine, University of Pennsylvania, Philadelphia, PA 19104



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George Hajishengallis, D.D.S., Ph.D.
Thomas W. Evans Centennial Professor

October 10, 2025

Dear Review Committee Members,

It is with great pleasure and unwavering support that I endorse Dr. Hardik Makkar's NIDCR K99/R00 application, titled **"Probing the Mechanical Regulation of Inflammation in Periodontal Health and Disease"**. I also commit to **serving as a member of his Advisory Committee**. Dr. Makkar's proposed research addresses an underexplored area in periodontal disease pathogenesis, specifically focusing on the innovative concept of how gingival tissue mechanics and ECM stiffness regulate immune responses as the disease progresses. His work promises to provide fresh perspectives and transformative insights into the biomechanical regulation of gingival tissue homeostasis, with the potential to drive the development of novel therapeutic approaches for managing periodontal diseases.

I am the Thomas W. Evans Centennial Professor in the Department of Basic and Translational Science at the University of Pennsylvania's School of Dental Medicine, where my lab uses diverse and complementary in vitro and in vivo preclinical models and multidisciplinary research approaches to understand the mechanisms that regulate immunity and inflammation in the oral mucosa and how this in turn impacts systemic health and disease. In this manner, the laboratory and its collaborators have made significant novel contributions that challenged earlier paradigms and provided implications and applications above and beyond the oral immunology field. These include, but are not limited to, the introduction of the keystone pathogen concept; the polymicrobial synergy and dysbiosis model in inflammatory disease; the inflammophilic nature of the dysbiotic oral microbiota; the location-dependent homeostatic principle; the DEL-1-IL-17 balance principle; and the establishment of maladaptive trained myelopoiesis as a mechanistic basis of inflammatory comorbidities. Some of our basic discoveries in model organisms have found application in the treatment of human disease.

During the K99 phase, Dr. Makkar will leverage my lab's expertise to learn the workflow for murine models of ligature-induced periodontitis and develop technical proficiency in processing animal tissues for downstream molecular and protein-based assays. My guidance will be critical for the successful execution of his in vitro workflow under Aim 2 (stromal-myeloid crosstalk using the hydrogel model) and in vivo immunology studies in mouse models, including the interpretation of the generated data. I will provide regular and biannual advisory meetings, one-on-one mentorship as needed, and access to my laboratory resources and research team. Additionally, Dr. Makkar will benefit from opportunities to present and network at specialized meetings, such as the Aegean Conference on Oral Mucosal Immunity and Microbiome, which I co-organize annually. As a dentist-scientist, I will also provide valuable career guidance and mentoring to support his transition toward research independence.

Dr. Makkar's preliminary findings are highly promising, demonstrating his ability to pioneer research in an underexplored area of Periodontology. I am confident that his research will significantly broaden our understanding of periodontal diseases, extending beyond, but not limited to, microbial etiopathogenesis. His dedication and interdisciplinary approach make him an exceptional candidate for the K99/R00 award.

In conclusion, I wholeheartedly support Dr. Makkar's application and recognize his potential as a leading independent researcher. Please do not hesitate to reach out should you require any additional information or clarification.

Yours sincerely,

A handwritten signature in black ink, appearing to read "G. Hajishengallis".

George Hajishengallis, D.D.S., Ph.D.
Thomas W. Evans Centennial Professor

UNIVERSITY of PENNSYLVANIA



Michael C. Abt, Ph.D.
Assistant Professor

Department of Microbiology 22 October 2025

RE: Dr. Hardik Makkar K99/R00 application

Dear Hardik,

I am delighted to write this letter to confirm my strongest support and participation on your mentoring committee for your forthcoming K99/R00 application entitled "*Probing the Mechanical Regulation of Inflammation in Periodontal Health and Disease*." Your proposed research, exploring how extracellular matrix (ECM) stiffness regulates gingival fibroblast epigenetic states and stromal–myeloid cell interactions, has the potential to uncover key mechanobiological mechanisms underlying periodontitis and chronic oral inflammation more broadly.

As an Assistant Professor in the Department of Microbiology at the University of Pennsylvania with significant experience in NIH-funded research, my lab investigates how microbial communities modulate immune responses in infectious diseases. Your project aligns closely with our focus on immune–microbiome interactions, and I am enthusiastic about the opportunity to contribute to your training and scientific growth.

As an Advisor, I will provide analytical and technical guidance on animal workflows, focusing on mucosal immunology and immune–microbiome crosstalk relevant to Aim 2 of your proposal. This includes both the in vivo ligature-induced periodontitis model and in vitro experiments investigating stromal–immune crosstalk using tunable gingival ECM hydrogels. We will meet quarterly, supplemented by one-on-one discussions as needed, to review progress and refine experimental approaches. My lab's expertise in microbiology and mucosal immunity will support your efforts to investigate how gingival matrix stiffness impacts microbiome and immune dynamics in vivo. I will also assist in data interpretation and provide an immunological perspective to strengthen your mechanistic insights.

Beyond experimental mentorship, I am committed to supporting your professional development. I will facilitate your active participation in the Mucosal Immunology and Inflammation Research in Progress Group (MiiRIP), now organized by the UPenn Center for Molecular Studies in Digestive and Liver Disease. This forum provides trainees with valuable opportunities to present research, exchange technical insights, and foster interdisciplinary collaborations in mucosal immunology. I believe your involvement in this community will enhance your scientific visibility and collaborative potential.

Your preliminary data are compelling and reflect a thoughtful, rigorous approach to a challenging and understudied question. The integration of mechanobiology, immunology, and oral health in your work represents an exciting and innovative direction. I am confident that your research will lead to important advances and establish you as a leader in the field.

In summary, I strongly support your K99/R00 application and am committed to your continued scientific and career development. Please do not hesitate to contact me with any questions.

Sincerely, 

Michael C. Abt, Ph.D.

Assistant Professor, Department of Microbiology

Perelman School of Medicine, University of Pennsylvania

303B Johnson Pavilion 3610 Hamilton Walk Philadelphia, PA 19104

Dr. Hyun (Michel) Koo, DDS, PhD (Penn Dental Medicine) and Dr. Kathleen Stebe, PhD (Penn Engineering)
Co-Directors, Center for Innovation & Precision Dentistry (CiPD)

Dear Colleagues,

As Co-Directors of the CiPD and the NIDCR T90/R90 Postdoctoral Training Program at the University of Pennsylvania, we strongly and enthusiastically support Dr. Hardik Makkar's K99/R00 application "**Probing the Mechanical Regulation of Inflammation in Periodontal Health and Disease**". Hardik, an exceptionally promising young investigator, is a R90 postdoctoral fellow. We are eager to continue supporting his growth under K99 support. An essential element of the R90 program is the Career Mentoring Committee (CMC) comprising the research primary mentor, co-mentor, and the two of us as co-directors. All CMCs must have at least one clinician scientist focused on oral-craniofacial health. We have committed with enthusiasm to continue to mentor him should he be awarded this K99/R00 grant. Furthermore, we will provide him full access to CiPD facilities, seminars, and resources, including a dedicate desk and space for collaborations, opportunities to apply for seed funds, as well as participation of our annual symposium to both present, network, and interact with Penn faculty, academia/industry leaders and guest speakers.

Our research experiences span the development of novel engineering tools to mitigate pathogenic biofilms and oral diseases (H.K.) and functional soft matter for fundamental study and for diverse application (K.S.). The aim of our program is to develop a new cohort of experts trained at the interface of oral health and engineering. Thus, we require that all T90 trainees be co-mentored and work collaboratively with clinician-scientists and engineers in order to cement their ability to bring new approaches across disciplines. Each trainee's CMC functions as a highly customized, results-oriented "research vision and career development committee" that actively monitors trainee progress and provides actionable feedback. Since his selection as an R90 Fellow, we have had extensive interactions with Hardik, discussing his professional goals, career plans, research vision, and planned grant submissions. As a result, we are well aware of his goal to bring state-of-the-art skills as a Dentist-Scientist specializing in Mechanobiology to address needs in oral and craniofacial health. We are excited about Hardik's potential as an academician at the intersection of these fields and will continue to support him through the CiPD.

While our direct research expertise is outside of Dr. Makkar's particular area of interest, we have extensive experience as mentors in our respective laboratories and in serving the University's research and training mission. For example, K.S. has served as Department Chair at Johns Hopkins University and at the University of Pennsylvania, and as Deputy Dean of Penn Engineering with oversight of research, innovation, doctoral and post-doctoral programs. H.K. has led several NIDCR-funded, multidisciplinary research in collaboration with Penn Engineering and the School of Medicine. We serve as co-directors of the CiPD whose mission is to bridge schools and unite researchers to advance new approaches to address clinical needs in dental medicine. In addition, H.K. is actively involved with the AADOCR and IADR scientific and mentoring committees that will be helpful for Hardik's career development in the oral & craniofacial area. We are confident that Hardik's training and mentoring consortium, with members with diverse expertise and viewpoints, will provide clear guidance as he navigates a successful transition to an independent research career at the intersection of disciplines. We will also assist Hardik's career transition by assisting him with future grants, reviewing his faculty application, attending practice talks, and helping to network with other colleagues in bioengineering and oral health sciences. Furthermore, as co-directors of CiPD, we have provided a dedicated desk and space with a computer and software, administrative support, and full access to all center resources.

In summary, Hardik Makkar has all the qualifications, scientific maturity, and unwavering commitment for an independent research career at the interface of bioengineering and craniofacial health. Thus, we recommend (and will further support) Dr. Makkar for the K99/R00 Award with our highest alacrity and strongest support without any reservation whatsoever. If you have additional questions, please do not hesitate to contact us.

Sincerely yours,

Hyun (Michel) Koo, DDS, PhD



Kathleen J. Stebe, PhD





Kai Tan, PhD

Professor of Pediatrics, Cell & Developmental Biology, Genetics
Richard & Sheila Sanford Endowed Chair

Director, Center for Single Cell Biology at Children's Hospital of Philadelphia
Co-Leader, Pediatric Oncology Program, Abramson Cancer Center

November 4, 2025

Hardik Makkar, PhD
NIDCR R90 Postdoctoral Fellow
Center for Innovation and Precision Dentistry (CiPD)
School of Dental Medicine
University of Pennsylvania
Philadelphia, PA 19104

Dear Hardik,

I am very pleased to provide this letter of support for your NIH (NIDCR) K99 grant. I am excited to assist your studies utilizing ATAC-sequencing and Spatial Transcriptomics to study the mechanical regulation of inflammation in periodontal health and disease. I lead the Center for Single Cell Biology (CSCB) at the Children's Hospital of Philadelphia (CHOP) that provides a unified research infrastructure for the single-cell research community at CHOP and the University of Pennsylvania. Single-cell-resolution measurements can be integrated to build phenotypic maps that span subcellular, cellular, and tissue and system scales, which can allow for detailed cell-specific functional observations to pinpoint the perturbations caused by diseases. The Service Core of the Center is equipped with state-of-the-art instruments, experienced research assistants and bioinformaticians to offer in-house experimental and computational support to your single-cell and spatial omics research.

Regarding your project, I look forward to providing my guidance and expertise by assisting your studies on nuclear mechano-transduction and stromal-myeloid crosstalk in periodontal health and disease.

Best of luck with the application and I look forward to the future success of this project!

Sincerely,
Kai

A handwritten signature in black ink that reads "Kai Tan".



Philadelphia, PA
November 04, 2025

Hardik Makkar
NIDCR R90 Postdoctoral Fellow
Center for Innovation and Precision Dentistry (CiPD)
School of Dental Medicine
University of Pennsylvania
Philadelphia, PA 19104

Dear Hardik,

It is my pleasure to send this letter of support for your NIH (NIDCR) K99 grant. I am excited to assist your studies utilizing super-resolution microscopy to measure changes in chromatin structure of Gingival fibroblasts. My lab is an expert in nuclear mechanobiology. We employ super-resolution stochastic optical reconstruction microscopy (STORM) to characterize nanoscale regulation of cellular behaviors by biophysical cues. We have established these systems to investigate musculoskeletal cells. I am excited to apply these technologies to study pathogenesis of periodontal diseases. I look forward to expanding the scope of its application and will provide my guidance and expertise by assisting your studies on nuclear mechanotransduction in periodontal health and disease.

I look forward to the future success of this project.

Sincerely,

A handwritten signature in black ink, appearing to read "Su Chin Heo".

Su Chin Heo, Ph.D.
Assistant Professor
Department of Orthopaedic Surgery
Department of Bioengineering
University of Pennsylvania
375A Stemmer Hall
3450 Hamilton Walk
Philadelphia, PA 19104-6081

DESCRIPTION OF INSTITUTIONAL ENVIRONMENT

The University of Pennsylvania School of Dental Medicine (Penn Dental Medicine) and the Center for Innovation & Precision Dentistry (CiPD) are fully committed to supporting the training and the proposed work described in this application (See ***Institutional Commitment to Candidate's Research Career Development and Primary/Co-Mentor Statement of Support***). The CiPD is Co-led by Dr. Stebe and Dr. Koo and includes research faculty from across Penn Dental Medicine and Penn Engineering, both located in the Penn campus in Philadelphia. The CiPD houses the NIDCR T90/R90 Post-doctoral training program “*Advanced Training at the Interface of Engineering and Oral-Craniofacial Sciences*” (2.5M Postdoc Training Grant). Dr. Hardik Makkar is a member of the cohort of trainees under this program. As detailed below, these entities offer a wide diversity of high-quality training opportunities and world-class research facilities.

Through formal seminars and informal opportunities, Dr. Hardik will have the opportunities to interact with a number of leading scientists in Mechanobiology, Immunology, single-cell biology, and other dental, biological, and engineering sciences. Penn Dental Medicine holds weekly seminars (e.g., *Basic & Translational Sciences CrossTalk*, *Frontiers in Science*), which serve as an important stage for both early-stage researchers (e.g., postdocs) and faculty investigators to share their work and offer opportunities for or interdisciplinary networking, which is critical for building cross-disciplinary collaborations. In addition, Penn Dental Medicine also organizes yearly *Research Day* and *CiPD Annual Symposium* with invited distinguished speakers, further facilitating high-level research collaboration between dentists, scientists, and engineers from broad research communities. Furthermore, the school and CiPD provides Travel Awards/Funds that support participation of early-career investigators in national/international conferences and professional meetings (e.g., AADOCR, IADR meetings).

To augment these training opportunities, the University of Pennsylvania has a number of important programs for enhancing the postdoctoral academic/professional development. Penn's Biomedical Postdoctoral Program (BPP) offers coursework, seminars and workshops in a range of professional development-related topics including *Presentation and Public Speaking Skills*, *Basic Job Search Skills*, *Career Paths for Biomedical Scientists*, *K & F Awards Workshops*, *Scientific Writing*, *Grant Writing and the Peer Review Process*, *Laboratory Management*. Also, Penn BPP and other Penn Centers hold research symposia, e.g., *Biomedical Postdoctoral Research Symposium* (BPP). These events also include networking opportunities and professional development sessions. In addition, faculty-led, discussion-based courses and certification programs in the *Responsible Conduct of Research* (RCR) are organized by Penn BPP. This proposed research project is also supported by world-class laboratory and core facilities (***Detailed under Facilities and Resources***) In addition, over 60 Penn research centers and core research facilities are available, including *Molecular Biology Core*, *Microscopy Core*, *Tissue Processing Core*, *Next Generation Sequencing Core*, and *Bioinformatics Core*.

Robert Schattner Center
 University of Pennsylvania
 School of Dental Medicine
 240 S. 40th Street
 Philadelphia, PA 19104-6030
 www.dental.upenn.edu

November 4, 2025

Dear Colleagues,

I am writing this letter to express my strongest support and enthusiasm for Dr. Hardik Makkar's K99/R00 application, **"Probing the Mechanical Regulation of Inflammation in Periodontal Health and Disease."** Dr. Makkar has put together a compelling training and career development program in the fields of Mechanobiology, Biomaterials, and Immunology. As one of the *NIDCR R90 Postdoctoral Fellows* in the Center for Innovation & Precision Dentistry (CiPD), a joint venture between Penn Dental Medicine and Penn Engineering, Hardik is uniquely qualified for the NIDCR K99 award that will accelerate his independent career as a dentist- scientist. Hardik has been exceptionally productive while actively pursuing academic growth as attested in his impressive CV and strong letters of support from leaders in the field of Mechanobiology, Immunology, and Material Scientists.

Dr. Makkar has surrounded himself with an exceptional mentorship team, including Dr. Kyle H. Vining (primary mentor) and Dr. Rebecca G. Wells (co-mentor), as well as an Advisory Committee of leaders in their fields (Dr. Kang I Ko, Dr. George Hajishengallis, Dr. Michael C. Abt, Dr. Hyun (Michel) Koo, and Dr. Kathleen Stebe). Both primary mentor and co-mentor have immense experience running highly productive/funded research labs and a proven track record of mentoring graduate/postdoc fellows and junior faculty. Dr. Makkar's proposed project investigates the mechanobiology of extracellular matrix (ECM) changes in periodontal tissue in health and disease, an important but under-researched area. By examining how alterations in the mechanical properties of gingival ECM affect the host's immune responses, Dr. Makkar seeks to address a critical gap in our understanding of periodontal disease pathogenesis. This novel approach could offer groundbreaking insights into the molecular and biomechanical mechanisms behind chronic oral inflammatory diseases like periodontitis. Additionally, his research may identify new therapeutic targets and strategies for treating this global health issue. Dr. Makkar will have the opportunity to participate in the weekly Cross-Talk Interdisciplinary seminar series, Penn Dental Annual Research Day, and CiPD Symposium events to present his research findings to our faculty, students/residents, and post-doctoral fellows and will have ample opportunities to meet invited speakers. In addition, Dr. Makkar will be able to enroll in workshops/seminars offered by our nationally recognized Biomedical Postdoctoral Program.

As the Vice Dean for Scholarship and Research, I am committed to providing Dr. Makkar with a truly exceptional scientific and training environment and any additional support necessary for a successful mentored phase and eventual transition to a faculty position. He will have full access to all research equipment and facilities in Penn Dental Medicine with dedicated laboratory space and office space within the CiPD. In addition, Dr. Makkar will have complete access to all UPenn core facilities, animal resources, and administrative support from our grants managers. I will work closely with all involved to ensure that he has protected time to successfully complete his proposed research and career development program.

In summary, Dr. Vining's and Dr. Well's mentorship, the support of the Advisory Committee members, and the School's commitment will ensure that Dr. Makkar's training and career development are productive and successful. Dr. Makkar has all the qualifications and commitment that will strongly position him to fill critically needed gaps of knowledge and workforce at the dentistry-engineering interface. I believe he will be highly successful and achieve an exemplary career as an independent dentist-scientist. As such, I fully endorse this proposal without reservation and recommend him with the greatest enthusiasm.

Sincerely,



Dana T. Graves, DDS, DMSc
 Professor and Interim Chair, Department of Periodontics Vice Dean for Scholarship and Research
 Director, Doctor of Science in Dentistry Program

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.

JUSTIFICATION FOR THE USE OF HUMAN SPECIMENS.pdf

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

JUSTIFICATION FOR THE USE OF HUMAN SPECIMENS:

1. Information on who is providing the data/biological specimens and their role in the proposed research:
 - Fred Hutch Cancer Center, Cooperative Center of Excellence in Hematology (CCEH) – provides fee-for-service mobilized CD34+ primary human hematopoietic stem and progenitor cells.
 - University of Pennsylvania , School of Dental Medicine, IRB approved protocol #844933 – provides Gingival tissues from donors for the isolation of Gingival Fibroblasts.

2. Description of the identifiers that will be associated with the human specimens and data:

The human cells have no identifiers associated with them. There is no data shared on the human donors from the Fred Hutch Cancer Center and Penn Dental Medicine. Investigators may seek prior approval to obtain key, de-identified information on their withdrawn specimen.

3. Who has access to subjects' identities?

No personnel have access to subjects' identities.

4. Information about the manner in which the privacy of research participants and confidentiality of data will be protected:

There are no research participants in this proposal. All data remains confidential.

Delayed Onset Studies

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

VERTEBRATE ANIMALS

All experiments will be carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All the animal related procedures related to this proposal, including topical applications of the agents, were previously reviewed and approved by the University of Pennsylvania Institutional Animal Care and Use Committee. My mentor's labs **Dr. Kyle Vining**, **Dr. Rebecca Wells** and Advisor Labs **Dr. George Hajishengallis** and **Dr. Kang I Ko** have extensive experience in using these model systems, which allows me to ensure reproducibility and optimization of the model. We are making every effort to conserve our use of animals, as evidenced by our use of in vitro models when appropriate. Dr. Kyle Vining has access to procedure rooms and rodent housing rooms dedicated to the use of his laboratory, and the researchers in his labs have full access to all resources of the animal care facility. Veterinary care is available 24 hours a day, seven days a week.

Description of procedures and justification: We propose to use equally distributed male and female WT C57BL/6 in our animal studies. Statistical analysis and biological variables. Based on our preliminary data, power analysis with $\alpha=0.05$, $\beta=0.2$ (power of 0.8), and ~ 1.2 effect size, we expect $n=10$ for each group to be sufficient to achieve statistical significance at $p<0.05$. All experiments will be initiated when mice are 12wks old. Normal data distribution will be tested using Wilk-Shapiro test prior to parametric analysis, and two-way ANOVA followed by Tukey's post-hoc test will be used to compare control and transglutaminase treatment group. Each individual animal will be used as a unit of measurement for statistical analysis.

All procedures and housing of animals will be in approved facilities at University of Pennsylvania. A fully staffed animal facility, including housing and surgical suites and veterinary support, is located on the campus. All animal experiments will be performed in a pathogen-free animal facility with approval by a AAALAC accredited Animal Studies committee. Mice will be anesthetized by inhalation isoflurane (1.5-2%) prior to all surgical procedures. The animal will be anesthetized and monitored for adequate surgical plan by observing that the animal is immobilized, regular deep breathing, and loss of toe reflex. During post-operative monitoring, animals will be examined for signs of bleeding, infection, dehiscence of surgical wounds, and for lethargy, lack of eating or production of urine/feces. We specifically will utilize a scoring system to determine a humane end point for these studies. Following ligature placement, the mice will be monitored 3 times a week. Animals that at any time in the experiment become moribund will be immediately euthanized. Experimental Illness Report forms will be used to track the health of the animals.

Minimization of Pain and Distress and method for euthanasia: These experiments have been conscientiously designed to evaluate as many parameters per mouse as possible. Procedures to reduce discomfort, distress, pain, and injury have been put in place. CO₂ inhalation and cervical dislocation euthanasia will be used in all studies; this method is consistent with the Recommendations of the Panel on Euthanasia of the American Veterinary Medical Association. The facilities and programs of the vivarium, the division of Laboratory Animal Medicine and the University Laboratory Animal Resources of the University of Pennsylvania are fully accredited by the Accreditation of Laboratory Animal Care International (AAALAC) and are in full compliance with state law, federal statute, and NIH policy. The University of Pennsylvania's Division of Laboratory Animal Medicine and Laboratory Animal Resources staff consists of more than 100 persons with board-certified laboratory animal veterinarians, clinical laboratory animal veterinarians, a veterinary pathologist and 10 veterinary technicians with expertise in anesthesia of a wide variety of species. They are assisted by trained and licensed animal health technicians as well as animal care technicians. The Division is responsible for preventive, diagnostic, and clinical services to all laboratory animals, providing guidance to investigators regarding handling, immobilization, anesthesia, analgesia, and euthanasia as well as monitoring of all surgery programs and the provision of appropriate postsurgical care. A veterinarian is always available during and after normal working hours, weekends, and holidays. Staff is trained to recognize signs of ill health, and a well-defined reporting mechanism is in place to ensure that such animals are examined by appropriate Division personnel in a timely manner. The Division's Animal Disease Diagnostic Laboratory supports the efforts of the programs that provide veterinary care.

NIH Generated message:

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

AUTHENTICATION OF KEY BIOLOGICAL AND/OR CHEMICAL RESOURCES

Chemicals

All acquired compounds and reagents will be authenticated for both identity and purity, based on certificate of analysis from manufacturers. This information will be included in peer-reviewed publications on the project, along with details on commercial sources for precursors and any necessary purification, handling and storage of reagents and products (e.g., under an inert atmosphere).

Antibodies

Antibodies for proteins in proposed studies will be obtained from commercial manufacturers providing hybridoma clone identification, lot number and appropriate references. The specificity of the antibodies employed in this study will be authenticated by immunoblot analysis (including knockdown samples when possible) and appropriate controls are included in every experiment. In addition, we will monitor the Antibody Registry database to be aware of any issues observed by other investigators with antibodies in use in our laboratory.

Microscopy and associated reagents

The fluorescent probes, laser and filter parameters related to the confocal microscopy have been extensively tested and selected to minimize potential cross talk between different excitation and emission channels. We will use standard and well-published fluorescence probes (commercially available) and rigorously established experimental protocols to ensure reproducibility.

Cell lines

Primary human cells for in vitro experimentation will be characterized by qPCR and flow cytometry for each donor to confirm starting cell population. Primary cells will also be tested routinely for mycoplasma and bacterial contaminations. Each primary cell culture is passaged less than 20 times. Quality of commercially obtained reagents for cell-based assays (e.g., MTT, 2',7'-dichlorodihydrofluorescein) will be verified by expiration dates, lot numbers, and certificate of analyses from manufacturers.