

Electronic Cover Sheet		
PI: <b>Pandya, Mirali</b>	Title: Probing the Role of a Succinate-Related Metabolic Reprogram in Periodontal Pathogenesis	
Received: 10/13/2025	Opportunity: PA-24-182	Council: 05/2026
Competition ID: FORMS-I	FOA Title: Mentored Clinical Scientist Research Career Development Award (Parent K08 Independent Clinical Trial Not Allowed)	
<b>1K08DE036343-01</b>	Dual:	Accession Number: 5206029
IPF: 7636101	Organization: UNIVERSITY OF SOUTHERN CALIFORNIA	
Former Number:	Department: Department of Endodontics and Periodontics	
IRG/SRG: ZRG1 MSOS-F (22)S	AIDS: N	Expedited: N
<u>Subtotal Direct Costs</u> <u>(excludes consortium F&amp;A)</u> Year 1: 124,996 Year 2: 124,996 Year 3: 124,996 Year 4: 124,996 Year 5: 124,996	Animals: N Humans: Y Clinical Trial: N Current HS Code: 20 HESC: N HFT: N Special Topics: Data Management Sharing	New Investigator: Early Stage Investigator:
<i>Senior/Key Personnel:</i>	<i>Organization:</i>	<i>Role Category:</i>
Mirali Pandya	University of Southern California	PD/PI
De-Chen Lin	University of Southern California	Other (Specify)-Advisory Committee
Pinghui Feng	University of Southern California	Other (Specify)-Co-Mentor
Chao Qin	University of Southern California	Other (Specify)-Collaborator
Casey Chen	University of Southern California	Other (Specify)-Mentor

*Reference Letters*

Kathy Svoboda	Texas A&M College of Dentistry	10/13/2025
MICHAEL PAINE	University of Southern California	10/13/2025
Thomas Diekwisch	University of Rochester	10/13/2025

## APPLICATION FOR FEDERAL ASSISTANCE

## SF 424 (R&amp;R)

3. DATE RECEIVED BY STATE		State Application Identifier	
1. TYPE OF SUBMISSION*		4.a. Federal Identifier	
<input type="radio"/> Pre-application <input type="radio"/> Application <input checked="" type="radio"/> Changed/Corrected Application		b. Agency Routing Number	
2. DATE SUBMITTED 2025-10-13	Application Identifier		c. Previous Grants.gov Tracking Number GRANT14520762
5. APPLICANT INFORMATION			UEI*: G88KLJR3KYT5
Legal Name*: University of Southern California			
Department:			
Division:			
Street1*: Department of Contracts and Grants			
Street2*: 3720 South Flower Street			
City*: Los Angeles			
County:			
State*: CA: California			
Province:			
Country*: USA: UNITED STATES			
ZIP / Postal Code*: 90089-0701			
Person to be contacted on matters involving this application			
Prefix:	First Name*: Caitlin	Middle Name:	Last Name*: Roberts      Suffix:
Position/Title: Contracts and Grants Officer			
Street1*: 3720 South Flower Street			
Street2*: CUB 303			
City*: Los Angeles			
County: Los Angeles			
State*: CA: California			
Province:			
Country*: USA: UNITED STATES			
ZIP / Postal Code*: 90089-0701			
Phone Number*: 2138216819		Fax Number:      Email: cr14685@usc.edu	
6. EMPLOYER IDENTIFICATION NUMBER (EIN) or (TIN)*		1-951642394-A1	
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 checked="" type="radio"/> New <input type="radio"/> Resubmission		<input type="radio"/> A. Increase Award <input type="radio"/> B. Decrease Award <input type="radio"/> C. Increase Duration	
<input type="radio"/> Renewal <input type="radio"/> Continuation <input type="radio"/> Revision		<input type="radio"/> D. Decrease Duration <input type="radio"/> E. Other (specify):	
Is this application being submitted to other agencies?* <input type="radio"/> Yes <input checked="" type="radio"/> No      What other Agencies?			
9. NAME OF FEDERAL AGENCY* National Institutes of Health		10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER TITLE:	
11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT* Probing the Role of a Succinate-Related Metabolic Reprogram in Periodontal Pathogenesis			
12. PROPOSED PROJECT Start Date*      Ending Date* 07/01/2026      06/30/2031		13. CONGRESSIONAL DISTRICTS OF APPLICANT CA-037	

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

Prefix: First Name\*: Mirali Middle Name: Last Name\*: Pandya Suffix:

Position/Title: Assistant Professor of Clinical Dentistry

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**15. ESTIMATED PROJECT FUNDING**

a. Total Federal Funds Requested\* \$674,980.00

b. Total Non-Federal Funds\* \$0.00

c. Total Federal & Non-Federal Funds\* \$674,980.00

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\*: Caitlin Middle Name: Last Name\*: Roberts Suffix:

Position/Title\*: Contracts and Grants Officer

Organization Name\*: University of Southern California

Department: Office of Research Contracts a

Division: Office of Research (Level 5)

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City\*: Los Angeles

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Country\*: USA: UNITED STATES

ZIP / Postal Code\*: 90089-0701

Phone Number\*: 2138216819 Fax Number: Email\*: cr14685@usc.edu

**Signature of Authorized Representative\***

Caitlin Roberts

**Date Signed\***

10/13/2025

**20. PRE-APPLICATION** File Name:**21. COVER LETTER ATTACHMENT** File Name: Cover\_Letter\_K08\_Final1052469828.pdf

## 424 R&amp;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: University of Southern California  
UEI: G88KLJR3KYT5  
Street1\*: 925 W. 34th Street  
Street2: DEN 4107  
City\*: Los Angeles  
County:  
State\*: CA: California  
Province:  
Country\*: USA: UNITED STATES  
Zip / Postal Code\*: 90089-0641  
Project/Performance Site Congressional District\*: CA-037

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**Additional Location(s)**

File Name:

**RESEARCH & RELATED Other Project Information**

<b>1. Are Human Subjects Involved?*</b> <input checked="" type="radio"/> Yes <input type="radio"/> No	
1.a. If YES to Human Subjects	
Is the Project Exempt from Federal regulations? <input type="radio"/> Yes <input checked="" type="radio"/> No	
If YES, check appropriate exemption number: <input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3 <input type="radio"/> 4 <input type="radio"/> 5 <input type="radio"/> 6 <input type="radio"/> 7 <input type="radio"/> 8	
If NO, is the IRB review Pending? <input type="radio"/> Yes <input checked="" type="radio"/> No	
IRB Approval Date:    02-01-2019	
Human Subject Assurance Number    00005906	
<b>2. Are Vertebrate Animals Used?*</b> <input type="radio"/> Yes <input checked="" type="radio"/> No	
2.a. If YES to Vertebrate Animals	
Is the IACUC review Pending? <input type="radio"/> Yes <input type="radio"/> No	
IACUC Approval Date:	
Animal Welfare Assurance Number	
<b>3. Is proprietary/privileged information included in the application?*</b> <input type="radio"/> Yes <input checked="" type="radio"/> No	
<b>4.a. Does this project have an actual or potential impact - positive or negative - on the environment?*</b> <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:	
<b>5. Is the research performance site designated, or eligible to be designated, as a historic place?*</b> <input type="radio"/> Yes <input checked="" type="radio"/> No	
5.a. If yes, please explain:	
<b>6. Does this project involve activities outside the United States or partnership with international collaborators?*</b> <input type="radio"/> Yes <input checked="" type="radio"/> No	
6.a. If yes, identify countries:	
6.b. Optional Explanation:	
<b>7. Project Summary/Abstract*</b>	Filename Abstract_Final1052469786.pdf
<b>8. Project Narrative*</b>	ProjectNarrative1052470118.pdf
<b>9. Bibliography &amp; References Cited</b>	Bibliography1052469781.pdf
<b>10. Facilities &amp; Other Resources</b>	Facilities1052469790.pdf
<b>11. Equipment</b>	Major_Equipment_Final1052469816.pdf

## Abstract

As a major mediator of inflammation and tissue destruction, succinate's role as a key component of metabolic reprogramming in periodontitis has recently emerged[1, 2]. Periodontitis is a chronic inflammatory disease that affects almost half of the adult population over the age of 65[3] and is associated with serious systemic issues such as diabetes[4] and cardiovascular diseases[5] likely connected due to chronic systemic inflammation. Nevertheless, there are still important unknowns about the biomarker potential of succinate, its relationship to mitochondrial dysfunction, and its effects on inflammation that are distinct to individual cells. Establishing succinate's function in the periodontal pathogenesis while facilitating the transition from mentored training to an independent clinician-scientist in the field of periodontal metabolomics is the goal of this K08 project. This study will build upon our preliminary findings from nine patient samples demonstrating approximately 2-fold succinate elevation and mitochondrial complex I reductions in gingival tissues from patients with periodontitis. Through integrated proteomics (LC-MS/MS) and metabolomics analyses of gingival tissue, crevicular fluid, and saliva from 105 participants (30 healthy, 75 diseased), aim 1 will assess the potential of succinate as a noninvasive biomarker for periodontal disease severity and progression. We will use a multivariate correlation model to correlate protein and metabolite levels with the clinical parameters such as probing depths, clinical attachment loss and bleeding on probing. By using *ex vivo* cocultures of human oral keratinocytes with *Porphyromonas gingivalis* or *Aggregatibacter actinomycetemcomitans*, aim 2 will examine the causal role of mitochondrial complex I downregulation in succinate accumulation. This will be accomplished through the use of seahorse metabolic profiling, rescue experiments with rotenone and dimethyl malonate to probe inflammatory cytokines via qPCR/ELISA, and shRNA knockdown of NDUFS8, NDUFB1, and NDUFA12 to mimic mitochondrial complex I downregulation. By using single-cell RNA sequencing on patient tissues, comparing with succinate levels, and validating by SDH modulation in an *ex vivo* coculture model and sorted immune cell subsets, Aim 3 will ascertain the inflammatory contributions of succinate while integrating multi-omics for pathway mapping. Predicted outcomes include confirming succinate as a non-invasive biomarker, achieve mechanistic insights into succinate-driven inflammation, and identifying cell-specific targets like SUCNR1 for potential therapy for future research. We believe this work aligns well with NIDCR priorities on oral health disparities, while allowing me foster skills in multi-omics and bioinformatics for future R01 independence.

## **Project Narrative**

This K08 project highlights succinate's pivotal role in periodontal pathogenesis, a disease impacting ~ 50% of adults and tied to multiple systemic diseases, by addressing gaps in its biomarker utility, mitochondrial-driven accumulation, and unexplored inflammatory mechanisms through integrated multi-omics approach and single cell RNA sequencing.

## References:

1. Guo, Y., et al., Targeting the succinate receptor effectively inhibits periodontitis. *Cell Rep*, 2022. 40(12): p. 111389.
2. Xu, F., et al., Succinate modulates oral dysbiosis and inflammation through a succinate receptor 1 dependent mechanism in aged mice. *Int J Oral Sci*, 2025. 17(1): p. 47.
3. Eke, P.I., et al., Prevalence of periodontitis in adults in the United States: 2009 and 2010. *J Dent Res*, 2012. 91(10): p. 914-20.
4. Mealey, B.L. and T.W. Oates, Diabetes mellitus and periodontal diseases. *J Periodontol*, 2006. 77(8): p. 1289-303.
5. Febbraio, M., C.B. Roy, and L. Levin, Is There a Causal Link Between Periodontitis and Cardiovascular Disease? A Concise Review of Recent Findings. *International Dental Journal*, 2022. 72(1): p. 37-51.
6. Martínez-García, M. and E. Hernández-Lemus, Periodontal Inflammation and Systemic Diseases: An Overview. *Front Physiol*, 2021. 12: p. 709438.
7. Hajishengallis, G. and T. Chavakis, Local and systemic mechanisms linking periodontal disease and inflammatory comorbidities. *Nature Reviews Immunology*, 2021. 21(7): p. 426-440.
8. Pirih, F.Q., et al., Association between metabolic syndrome and periodontitis: The role of lipids, inflammatory cytokines, altered host response, and the microbiome. *Periodontol* 2000, 2021. 87(1): p. 50-75.
9. Zhang, Y., et al., Immunometabolic rewiring in macrophages for periodontitis treatment via nanoquercetin-mediated leverage of glycolysis and OXPHOS. *Acta Pharmaceutica Sinica B*, 2024. 14(11): p. 5026-5036.
10. Zhang, Z., et al., Unraveling the Complex Nexus of Macrophage Metabolism, Periodontitis, and Associated Comorbidities. *J Innate Immun*, 2025. 17(1): p. 211-225.
11. Zhang, W. and R. Lang, Succinate metabolism: a promising therapeutic target for inflammation, ischemia/reperfusion injury and cancer. *Front Cell Dev Biol*, 2023. 11: p. 1266973.
12. Harber, K.J., et al., Succinate Is an Inflammation-Induced Immunoregulatory Metabolite in Macrophages. *Metabolites*, 2020. 10(9).
13. Zhang, Y., et al., Succinate accumulation induces mitochondrial reactive oxygen species generation and promotes status epilepticus in the kainic acid rat model. *Redox Biol*, 2020. 28: p. 101365.
14. Tannahill, G.á., et al., Succinate is an inflammatory signal that induces IL-1 $\beta$  through HIF-1 $\alpha$ . *Nature*, 2013. 496(7444): p. 238-242.
15. Huang, H., et al., Cellular succinate metabolism and signaling in inflammation: implications for therapeutic intervention. *Front Immunol*, 2024. 15: p. 1404441.
16. Mills, E. and L.A.J. O'Neill, Succinate: a metabolic signal in inflammation. *Trends in Cell Biology*, 2014. 24(5): p. 313-320.

17. Papapanou, P.N., Periodontal diseases: epidemiology. *Ann Periodontol*, 1996. 1(1): p. 1-36.
18. Tonetti, M.S., H. Greenwell, and K.S. Kornman, Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. *J Periodontol*, 2018. 89 Suppl 1: p. S159-s172.
19. Hajishengallis, G., R.P. Darveau, and M.A. Curtis, The keystone-pathogen hypothesis. *Nat Rev Microbiol*, 2012. 10(10): p. 717-25.
20. Pussinen, P.J., et al., Systemic exposure to *Porphyromonas gingivalis* predicts incident stroke. *Atherosclerosis*, 2007. 193(1): p. 222-228.
21. Shiheido, Y., et al., *Porphyromonas gingivalis*, a periodontal pathogen, enhances myocardial vulnerability, thereby promoting post-infarct cardiac rupture. *Journal of Molecular and Cellular Cardiology*, 2016. 99: p. 123-137.
22. Kang, N., et al., Periodontitis induced by *Porphyromonas gingivalis* drives impaired glucose metabolism in mice. *Front Cell Infect Microbiol*, 2022. 12: p. 998600.
23. Ryder, M.I., *Porphyromonas gingivalis* and Alzheimer disease: Recent findings and potential therapies. *J Periodontol*, 2020. 91 Suppl 1(Suppl 1): p. S45-s49.
24. Li, Y., et al., The Relationship Between *Porphyromonas Gingivalis* and Rheumatoid Arthritis: A Meta-Analysis. *Front Cell Infect Microbiol*, 2022. 12: p. 956417.
25. Arigbede, A.O., B.O. Babatope, and M.K. Bamidele, Periodontitis and systemic diseases: A literature review. *J Indian Soc Periodontol*, 2012. 16(4): p. 487-91.
26. Ishihara, C., et al., Involvement of propionate, citrulline, homoserine, and succinate in oral microbiome metabolite-driven periodontal disease progression. *Scientific Reports*, 2025. 15(1): p. 7149.
27. Collins, N.L., F. Van den Bergh, and D.A. Beard, Causes and consequences of succinate accumulation during cardiac ischemia. *Biophysical Journal*, 2023. 122(3): p. 303a.
28. Xiao, M., et al., Inhibition of  $\alpha$ -KG-dependent histone and DNA demethylases by fumarate and succinate that are accumulated in mutations of FH and SDH tumor suppressors. *Genes Dev*, 2012. 26(12): p. 1326-38.
29. Kim, Y.G., et al., Transcriptome sequencing of gingival biopsies from chronic periodontitis patients reveals novel gene expression and splicing patterns. *Hum Genomics*, 2016. 10(1): p. 28.
30. Lundmark, A., et al., Gene expression profiling of periodontitis-affected gingival tissue by spatial transcriptomics. *Sci Rep*, 2018. 8(1): p. 9370.
31. Chen, Y., et al., Single-cell RNA landscape of the osteoimmunology microenvironment in periodontitis. *Theranostics*, 2022. 12(3): p. 1074-1096.
32. Alghamdi, B., et al., Single-cell RNA profiling identifies immune cell population shifts in diabetes associated mucosal inflammation. *Mucosal Immunology*, 2025. 18(5): p. 1082-1097.
33. Lee, H., et al., Single-cell RNA sequencing reveals rebalancing of immunological response in patients with periodontitis after non-surgical periodontal therapy. *Journal of Translational Medicine*, 2022. 20(1): p. 504.

34. Park, N.-H., et al., Immortalization of normal human oral keratinocytes with type 16 human papillomavirus. *Carcinogenesis*, 1991. 12(9): p. 1627-1631.
35. Zhao, J., et al., Deamidation Shunts RelA from Mediating Inflammation to Aerobic Glycolysis. *Cell Metab*, 2020. 31(5): p. 937-955.e7.
36. Qin, C., et al., SARS-CoV-2 couples evasion of inflammatory response to activated nucleotide synthesis. *Proceedings of the National Academy of Sciences*, 2022. 119(26): p. e2122897119.
37. Rao, Y., et al., Pyrimidine synthesis enzyme CTP synthetase 1 suppresses antiviral interferon induction by deamidating IRF3. *Immunity*, 2025. 58(1): p. 74-89.e6.
38. Feng, S., et al., Cryptic phosphoribosylase activity of NAMPT restricts the virion incorporation of viral proteins. *Nature Metabolism*, 2024. 6(12): p. 2300-2318.
39. Massa, D., et al., PYHIN1 regulates pro-inflammatory cytokine induction rather than innate immune DNA sensing in airway epithelial cells. *Journal of Biological Chemistry*, 2020. 295(14): p. 4438-4450.
40. Sari, A., P. Santamaria, and L. Nibali, The effect of non-surgical periodontal treatment on progranulin levels. *J Periodontol*, 2025.

## **Facilities and Other Resources**

### **Laboratory**

Research will be conducted in laboratories on the 4th floor of the Norris Dental Science Building.

- **Dr. Feng's laboratories** (Rooms 4101, 4103, 4105, 4111, 4111A, 4114) encompass ~3,000 sq. ft., with bench space for 25 researchers, a 400 sq. ft. BSL2+ tissue culture suite, a walk-in cold room, and a walk-in freezer for storage and refrigerated experiments.
- **Dr. Chen's assigned laboratory** (~1,000 sq. ft.) and access to ~4,000 sq. ft. of shared space support tissue culture, aerobic/anaerobic microbiology, molecular biology, cell biology, and protein chemistry research.

Available instrumentation includes: FACScalibur flow cytometer, iCycler iQ Real-Time PCR system, multiple thermocyclers, confocal and fluorescence microscopes, Leica dissecting microscopes, Zeiss Axioplan 2 microscope with epifluorescence, histology equipment, freezers, incubators, spectrophotometers, and densitometers. Shared equipment also includes an Olympus FV101-VU confocal system and core-access imaging technologies.

### **Animals**

The Irani Building at USC provides state-of-the-art animal facilities. The investigators have assigned space for 200 cages of mice and access to a BSL2 suite (100-cage capacity) for microbial/viral infection experiments, supported by veterinary and technical staff.

### **Clinical Facilities**

The Ostrow School of Dentistry clinic facilities include over 140 chairs, with 32 dedicated to the Predoctoral Periodontics program and 12 to the Advanced Periodontics program. The School admits and provides care to over 4,000 new patients annually. These fully staffed facilities support research efforts in clinical periodontics.

### **Computational and Bioinformatics Resources**

Drs. Feng and Chen's groups have access to multiple personal computers and servers equipped with software for data management, word processing, statistical analysis, nucleic acid/protein sequence analysis, PCR primer design, densitometry, morphometric analysis, and image analysis. All systems are networked with high-speed internet, secure storage, and central computing facilities.

Through USC Libraries Bioinformatics Service Program (Norris Medical Library), investigators also have full access to advanced bioinformatics software and hardware resources.

### **Core Facilities and Other Resources**

The Ostrow School of Dentistry and CCMB provide access to advanced core facilities and shared equipment, including:

- **Molecular and Imaging:** Flow cytometry, confocal microscopy, electron microscopy, live-cell imaging, pathology analysis, micro-CT scanner, micro-MRI, microarray analysis.
- **Molecular Biology:** DNA sequencing, mass spectrometry, antibody production, transgenic mouse facility, shRNA and expression libraries.
- **Histology & Tissue:** Primary oral tissue culture, histology-pathology cores, organ culture facilities.



- Equipment: Ultracentrifuges, PCR thermocyclers, spectrophotometers, scintillation counters, HPLC, laminar flow hoods, and cold storage facilities.

### **Administrative and Office Resources**

Both investigators have personal office space in close proximity to their laboratories, with shared office areas for students and postdoctoral fellows. Administrative support is available for grant submissions, manuscript preparation, ordering, and financial management.

## **EQUIPMENT**

### **DR. PINGHUI FENG'S LAB:**

The laboratory of the mentor Dr. Pinghui Feng is equipped to perform modern molecular virology, genetics, cell biology, and cell culture, including three double-decked CO<sub>2</sub> incubators, five biosafety cabinets for cell culture, one Nikon eclipse Ti2 fluorescence microscope with CCD camera, multiple PCR machines, a gel documentation system, multiple minigel systems for protein electrophoresis, two IPGphor isoelectric focusing units, an I-26 Incubator shaker system, a Sorvall RC-6 refrigerated high speed centrifuge, an Optima XPN-80 ultracentrifuge, a BIO-RAD real-time PCR machine, a LI-COR Odyssey Infrared system, a FLUOStar Omega multi-wave length plate reader, six semidry transfer units, two Allegra X-14R table centrifuge, various power supplies and DNA electrophoresis apparatuses. The department has common warm rooms, cold rooms, and dark rooms, etc. Additionally, the Herman Ostrow School of Dentistry also provides equipments such as Phosphoimager, Ultracentrifuge, Nondrop spectrophotometer, multiple fluorescent microscopes and one live cell imaging system, FACS and sorting, an extraordinary shRNA library and mammalian gene expression library, and scintillation counter.

The Feng laboratory also has an inhouse mass spectrometer (Q-Executive, Orbitrap, Thermo Fisher) that is used for both protein and metabolite analyses. Additionally, instruments for histological analysis, including a cryostat (CM3050 S, Leica) and HistoCore (Leica), are available in the Feng lab. One table-top ultracentrifuge (Optima- MX TL, Beckman Coulter) is available for viral centrifugation using small volume (<2.0 ml).

### **DR. CASEY CHEN'S LAB:**

For the mentor Dr. Chen's lab, available on a shared basis are the contiguous facilities including ultra-low 80°C freezers, Milli-Q Plus UF and Milli-RO ultrapure water. Beckman L-80 ultracentrifuge, Sorvall RC-5B centrifuge, Beckman DU-650 spectrophotometer, and Beckmann Beta and Gamma liquid scintillation spectrometers. A fully equipped facility for washing and sterilization is available as well as walk-in cold room and darkroom facilities including a Kodak automatic film processor. Also available on a fee-for-service basis are the following University operated facilities and/or services: oligonucleotide synthesis, and protein and nucleic acid sequence determination. Dr. Chen also has the culture hood for the bacterial coculture experiments, incubator for bacterial growth and bench space for all bacteria related experiments.

Researchers also share an administrative manager dedicated to help in grant submissions, production of manuscripts and presentation materials, and assistance in ordering and accounting on grant accounts. Additional help is available from the administrative office of the Division of Periodontology, Diagnostic Sciences and Dental Hygiene

**RESEARCH & RELATED Senior/Key Person Profile (Expanded)**

PROFILE - Project Director/Principal Investigator				
Prefix:	First Name*: Mirali	Middle Name	Last Name*: Pandya	Suffix:
Position/Title*:	Assistant Professor of Clinical Dentistry			
Organization Name*:	University of Southern California			
Department:	Department of Endodontics and Periodontics			
Division:				
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Street2:				
City*:	Los Angeles			
County:				
State*:	CA: California			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	90089-0641			
Phone Number*:	972 408-7673	Fax Number:		
E-Mail*:	miralipa@usc.edu			
Credential, e.g., agency login: MPANDYA				
Project Role*:	PD/PI	Other Project Role Category:		
Degree Type:	PhD	Degree Year:	2019	
<b>Attach Biographical Sketch*:</b>	File Name:	Mirali_NIH_Biosketch1052469782.pdf		
<b>Attach Current &amp; Pending Support:</b>	File Name:			

PROFILE - Senior/Key Person				
Prefix: Dr.	First Name*: Casey	Middle Name	Last Name*: Chen	Suffix:
Position/Title*:	"Professor and Division Chair, Periodontology			
Organization Name*:	University of Southern California			
Department:	Academic Leadership			
Division:	DEN Leadership and Administrat			
Street1*:	925 West 34Th St.			
Street2:				
City*:	Los Angeles			
County:	Los Angeles			
State*:	CA: California			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	90089-0000			
Phone Number*: +1 213 740 1075		Fax Number: +1 213 740 6778		
E-Mail*: ccchen@usc.edu				
Credential, e.g., agency login: ccchen				
Project Role*: Other (Specify)		Other Project Role Category: Mentor		
Degree Type: Ph.D.		Degree Year: 1990		
<b>Attach Biographical Sketch*:</b>	File Name:	BIO_CHEN_C_1008251052469871.pdf		
<b>Attach Current &amp; Pending Support:</b>	File Name:	other_support2025_CC1052470096.pdf		

PROFILE - Senior/Key Person				
Prefix: Dr.	First Name*: Pinghui	Middle Name	Last Name*: Feng	Suffix:
Position/Title*:	Professor			
Organization Name*:	University of Southern California			
Department:	Infection and Immunity			
Division:	Perio-Diagnostic Sciences-DH			
Street1*:	925 W 34th St			
Street2:	DEN 4108			
City*:	Los Angeles			
County:	Los Angeles			
State*:	CA: California			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	90089-0641			
Phone Number*: 16262486116		Fax Number:		
E-Mail*: pinghuif@usc.edu				
Credential, e.g., agency login: PHFENG				
Project Role*: Other (Specify)		Other Project Role Category: Co-Mentor		
Degree Type: PhD		Degree Year: 2001		
<b>Attach Biographical Sketch*:</b>	File Name:	biosketch_Feng_2025_1052469787.pdf		
<b>Attach Current &amp; Pending Support:</b>	File Name:	other_support_Feng1052470097.pdf		

PROFILE - Senior/Key Person				
Prefix:	First Name*: De-Chen	Middle Name	Last Name*: Lin	Suffix:
Position/Title*:	"Assistant Professor"			
Organization Name*:	University of Southern California			
Department:	Lab for Developmental Biology			
Division:				
Street1*:	2250 Alcazar St.			
Street2:				
City*:	Los Angeles			
County:				
State*:	CA: California			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	90033-9062			
Phone Number*: (323) 442-3170	Fax Number:			
E-Mail*: dechenli@usc.edu				
Credential, e.g., agency login: DECHENLIN				
Project Role*: Other (Specify)		Other Project Role Category: Advisory Committee		
Degree Type: PhD		Degree Year: 2010		
<b>Attach Biographical Sketch*:</b>	File Name:	Bios_Lin_PI1052469954.pdf		
<b>Attach Current &amp; Pending Support:</b>	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Chao	Middle Name	Last Name*: Qin	Suffix:
Position/Title*:	Research Assistant Professor of Dentistry			
Organization Name*:	University of Southern California			
Department:	Infection and Immunity			
Division:	Perio-Diagnostic Sciences-DH			
Street1*:	925 W. 34th Street			
Street2:	DEN 4109			
City*:	Los Angeles			
County:				
State*:	CA: California			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	90089-0641			
Phone Number*: 213 608-7208	Fax Number:			
E-Mail*: chaochin@usc.edu				
Credential, e.g., agency login: qinchao				
Project Role*: Other (Specify)		Other Project Role Category: Collaborator		
Degree Type: PhD		Degree Year: 2019		
<b>Attach Biographical Sketch*:</b>	File Name:	Biosketch_Chao_v1052469955.pdf		
<b>Attach Current &amp; Pending Support:</b>	File Name:			

**BIOGRAPHICAL SKETCH**

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

NAME: Mirali Pandya

eRA COMMONS USER NAME : MPANDYA

POSITION TITLE: Assistant Professor of Clinical Dentistry

**EDUCATION/TRAINING**

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Mauras College of Dentistry / Maharaja Krishnakumarsinhji Bhavnagar University, India	BDS	05/2012	Dentistry
Texas A&M College of Dentistry, Texas, USA	PhD	08/2019	Oral Biology
Texas A&M College of Dentistry, Texas, USA	MS	05/2024	Oral Biology
Texas A&M College of Dentistry, Texas, USA	Residency	05/2024	Periodontology and Implantology

**A. Personal Statement**

I am a clinician-scientist with specialty training in periodontics and a strong commitment to advancing oral health through translational research that bridges basic science with clinical applications in periodontal diseases. My passion for oral biology began during my dental school where my rigorous dental training crystallized my firm grasp of the functional, structural and molecular aspects of the head and neck region including their interactions to maintain the integrity of orofacial complex. After graduation, I practiced briefly as a dental surgeon with a focus on treating rare oral disease. Subsequently, motivated by a desire to systematically investigate oral phenomena, I concentrated on gaining diverse research experience while establishing manifold and global scientific collaborations. At National University of Singapore College of Dentistry, I investigated novel hydrogels and dental pulp stem cells to discover innovative ways for dental pulp regeneration. After perfecting my stem cell culture and tissue engineering skills, I continued my research journey as a PhD student at Texas A&M College of Dentistry under the able guidance of Dr. Thomas Diekwisch. During PhD, my research focused on investigating the intricate mechanisms underlying tooth enamel formation and the role of amelogenin fragments in the organization of enamel prism structure. In addition, I pursued developing the first successful Clathrin mouse model deposited in the MMRRC repository for the research community to further investigate under a UG3 grant awarded to my mentor, and novel 3D systems, which would be a critical milestone in enamel research over 2D culture system shortcomings, to culture ameloblast cells using a combination of multiple growth factors and scaffolds.

Till date, I have published nine first author papers in high impact factor journals and eleven co-author papers, a JOVE protocol for the bioreactor cell culture, and several first author publications currently in the pipeline. In addition, during my role as a clinical assistant professor in the Department of Periodontics at Texas A&M from 2019 to 2021, I guided several periodontics residents to conceptualize and conduct experiments for key clinical research projects, including testing novel scaffolds for alveolar ridge augmentation, understanding the causes of peri-implantitis related implant failures, exploring the role of the novel hippo pathway in periodontium and investigating the complexities of temporomandibular joint disorders which has further developed my understanding for the need of oral disease diagnosis, treatment advancement and innovation in the field of Periodontics.

I firmly think of my PhD, my Clinical Assistant Professor experience, and the Periodontics specialty training as an important integration of science, clinical expertise, and technology for alleviation of human suffering and am confident that it has strongly positioned me for a rewarding career in an academic setting as a Periodontist who can treat a diverse patient population, participate in an interdisciplinary care approach, and contribute to the knowledge base through research. Currently, as a full time Assistant Professor of Clinical Dentistry at University of Southern California, I have 50% of my time dedicated to teaching and clinical appointment in the Department of Periodontics and I dedicate the remaining time to research, supported currently by Dr. Casey Chen and Dr. Pinghui Feng. Furthermore, I am convinced that the dynamic environment at USC has presented me the opportunity to collaborate with health professionals within and outside the school of dentistry in the prevention, diagnosis, evaluation and treatment of different periodontal disease and conditions, viral pathogenesis, microbiology, diagnostic biomarkers and explore the wonderful science for further betterment of the dental patients. My prior experiences have equipped me with great clinical expertise and foundational research skills in oral biology. I became board-certified Periodontist in 2025, and plan to advance my training in multi-omics, molecular techniques and bioinformatics to transition fully to an independent investigator role addressing the translational gaps in periodontal therapy

## B. Positions, Scientific Appointments, and Honors

### **Positions:**

2025– Present	Board Certification in Periodontology and Implantology by American Board of Periodontology
2024– Present	Assistant Professor of Clinical Dentistry, Department of Endodontics and Periodontics, University of Southern California, Los Angeles, CA
2021 – 2024	Periodontics Resident/Graduate Teaching Assistant, Department of Advanced Periodontics, Texas A&M College of Dentistry, Dallas, TX
2019 – 2021	Clinical Assistant Professor, Department of Periodontics, Texas A&M College of Dentistry, Dallas, TX
2015 – 2019	Graduate Research Assistant, PhD in Oral Biology, Department of Biomedical Sciences, Texas A&M College of Dentistry, Dallas, TX

### **Professional Appointments and Honors:**

- American Board of Periodontology, Diplomate
- American Academy of Periodontology, Member
- International Association for Dental Research, Member
- American Association for Dental and Craniofacial Research, Member
- American Dental Association, Member
- California Dental Association, Member
- Los Angeles County Dental Society, Member
- First place for oral presentation in graduate category at the Research day (April 2018) at Texas A&M College of Dentistry.

## C. Contributions to Science

**Current Citations - 796; I10 index – 16; H index – 14**

1. **To investigate the mechanisms underlying enamel prism formation and the transportation of mineral ions during amelogenesis:** During my PhD and following immediately after, I developed a novel *invitro* model system for understanding tooth enamel formation using 3D culture system, paving the path for future enamel research
  - Diekwisch TG, Zhang Y, Jin T, Zhu W, Pandya M, Gopinath G, Allen MJ, Reed DA, Keiderling T, Liao X. (2022). Highly acidic pH facilitates enamel protein self-assembly, apatite crystal growth and enamel protein interactions during amelogenesis. **Frontiers in Physiology**.:2550.

- Pandya, M. and Diekwisch, T.G., (2021). Amelogenesis: Transformation of a protein-mineral matrix into tooth enamel. **Journal of Structural Biology**, 213(4), p.107809.
  - Pandya, M., Ma, W., Lyu, H., Luan, X., Diekwisch, T. G. H. (2021) Propagation of Dental and Respiratory Cells and Organs in Microgravity. **J. Vis. Exp.** (171), e62690, doi:10.3791/62690.
  - Pandya, M., Lyu, H., Luan, X., & Diekwisch, T. G. (2021). Polarized, amelogenin expressing ameloblast-like cells from cervical loop/dental pulp co-cultures in bioreactors. **Stem Cells and Development**, (ja).
  - Pandya M. and Diekwisch, TGH., (2019). Enamel Biomimetics – fiction or future of dentistry. **International Journal of Oral sciences**.
  - Pandya, M., Lin, T., Li, L., Allen, M., Jin, T., Luan, X., and Diekwisch, T.G.H. (2017). Posttranslational amelogenin processing and changes in matrix assembly during enamel development. **Front. Physiol.**
  - Pandya, M., Liu, H., Dangaria S.J., Zhu, W., Li, L.L. Pan, S., Abufarwa, M., Davis, R.G., Guggenheim, S., Keiderling, T., Luan, X., and Diekwisch, T.G.H. (2017). Integrative temporo-spatial, mineralogic, spectroscopic, and proteomic analysis of postnatal enamel development in teeth with limited growth. **Front. Physiol.**
  - Pandya, M., Rosene, L., Farquharson C., Millán, J.L., and Diekwisch, T.G.H. (2017). Intravesicular phosphatase PHOSPHO1 function in enamel mineralization and prism formation. **Front. Physiol.**
  - Liu, H., Yan, X., Pandya, M., Luan, X., and Diekwisch, T.G.H. (2016). Daughters of the Enamel Organ: Development, Fate, and Function of the Stratum Intermedium, Stellate Reticulum, and Outer Enamel Epithelium. **Stem Cells Dev.**
- 2. Exploring the MicroRNAs, homeostasis, role of Hippo pathway in Periodontal Pathogenesis and Peri-implantitis:** As a periodontist, exploring the epigenetics aspect of periodontal disease and exploring the role of Hippo pathway in periodontal homeostasis is the line of research I would continue to develop and also add experience in multi-omics which will play an important role in precision medicine, the future of oral healthcare
- Pandya M, Gopinathan G, Tillberg C, Wang J, Luan X, Diekwisch TGH. (2022). The Hippo Pathway Effectors YAP/TAZ Are Essential for Mineralized Tissue Homeostasis in the Alveolar Bone/Periodontal Complex; **Journal of Developmental Biology**; 10(1):14.
  - Lyu, H., Zhou, X., Qian, Y., Liu, X., Gopinathan, G., Pandya, M., Qin, C., Luan, X. and Diekwisch, T.G., (2022). Long-acting PFI-2 small molecule release and multilayer scaffold design achieve extensive new formation of complex periodontal tissues with unprecedented fidelity. **Biomaterials**, 290, p.121819.
  - Luan X, Zhou X, Fallah P, Pandya M, Lyu H, Foyle D, Burch D, and Diekwisch TGH (2021). MicroRNAs: Harbingers and shapers of periodontal inflammation. **Seminars in Cell & Developmental Biology**. Academic Press
  - Francis M., Pandya M., Gopinathan G., Lyu H., Ma W., Foyle D., Nares S., and Luan X. (2019). Histone methylation mechanisms modulate the inflammatory response of periodontal ligament progenitors. **Stem Cells Dev.**
  - Harrel SK., Wilson Jr TG., Pandya M., Diekwisch, TGH. (2018). Titanium particles generated during ultrasonic scaling of implants. **J Periodontol**.
- 3. Tissue Engineering and 3D printing:** I have an active interest in translational research that would provide cost effective novel solutions for patient oral healthcare.
- Ma W, Lyu H, Pandya M, Gopinathan G, Luan X, Diekwisch TGH (2021) Successful Application of a Galanin-Coated Scaffold for Periodontal Regeneration. **Journal of Dental Research**;100(10):1144-1152.
  - Pandya, M., Saxon, M, Bozanich, J, Tillberg, C, Luan, X, & Diekwisch, TGH. (2021). The Glycoprotein/Cytokine Erythropoietin Promotes Rapid Alveolar Ridge Regeneration In Vivo by Promoting New Bone Extracellular Matrix Deposition in Conjunction with Coupled Angiogenesis/Osteogenesis. **International Journal of Molecular Sciences.**, 22(6), 2788.
  - Isaac, A.; Jivan, F.; Xin, S.; Hardin, J.; Luan, X.; Pandya, M.; Diekwisch, T. G. H.; Alge, D. L. (2019) Microporous Bio-Orthogonally Annealed Particle Hydrogels for Tissue Engineering and Regenerative Medicine. **ACS Biomater. Sci. Eng.**, 5, 6395–6404. doi: 10.1021/acsbiomaterials.9b01205



- Maschio, F., Pandya, M., and Olszewski, R. (2016). Experimental Validation of Plastic Mandible Models Produced by a "Low-Cost" 3-Dimensional Fused Deposition Modeling Printer. **Med Sci Monit.**
- Yap, A.U., Pandya, M., and Toh, W.S. (2016). Depth of cure of contemporary bulk-fill resin-based composites. **Dent Mater J.**
- Lu, Q., Pandya, M., Rufaihah, A.J., Rosa, V., Tong, H.J., Seliktar, D., and Toh, W.S. (2015). Modulation of Dental Pulp Stem Cell Odontogenesis in a Tunable PEG-Fibrinogen Hydrogel System. **Stem Cells Int.**

**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: Chen, Casey

eRA COMMONS USER NAME: ccchen

POSITION TITLE: Professor

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
National Taiwan University, Taiwan	B.D.S.	05/1980	Dentistry
University of Pittsburgh, Pittsburgh, PA	Certificate	05/1984	Periodontology
State Univ. of New York at Buffalo, Buffalo, NY	Ph.D.	06/1990	Oral Biology
Loma Linda University, Loma Linda, CA	D.D.S.	06/1992	Dentistry

**A. Personal Statement**

I am a dentist with advanced training as a periodontist and have pursued related microbiology research themes. My research interests include microbial diagnosis, genomics, and metagenomics of periodontal bacteria, microbial markers for periodontitis, virulence determinants of periodontal pathogenic species, and clinical periodontics. I have published manuscripts that involve the microbial etiology of periodontitis, the detection of oral bacteria and viruses, the oral microbiome concerning periodontal health and disease, periodontal therapy, antimicrobial assays, and the host response to periodontitis. As a department chair, I am mindful of developing talented faculty for our future. The school is fortunate to recruit Dr. Mirali Pandya, a dual-degree clinician-scientist. We are committed to providing support to Mirali's career development. I will provide my expertise in three areas: 1) periodontal microbiology, 2) clinical periodontics, and 3) career advancement in academia. The research plan will test the hypothesis that succinate accumulation contributes to periodontitis pathogenesis by impairing mitochondrial electron transport and immune regulation. We have assembled a team of mentors with complementary skills to guide Mirali in completing the research plan. As a department chair and the Associate Dean of Applied Biomedical and Clinical Sciences, I will provide general support for all aspects of the research plan. I have mentored many clinicians and scientists in the past 30 years, and I will be able to guide Mirali in her growth to be an independent clinician-scientist.

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

R01 AG070904-01A2 (Feng)

07/01/2021-06/30/2026

NIH/NIA

Explore roles of HSV-1 in Alzheimer's disease using mouse models

To investigate how aging and herpesvirus infection interact in normal and AD model mouse, and how aging and herpesvirus infection (as an environmental factor) may synergize to induce neurodegeneration.

Role: Co-Investigator

R01 DE031307-02 (Jokerst)

09/01/2021-08/31/2026

NIDCR

A Miniaturized and High-frequency Acoustic Imaging System for Oral Health and Diseases of The Head and Neck

The applicant proposes the development of a miniaturized and high-frequency acoustic imaging system for oral health and diseases of the head and neck.

Role: Co-Investigator

1R56DE032903 (Contact PI, Tamerler, Other PIs, Snead, Spencer) 09/07/23-09/06/25 (NCE)  
 NIH/NIDR  
 Peptide-enabled tunable restorative interface.  
 This proposal will develop silver-binding peptides to work synergistically with SDF to arrest caries and remineralize the tooth.  
 Role: Co-investigator

R21 DE029917 (Jokerst) 09/09/2020-08/31/2022  
 NIDCR  
 Molecular Imaging of Gingipain Activity in Advanced Periodontitis.  
 The goal is to develop a device that will detect gingipains produced by *P. gingivalis* in subgingival plaque by ultrasound imaging.  
 Role: Co-Investigator

1R43DE031196-01 (Ali Hariri) 09/2021-08/2022  
 NIDCR  
 Periodontal Ultrasound/Photoacoustic Imaging  
 To develop an imaging device for periodontium for periodontal diagnosis  
 Role: Subaward PI

R01 CA221521-05 (Feng) 07/01/2021-06/30/2022  
 NCI  
 NFAT activation in kGPCR tumorigenesis  
 The goal is to examine the role of ASNS and IKK $\epsilon$  in NFAT activation and enabling tumorigenesis.  
 Role: Contact PI

## **B. Positions and Honors**

### **Positions and Employment**

2021-	Co-chair, Department of Endodontics & Periodontics
2021-	Associate Dean of Applied Biomedical and Clinical Sciences
2009-2021	Chair, Division of Periodontology, Diagnostic Sciences and Dental Hygiene
2008-	Professor, USC School of Dentistry
2006-2016	Section Chair and Director, Predoctoral Periodontology, USC School of Dentistry
2001-2009	Chair, Primary Oral Health Care Division, USC School of Dentistry
1998-2008	Associate Professor, USC School of Dentistry
1992-1998	Assistant Professor, Department of Periodontology, USC School of Dentistry
1990-1991	Postdoctoral Research Associate, Dept. of Oral Biology, SUNY at Buffalo

### **Academic Honors and Awards**

2019	Fellow, International College of Dentists
2005-2006	Ruth Kirstein Senior Scientist Fellow, visiting faculty, University of Washington, School of Dentistry
1991	OKU Dental Society, Loma Linda University
1984-1990	Periodontal Disease Training grant, National Institutes of Health

### **Editorial Board and Journal Reviewer**

2023-	Editorial board of Host and Microbe Associations, Frontiers in Microbiomes
2022-	Editorial Board, Extra-intestinal Microbiome, Frontiers in Cellular and Infection Microbiology
2020-	Topic Editor, Pathogens
2019-	Editorial Board, Scientific Reports
2018-	Editorial Board, Journal of Oral Microbiology
2012-2014	Editorial Board, Molecular Oral Microbiology
2002-2004	Editorial Board, Journal of Dental Research

**Journal Reviewer:** ISME Journal, Scientific Reports, Applied and Environmental Microbiology, International Journal of Biological Macromolecules, Journal of Bone and Mineral Research, Journal of Materials Science, Archives of Oral Biology, Molecular Oral Microbiology, Gene, J Medical Microbiology, Infection and Immunity, J Periodontology, J Clinical Microbiology, European Journal of Oral Sciences, International Journal of Systemic Bacteriology, Anaerobe, Oral Microbiology and Immunology, Clinical Infectious Diseases, PlosOne

### **Grant Reviewer**

2023	NIH Special Emphasis Panel/Scientific Review Group 2023/05 ZDE1 YM (18) R
2022	NIH, CSR, ZRG1 MOSS-L (02)(Chair)
2021	NIH, CSR, ZRG1 MOSS-L (02 & 03) (Co-chair and Chair)
2021	NIDCR SPECIAL GRANTS REVIEW COMMITTEE
2014-	NIH, CSR, Musculoskeletal, Oral and Skin Sciences (MOSS) Study Section
	NIH, CSR, Genes, Genomes, and Genetics (GGG) Study Section
2013-	Southern California Clinical and Translational Science Institute (CTSI)
2012	NIAID Special Emphasis Panel/Scientific Review Group
2008-	NIH, CSR, Oral, Dental and Craniofacial Sciences (ODCS) Study Section
2008	NIH, CSR, Small Business and Technology Transfer (SBIR/STTR) Study Section
2003, 2009	Kuwait University
2003	NIH Special Emphasis Panel
2002	Canadian Institutes of Health Research
2001, 2002	American Institute of Biological Sciences, US Army Combat Casualty Care Research Program
2000	National Medical Technology Testbed Applications

## **C. Contribution to Science**

**1. Developing novel approaches in the diagnosis and treatment of oral diseases.** I have an interest in developing novel diagnostic tools and dental materials for periodontitis, peri-implantitis and dental caries.

- Lei Fu, Reza Khazaeinezhad, Ali Hariri, Baiyan Qi, Casey Chen, Jesse V. Jokerst. Posterior photoacoustic/ultrasound imaging of the periodontal pocket with a compact intraoral transducer. Photoacoustics. Volume 28, 2022 <https://doi.org/10.1016/j.pacs.2022.1004082022>.
- Moore, Colman; Law, Jane; Pham, Christopher; Chang, Kai-Chiao J.; Chen, Casey; Jokerst, J. V. "High-resolution ultrasonography of gingival biomarkers for periodontal diagnosis in healthy and diseased subjects". Dentomaxillofacial Radiology 2022 May 12:20220044. doi: 10.1259/dmfr.20220044. Epub ahead of print. PMID: 35522698.
- Wisdom EC, Zhou Y, Chen C, Tamerler C, Snead ML. Mitigation of peri-implantitis by rational design of bifunctional peptides with antimicrobial properties. *ACS Biomater Sci Eng*. 2020;6(5):2682-2695. doi:10.1021/acsbiomaterials.9b01213
- Wisdom EC, Chen C, Yuca E, Zhou Y, Tamerler C, Snead ML. Repeatedly applied peptide film kills bacteria on dental implant. *JOM*, 2019, Apr;71(4):1271-1280.

**2. Evolution and genomic diversity of periodontal pathogens.** I have devoted my effort to a periodontal pathogenic species, *A. actinomycetemcomitans*, and examined its genomic and phenotypic diversity and its evolutionary divergence to lineages of high and low virulence. The effort has led to a greater understanding of bacterial adaptation to humans, and potential new virulence determinants.

- Kittichotirat, W., Bumgarner, R.E., Chen, C. Genomic Islands Shape the Genetic Background of Both JP2 and Non-JP2 *Aggregatibacter actinomycetemcomitans*. *Pathogens* 2022, 11, 1037.
- Kittichotirat W, Bumgarner RE, Chen C. Evolutionary Divergence of *Aggregatibacter actinomycetemcomitans*. *Journal of dental research*. 2016; 95(1):94-101. PubMed [journal] PMID: 26420795, PMCID: PMC4700661
- Tang-Siegel G, Bumgarner R, Ruiz T, Kittichotirat W, Chen W, Chen C. Human Serum-Specific Activation of Alternative Sigma Factors, the Stress Responders in *Aggregatibacter actinomycetemcomitans*. *PloS one*. 2016; 11(8):e0160018. PubMed [journal] PMID: 27490177, PMCID: PMC4973924
- Sun R, Kittichotirat W, Wang J, Jan M, Chen W, Asikainen S, Bumgarner R, Chen C. Genomic Stability of *Aggregatibacter actinomycetemcomitans* during Persistent Oral Infection in Human. *PloS one*. 2013; 8(6):e66472. PubMed [journal] PMID: 23824402, PMCID: PMC3688926

**3. Oral microbiota of periodontal health and disease.** My interest is in oral microbiome and bacterial community structure in periodontal health and disease. My effort has contributed to a greater understanding of the impact of periodontal treatment to the oral microbial community.

- a. Chen C, Hemme C, Beleno J, Shi ZJ, Ning D, Qin Y, Tu Q, Jorgensen M, He Z, Wu L, Zhou J. Oral microbiota of periodontal health and disease and their changes after nonsurgical periodontal therapy. The ISME journal. 2018; 12(5):1210-1224. PubMed [journal] PMID: 29339824, PMCID: PMC5932080
- b. Manrique P, Freire MO, Chen C, Zadeh HH, Young M, Suci P. Perturbation of the indigenous rat oral microbiome by ciprofloxacin dosing. Molecular Oral microbiology. 2013; 28(5):404-14. NIHMSID: NIHMS494115 PubMed [journal] PMID: 23844936, PMCID: PMC3767763
- c. Laksmana T, Kittichotirat W, Huang Y, Chen W, Jorgensen M, Bumgarner R, Chen C. Metagenomic analysis of subgingival microbiota following non-surgical periodontal therapy: a pilot study. The open dentistry journal. 2012; 6:255-61. PubMed [journal] PMID: 23341849, PMCID: PMC3547359

**4. Pathogenesis of *A. actinomycetemcomitans*.** I have used *A. actinomycetemcomitans* as a model organism to investigate the microbial pathogenesis of periodontitis. My effort has led to a greater understanding of the interplay between microbial virulence factors and host responses in periodontitis.

- a. Yang YA, Cheng YA, Chen C. Genomic integration and expression of the *Aggregatibacter actinomycetemcomitans* catalase gene in *Aggregatibacter aphrophilus*. Archives of oral biology. 2018; 86:116-122. NIHMSID: NIHMS926028 PubMed [journal] PMID: 29223024, PMCID: PMC5792192
- b. Mahabady S, Tjokro N, Aharonian S, Zadeh HH, Chen C, Allayee H, Sedghizadeh PP. The in vivo T helper type 17 and regulatory T cell immune responses to *Aggregatibacter actinomycetemcomitans*. Molecular oral microbiology. 2017; 32(6):490-499. NIHMSID: NIHMS945637 PubMed [journal] PMID: 28544588, PMCID: PMC5842142
- c. Freire MO, Devaraj A, Young A, Navarro JB, Downey JS, Chen C, Bakaletz LO, Zadeh HH, Goodman SD. A bacterial-biofilm-induced oral osteolytic infection can be successfully treated by immuno-targeting an extracellular nucleoid-associated protein. Molecular oral microbiology. 2017; 32(1):74-88. NIHMSID: NIHMS764780 PubMed [journal] PMID: 26931773, PMCID: PMC5010536
- d. Karched M, Ihalin R, Eneslätt K, Zhong D, Oscarsson J, Wai SN, Chen C, Asikainen SE. Vesicle-independent extracellular release of a proinflammatory outer membrane lipoprotein in free-soluble form. BMC microbiology. 2008; 8:18. PubMed [journal] PMID: 18226201, PMCID: PMC2257964

**Complete List of Published Work in MyBibliography:**

<https://www.ncbi.nlm.nih.gov/myncbi/123roOOG60QAQ/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: Feng, Pinghui

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

POSITION TITLE: Professor of Infection and Immunity

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
Hunan Normal University, Hunan, PR. China	B.S.	07/1994	Biology
Wuhan Institute of Virology, CAS, Hubei, PR. China	M.S.	07/1997	Microbial Genetics
University of Missouri, Kansas City, MO	Ph.D.	08/2001	Cell Biology and Biophysics
Harvard Medical School	Postdoc/ Instructor	09/2001- 08/2006	Microbiology and Mol. Genetics, Tumor Virol

A. Personal Statement

The PI believes that he is particularly well suited and strategically poised to perform the studies in this proposal. The group has made the original discovery that herpesviruses, such as herpes simplex viruses and gamma herpesviruses, encode pseudoenzymes and *bona fide* protein deamidases to deamidate and inactivate RIG-I, suggesting that protein deamidation is a powerful means to regulate signal transduction. Interesting, the gamma herpesvirus vGAT proteins usurp a cellular glutamine amidotransferase to deamidate RIG-I and glutamine amido transferases catalyze the synthesis of nucleotides, amino acids, glycoproteins and enzyme cofactors in mammalian cells, raising the possibility that metabolism is intrinsically coupled to signal transduction via protein deamidation. Using herpesvirus infection as a model system, the PI's group has uncovered fundamental roles of protein deamidation in key biological processes, e.g., infection and immunity (*Mol. Cell*, 2015; *Cell Host & Microbe*, 2016; 2018), transcription and protein nuclear import (*Sci. Adv.* 2019), metabolic reprogramming by cancer cells and viruses (*Cell Metab.*, 2020; *PNAS*, 2022; *Immunity*, 2025; *Nat. Metab.*, 2024). Overall, the Feng lab has developed a number of resources and protocols to interrogate the interaction between metabolism and immune response, all of which will be readily available for Dr. Mirali Pandya.

R01CA285192 (PI: Pinghui Feng)Proposal Period: 07/01/2023-06/30/2028

Title: Targeting IKKepsilon-mediated nucleotide synthesis in KSHV-associated lymphoma

Description: To examine the molecular mechanism and develop small molecule inhibitors of IKKepsilon that impedes KSHV-positive lymphoma.

R01AG070904 (PI: Pinghui Feng)Proposal Period: 07/15/2021-06/30/2026

Title: Roles of HSV-1 in Alzheimer's disease using mouse models

Description: To examine the role of HSV-1 in neurodegeneration of Alzheimer's disease using mouse models.

R21AI180537 (PI: Pinghui Feng)Proposal Period: 5/01/2024-04/30/2026

Title: Targeting the UL37 Deamidase to impede HSV-1 infection.

Description: To explore small molecule inhibitors that target the UL37 deamidase to impede capsid assembly and immune evasion of HSV-1.

R01AI184716 (Contact PI: Pinghui Feng) Proposal Period: 08/21/2025-07/31/2030

Title: Explore a key nucleotide synthesis enzyme to develop a broad-spectrum antiviral therapy.

Description: To examine the molecular mechanism that DNA and RNA viruses hijack a nucleotide synthetic enzyme to evade immune response and develop small molecule inhibitors for antiviral therapy.

RF1AG088625 (Contact PI: Pinghui Feng) Proposal Period: 09/15/2025-08/31/2029

Title: Regulated cGAS activation in HSV-1-associated neuropathogenesis.

Description: To determine the mechanism of activation of cGAS regulated by DNA ligands and deamidation during HSV-1 infection in the brain using mouse models for Alzheimer's disease.

## B. Positions, Scientific Appointments, and Honors

### Research and Professional Experience

2018-present	Professor and Chair, Section of Infection and Immunity, Herman Ostrow School of Dentistry, University of Southern California
2019-2020	Guest Professor, Hunan Normal University, State Key Lab for Freshwater Fish Research
2017-2018	Professor, Department of Molecular Microbiology and Immunology, University of Southern California, Los Angeles, CA
2011-2017	Associate Professor, Department of Molecular Microbiology and Immunology, University of Southern California, Los Angeles, CA
2006-2011	Assistant Professor, Department of Microbiology, UT Southwestern Medical Center, Dallas, TX
2004-2006	Instructor in Microbiology and Molecular Genetics, New England Primate Research Center, Harvard Medical School, Southborough, MA
2001-2004	Postdoctoral Fellow in Microbiology and Molecular Genetics, New England Primate Research center, Harvard Medical School, Southborough, MA

### Honors

2020	Member, National Academy of Inventors, Southern California Chapter
2019	Elected Fellow, American Academy of Microbiology
2018	Inaugural Chair, Section of Infection and Immunity, Herman Ostrow School of Dentistry, USC
2017	R35 Mid-career SOAR Award, NIDCR, NIH
2015	Research Scholar, Margaret Early Medical Research Foundation
2011	Research Scholar, American Cancer Society
2008	Alumni Achievement Award, University of Missouri-Kansas City
2006	Leukemia and Lymphoma Society Special Fellowship
2006	Endowed Scholar at UT Southwestern Medical Center
2003	Leukemia and Lymphoma Society Fellowship
1997-2001	Chancellor Non-resident Award, University of Missouri, Kansas City, MO
1992	Chancellor Fellowship, Hunan Normal University, Hunan, PR. China

## C. Contributions to Science

### 1. Discovery of protein deamidation in regulating signal transduction in mammalian system.

Innate immunity is the first line of defense in response to invading pathogens. To counteract host immune defense, viruses have evolved diverse intricate strategies to evade and exploit innate immune signaling cascades. Herpesviruses are notorious for the ability to manipulate host immune system. In studying the RIG-I and MAVS-dependent innate immune defense against herpesviruses, we uncovered surprising roles of the MAVS-dependent signaling cascade in evading antiviral response and promoting viral lytic gene expression. Interestingly, both activities are enabled by the viral replication transactivator (RTA), an essential transcription factor for KSHV and  $\gamma$ HV68. Subsequently, we have employed diverse biochemical, viral genomic

and genetic analysis to identify a group of viral pseudo enzyme (vGAT) and their cellular counterpart, phosphorylformyl- ribosylglycinamide synthetase (PFAS) to deamidate and concomitantly activate RIG-I. This work establishes the first example whereby a pattern recognition receptor is activated by an enzyme complex. Our study also identifies the protein-deamidating activity of PFAS that was known to deamidate free glutamine. Research in my laboratory is currently oriented to understand the regulation of deamidation in infection and immunity.

- 1) He S., Zhao J, He X, Minassian A, Zandi E, Liang C, Jung JU, Zhang X, and **Feng P.** (2015) Viral pseudo enzymes activate RIG-I via deamidation to evade cytokine production. *Mol Cell*. 58(1):134-46. Please also see preview by Daniel Kolakofsky and Dominique Garcin: <http://www.sciencedirect.com/science/article/pii/S1097276515002129>
- 2) Zhao J, Zeng Y, Xu SM, Chen J, Shen G, Yu C, Peng J, Knipe DM, Yuan WM, Xu WQ, Zhang C Xia Z, and **Feng P.** A viral deamidase targets RIG-I to block RNA-induced activation. *Cell Host & Microbe*. 2016. Dec 14;20(6):770-784. doi: 10.1016/j.chom.2016.10.011. Please also see preview by Dominique Garcin: [http://www.cell.com/cell-host-microbe/abstract/S1931-3128\(16\)30489-9](http://www.cell.com/cell-host-microbe/abstract/S1931-3128(16)30489-9).
- 3) Zhao J, Tian M, Zhang S, Delfarah A, Gao R, Rao Y, Savas AC, Bubb L, Lei X, Moshirian R, Zhu W, Peng C, Jiang T, Chen L, Graham NA, and **Feng P.** Deamidation Shunts RelA from Mediating Inflammation to Aerobic Glycolysis. *Cell Metabolism*. 31(5):937-955.e7. doi: 10.1016/j.cmet.2020.04.006. Epub 2020 Apr 22.
- 4) Rao Y, Qin C, Savas AC, Liu Q, Feng S, Hou G, Xie T, and Feng P. Pyrimidine synthesis enzyme CTP synthetase 1 suppresses antiviral interferon induction by deamidating IRF3. *Immunity*. 2025. 58(1):74-89.e6.

## 2. Discovering IKK $\epsilon$ as a key negative regulator of T cell response.

To understand the roles of IKK $\epsilon$  in viral GPCR-mediated signaling, we accidentally stumbled on discovering the critical roles of IKK $\epsilon$  in T cell activation and immune response. In summary, we have discovered that: 1) IKK $\epsilon$  potently phosphorylates NFAT and inhibits NFAT activation. 2) IKK $\epsilon$  is rapidly activated in response to T cell activation induced by TCR ligation or calcium influx; 3) depletion or pharmacological inhibition of IKK $\epsilon$  reduced NFAT phosphorylation and promote NFAT activation and T cell immune response. Finally, 4) loss of IKK $\epsilon$  elevated T cell antiviral immune response and conversely dramatically reduced viral persistent infection by >50-fold. Currently, we are developing small molecules that can inhibit IKK $\epsilon$  to boost T cell immunity for immunotherapy against infectious agents and cancers.

- 1) Zhang J, Feng H, Zhao J, Feldman ER, Chen SY, Yuan WM, Huang C, Akbari O, Tibbetts SA, and **Feng P.** IKK $\chi$  kinase is an NFATc1 kinase that inhibits T cell immune responses. *Cell Reports* 2016 Jul 12;16(2):405-18. doi: 10.1016/j.celrep.2016.05.083.
- 2) Wang Y, Lu X, Zhu L, Shen Y, Chengedza S, Feng H, Wang L, Jung JU, Gutkind JS, **Feng P.** (2013). IKKepsilon kinase is crucial for viral G protein-coupled receptor tumorigenesis. *Proc Natl Acad Sci U S A*. 2013 Jul 2;110(27):11139-44.

## 3. Defined a novel viral host shutoff mechanism enabled by a viral nuclease.

My graduate study focused on the molecular action by which the herpes simplex virus virion host shutoff (vhs) protein in degrading mRNA, while sparing tRNA and ribosomal RNA. We identified a translation initiation factor, eIF4H, which targets vhs to mRNA and to the regions of translation initiation. Subsequent biochemical and genetic analysis also revealed additional components, e.g., eIF4A, linking vhs to the translation initiation regions of mRNA. This work elucidated the molecular mechanism by which herpes simplex viruses target mRNA to shutoff host protein biosynthesis and established a new model of host shutoff.

- 1) **Feng, P.**, Everly, D.N., Jr., and Read, G.S. (2001). mRNA Decay During HSV Infections: Interaction Between a Viral Nuclease and a Cellular Translation Factor. *J. Virol*. 75: 10272-10280.
- 2) Everly, D.N., Jr., **Feng, P.**, and Read, G.S. (2002). mRNA Degradation by the Virion Host Shutoff Protein (UL41) of Herpes Simplex Virus: Genetic and Biochemical Evidence that UL41 is a Nuclease. *J. Virol*. 76: 8560-8571.
- 3) **Feng, P.**, Everly, D.N., Jr., and Read, G.S. (2005). Protein-protein interactions involving the virion host shutoff protein (UL41) and their implications in mRNA decay during HSV infection. *J. Virol*. 79:9651-9664.



Work from my postdoctoral research centered on delineating virus interactions with the host apoptosis and autophagy system, intrinsic immune response to restrict viral replication. Using human Kaposi's sarcoma-associated herpesvirus (KSHV) and murine gamma herpesvirus 68 (γHV68), we identified two mitochondrial proteins, KSHV K7 and γHV68 vMAP, which displayed distinct activities to block mitochondrion-dependent apoptotic signaling. In collaboration with Dr. Chengyu Liang, we have also discovered that viral Bcl-2 homologs inhibit autophagy via interacting with UVRAG, a positive regulator of autophagy. These studies expand our understanding in regulation of apoptosis and identified a new player in autophagy.

- 1). **Feng, P.**, Park, J., Lee, B.S., Lee, S.-H., Bram R., and Jung, J.U. (2002). Kaposi's Sarcoma-Associated Virus K7 Mitochondrial Protein Targets a Cellular CAML to Modulate Calcium Concentration and Inhibit Apoptosis. *J. Virol.* 76: 11491-11504.
- 2). **Feng, P.**, Scott C., Cho, N.-H., Nakayama, H., Chung, Y.-H., Monteiro, M.J., and Jung, J.U. (2004). Kaposi's sarcoma-associated virus K7 targets a UBL/UBA domain-containing protein to promote protein degradation. *Mol. Cell Biol.* 24: 3938-3948.
- 3). Liang, C.Y., **Feng, P.**, Ku, B., Dotan, I., Canaani, D., Oh, B.-H., Jung, J.U. (2006). Autophagic and tumor suppressor activity of a Beclin1-binding UVRAG. *Nat. Cell Biol.* 8:688-99.
- 4). **Feng P.**, Liang C, Shin YC, E X, Zhang W, Gravel R, Wu TT, Sun R, Usherwood E, Jung JU. (2007) A Novel Inhibitory Mechanism of Mitochondrion-Dependent Apoptosis by a Herpesviral Protein. *PLoS Pathog.* 3(12):e174.

## 1. Delineate the signal transduction and regulation of KSHV G protein-coupled receptor in tumorigenesis.

Kaposi's sarcoma-associated herpesvirus (KSHV) is an oncogenic DNA virus that causes various malignancies of endothelial or lymphoid tissues in immune compromised patients. One unique feature of KSHV-induced tumor formation is the involvement of lytic cycle and proteins expressed thereof. Interestingly, KSHV G protein-coupled receptor is a potent signaling molecule that, when expressed in mouse endothelium, *is sufficient to induce human KS-like lesion. Our work has delineated viral and cellular mechanisms governing KSHV GPCR signaling and tumorigenic activity.* Specifically, we have defined K7-induced ER-associated degradation, tyrosine sulfation in ligand association and inhibition of ER SERCA2 calcium channel in activating NFAT. These studies uncovered distinct cellular and viral mechanisms regulating KSHV and other herpesviral GPCR signaling and tumor formation.

- 1) Feng, H., Dong, X., Negaard, A., and **Feng P.** (2008) Kaposi's sarcoma-associated herpesvirus K7 induces viral G protein-coupled receptor degradation and reduces its tumorigenicity. *PLoS Pathog.* 4(9):e1000157.
- 2) Wang Y, Lu X, Zhu L, Shen Y, Chengedza S, Feng H, Wang L, Jung JU, Gutkind JS, **Feng P.** (2013) IKK epsilon kinase is crucial for viral G protein-coupled receptor tumorigenesis. *Proc Natl Acad Sci U S A.* 2013 Jul 2;110(27):11139-44.
- 3) Zhang J, He S, Brulois K, Feng H, Lan K, Jung JU, and **Feng P.** (2015) Herpesviral G protein-coupled receptors activate NFAT to induce tumor formation via inhibiting the sarco/endoplasmic calcium ATPase. *PLoS Pathog.* 11(3): e1004768.
- 4) Zhang J, Zhu L, Lu X, Feldman ER, Keyes LR, Wang Y, Fan H, Feng H, Xia Z, Sun J, Jiang T, Gao SJ, Tibbetts SA, **Feng P.** (2015) Recombinant Murine Gamma Herpesvirus 68 Carrying KSHV G Protein-Coupled Receptor Induces Angiogenic Lesions in Mice. *PLoS Pathog.* 11(6):e1005001.

### **Complete List of Published Work in MyBibliography:**

<http://www.ncbi.nlm.nih.gov/sites/myncbi/1v9izoWd7wg5L/bibliography/43604579/public/?sort=date&direction=ascending>

Or on PubMed: <https://pubmed.ncbi.nlm.nih.gov/?term=Pinghui+Feng>

**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: Lin, De-Chen

eRA COMMONS USER NAME: DECHENLIN

POSITION TITLE: Assistant Professor (Tenure-Track), Ostrow School of Dentistry; Associate Director, USC Head and Neck Center, University of Southern California

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
Nanjing University, China	B.S.	06/2005	Biology
Chinese Academy of Medical Sciences, China	Ph.D.	06/2010	Cell Biology
National University of Singapore	Postdoctoral	09/2013	Cancer Genomics
Cedars-Sinai Medical Center, CA	Postdoctoral	02/2014	Cancer Genomics

**A. Personal Statement**

I have a passion for cancer genomics/epigenomics, and I obtained training and expertise in both experimental and computational biology. I oversee the basic research at USC Head and Neck Center as Associate Director. The major interest of my laboratory is investigating genomic and epigenomics abnormalities in **squamous cell carcinoma, including head neck cancers**, which has been my research focus since my graduate training. Over the last 18 years, I have developed strong research expertise in squamous cancer, and established patient biobanks, model systems and innovative methodologies for the study of squamous cancer. For example, I am among the first in the world to demonstrate the genomic landscape of head and neck nasopharynx cancer (*Nat Genet*, 2014, citation=406) and esophageal squamous cancer (*Nat Genet* 2014, citation=688). I delineated for the first time the clonal evolution of squamous cancer (*Nat Genet* 2016, citation=269). I have also developed novel mathematical methods and computational pipelines for the analyses of genomic sequencing (*Genome Res* 2018, citation=4,091) and epigenomic data (*Bioinformatics*, 2020).

More recently, to study early neoplastic evolution and intratumoral heterogeneity, I have developed a novel organoid modeling (*Sci Transl Med* 2022; *J Exp Med* 2025) and bioinformatic framework (*Genome Biol*, 2023), serving as the bedrock for the current proposal. As PI or M-PI on several Institutional and NIH grants, I have laid the groundwork for the proposed research by pioneering and establishing **CRISPR-edited, cross-species premalignant organoid modeling**. Moreover, we have established human **patient-derived organoids (PDO)** for various upper aerodigestive tissues, such as oral cavity, Barrett's esophagus and gastroesophageal junction. Therefore, the current application builds logically and strongly on my prior work. In addition, as Associate Director of the USC Head and Neck Center, I direct HNSCC basic research at USC and run the organoid bank.

In summary, I have the expertise, leadership, training, resource as well as motivation necessary to successfully lead the proposed research on head neck squamous cancer.

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

1) 1R01DE033648 Lin (PI) 03/01/24-02/28/29  
Agency: NIH/NIDCR  
Title: Pharmacological and dietary inhibition of a novel metabolic-epigenetic crosstalk in head and neck cancer

2) Concern Foundation for Cancer Research Lin (PI) 02/01/24-01/31/25

Agency: Concern Foundation

Title: Investigation of head neck cancer immune response using patient tumor-derived organoid models

Selected Recent Representative Citations (my Correspondence authorship is denoted by \*):

- 1) **Lin DC\***, Hao JJ, Nagata Y, Xu L, Shang L, Meng X, Sato Y, Okuno Y, Varela AM, Ding LW, Garg M, Liu LZ, Yang H, Yin D, Shi ZZ, Jiang YY, Gu WY, Gong T, Zhang Y, Xu X, Kalid O, Shacham S, Ogawa S, Wang MR, Koeffler HP. *Genomic and molecular characterization of esophageal squamous cell carcinoma*. **Nat Genet.** 2014 May;46(5):467-73. PMID: PMC4070589
- 2) Zhao H, Cheng Y, Kalra A, Ma K, Zheng Y, Ziman B, Tressler C, Glunde K, Shin EJ, Ngamruengphong S, Khashab M, Singh V, Anders RA, Jit S, Wyhs N, Chen W, Li X, **Lin DC\***, Meltzer SJ. *Generation and multiomic profiling of a TP53/CDKN2A double-knockout gastroesophageal junction organoid model*. **Sci Transl Med.** 2022 Nov 30;14(673):eabq6146. PMID: PMC10026384
- 3) Zheng Y, Ziman B, Ho AS, Sinha UK, Xu LY, Li EM, Koeffler HP, Berman BP, **Lin DC\***. *Comprehensive analyses of partially methylated domains and differentially methylated regions in esophageal cancer reveal both cell-type- and cancer-specific epigenetic regulation*. **Genome Biol.** 2023 Aug 24;24(1):193. PMID: PMC10463844
- 4) Nam C, Li LY, Yang Q, Ziman B, Zhao H, Hu B, Collet C, Jing P, Lei Q, Xu LY, Li EM, Koeffler HP, Sinha UK, **Lin DC\***. *A druggable cascade links methionine metabolism to epigenomic reprogramming in squamous cell carcinoma*. **Proc Natl Acad Sci U S A.** 2024 Jun 25;121(26):e2320835121. PMID: PMC11214090
- 5) Sinha UK, Park YM, **Lin DC\***. *A High-Power Spatial, Single-Cell View of Pericytes in Esophageal Cancer Metastasis*. **Nat Genet.** 2025. In Press

## B. Positions, Scientific Appointments, and Honors

### Positions and Scientific Appointments

2022-Present	Associate Director, USC Head and Neck Center, Keck School of Medicine, University of Southern California, Los Angeles, CA
2022-Present	Assistant Professor (Tenure-Track), Center for Craniofacial Molecular Biology, Herman Ostrow School of Dentistry, University of Southern California, Los Angeles, CA
2020-2021	Adjunct Associate Professor, UCLA School of Medicine, Los Angeles, CA
2018-2021	Member, UCLA Molecular Biology Institute
2017-2021	Member, Samuel Oschin Comprehensive Cancer Institute
2016-2021	Faculty, Research Scientist I, Cedars-Sinai Medical Center, Los Angeles, CA
2016-2021	Member, UCLA Jonsson Comprehensive Cancer Center
2014-2015	Project Scientist, Cedars-Sinai Medical Center, Los Angeles, CA

### Grant reviews

2025	NIH/NCI Program Project (P01) Study Section (PAR-23-059), ZRG1 CTH-H (45) Jan 2026 Council, Temporary Member
2025-present	NIH Mechanisms of Cancer Therapeutics B (MCTA) Study Section, Standing Member (5 year term)
2025	NIH Mechanisms of Cancer Therapeutics B (MCTB) Study Section (May 2025 Council), Temporary Member
2024	NIH Mechanisms of Cancer Therapeutics A (MCTA) Study Section (May 2024 Council), Temporary Member
2024	NIH/NCI Program Project (P01) Study Section (PAR-23-059), SEP-B ZCA1 RPRB-T (O1) Temporary Member
2024	NIH Mechanisms of Cancer Therapeutics A (MCTA) Study Section (Jan 2025 Council), Temporary Member
2023	WF Kankerbestrijding – Dutch Cancer Society (DCS), reviewer
2023	DOD Rare Cancer Research Program (RCRP) Concept Award for Gastrointestinal Cancers (Con-GIC) Study Section

2023	DOD Peer Review Cancer Research Program Gastrointestinal Cancers (GIC) Study Section
2022-present	USC Norris Scientific Review Committee, Sitting Member
2022	NIH Cancer Genetics (CG) study section, Temporary Member
2022	DOD Peer Review Cancer Research Program Gastrointestinal Cancers (GIC) study section
2021	DOD Peer Review Cancer Research Program Gastrointestinal Cancers (GIC) study section
2020	NIH Cancer Genetics (CG) study section, Temporary Member
2018	Wellcome Trust United Kingdom, external reviewer
2016-present	Research Grants Council (RGC) of Hong Kong, external reviewer

### **Selected Honors**

2024	Elected Member, Sigma Xi
2023	USC School of Dentistry Innovative Scientific Achievement Award (Mentor)
2020	Readers' Choice: The best of Oncogene 2019
2019	Best of AACR Award: most-cited research article in <i>Cancer Research</i> (2019)
2018	DeGregorio Foundation Award
2017	Tower Foundation Career Development Award
2016	CURE:CTSI Pilot and Feasibility Award
2015	Donna and Jesse Garber Awards for Cancer Research
2015	American Society of Hematology (ASH) Fellow Scholar Award
2015	MDS Young Investigator Award
2014	Incorporated Scholar-in-Training Award, AACR-Aflac

### **C. Contribution to Science** (my Correspondence authorship is denoted by \*)

**I. Characterization of regulatory mechanisms during cancer evolution.** To study early neoplastic evolution, I have recently developed a novel organoid modeling from normal human samples and performed CRISPR/Cas9 genomic editing to double knockout early founding drivers (TP53/CDKN2A), leading to cell metaplasia, dysplasia and neoplastic evolution (a). I also have devised and led studies demonstrating that malignant transformation is accompanied by epigenome-wide alterations, which are regulated by epigenetic regulators (b-c). More recently, I have further developed CRISPR-edited organoid modeling of early malignant transformation by triple knockout of TP53/CDKN2A/MLL3 (also known as MKT2C), published in d.

- a. Zhao H, Cheng Y, Kalra A, Ma K, Zheng Y, Ziman B, Tressler C, Glunde K, Shin EJ, Ngamruengphong S, Khashab M, Singh V, Anders RA, Jit S, Wyhs N, Chen W, Li X, **Lin DC\***, Meltzer SJ. *Generation and multiomic profiling of a TP53/CDKN2A double-knockout gastroesophageal junction organoid model. Sci Transl Med.* 2022 Nov 30;14(673):eabq6146. PMID: PMC10026384
- b. Chen L, Huang M, Plummer J, Pan J, Jiang YY, Yang Q, Silva TC, Gull N, Chen S, Ding LW, An O, Yang H, Cheng Y, Said JW, Doan N, Dinjens WN, Waters KM, Tuli R, Gayther SA, Klempner SJ, Berman BP, Meltzer SJ, **Lin DC\***, Koeffler HP. *Master transcription factors form interconnected circuitry and orchestrate transcriptional networks in oesophageal adenocarcinoma. Gut* 2020 Apr;69(4):630-640. PMID: PMC8108390
- c. Jiang YY, Jiang Y, Li C, Zhang Y, Dakle P, Kaur H, Deng J, Lin R, Han L, Xie JJ, Yan Y, Doan N, Zheng Y, Mayakonda A, Hazawa M, Xu L, Li Y, Aswad L, Jeitany M, Kanojia D, Guan X, Said JW, Yang W, Fullwood MJ, Koeffler HP & **Lin DC\***. *TP63, SOX2, and KLF5 Establish a Core Regulatory Circuitry That Controls Epigenetic and Transcription Patterns in Esophageal Squamous Cell Carcinoma Cell Lines. Gastroenterology.* 2020 Oct;159(4):1311-1327.e19. PMC Journal In Process
- d. Nam, C., Huang, G., Zheng, Y., Zhao, H., Pan, Y., Hu, B., Wenger, T., Van, H., Xu, L.-Y., Li, E.-M., Koeffler, H.P., Ge, K., Dou, Y., Sinha, U., Park, Y.M., **Lin DC\***. *The MLL3/GRHL2 complex regulates malignant transformation and anti-tumor immunity in squamous cancer. J Exp Med.* 2025 Apr 7;222(4):e20240758. PMID: PMC11834937

**II. Genomic landscapes and evolution of squamous cancer.** As a pioneer in the field, I am among the first in the world to establish genomic landscapes and evolution in different types of squamous cancers, including esophageal squamous cancer (a, citation=654) and head neck cancer (b, citation=382). Moreover, I led a study which delineated for the first time the clonal evolution of esophageal squamous cancer (c, citation=253). We have also comprehensively contrasted driver genes and pathways between esophageal squamous cancer and adenocarcinomas (d, citation=115).

- a. **Lin DC\***, Hao JJ, Nagata Y, Xu L, Shang L, Meng X, Sato Y, Okuno Y, Varela AM, Ding LW, Garg M, Liu LZ, Yang H, Yin D, Shi ZZ, Jiang YY, Gu WY, Gong T, Zhang Y, Xu X, Kalid O, Shacham S, Ogawa S, Wang MR, Koeffler HP. *Genomic and molecular characterization of esophageal squamous cell carcinoma*. **Nat Genet**. 2014 May;46(5):467-73. PMCID: PMC4070589
- b. **Lin DC\***, Meng X, Hazawa M, Nagata Y, Varela AM, Xu L, Sato Y, Liu LZ, Ding LW, Sharma A, Goh BC, Lee SC, Petersson BF, Yu FG, Macary P, Oo MZ, Ha CS, Yang H, Ogawa S, Loh KS, Koeffler HP. *The genomic landscape of nasopharyngeal carcinoma*. **Nat Genet**. 2014 Aug;46(8):866-71. PMC Journal In Process
- c. Hao JJ, **Lin DC\***, Dinh HQ, Mayakonda A, Jiang YY, Chang C, Jiang Y, Lu CC, Shi ZZ, Xu X, Zhang Y, Cai Y, Wang JW, Zhan QM, Wei WQ, Berman BP, Wang MR, Koeffler HP. *Spatial intratumoral heterogeneity and temporal clonal evolution in esophageal squamous cell carcinoma*. **Nat Genet**. 2016 Dec;48(12):1500-1507. PMCID: PMC5127772
- d. **Lin DC\***, H Q. Dinh, J Xie, A Mayakonda, T Silva, YY Jiang, LW Ding, JZ He, XE Xu, J Hao, MR Wang, C Li, L Xu, E Li, B Berman, HP Koeffler. *Identification of distinct mutational patterns and new driver genes in oesophageal squamous cell carcinomas and adenocarcinomas*. **Gut**. 2018 Oct;67(10):1769-1779. PMCID: PMC5980794

**III. Identification of key signaling pathways and driver genes in squamous cancer.** I pioneered the studies of the super-enhancer-mediated epigenomic dysregulation in esophageal squamous cancer (a). I have also identified and characterized master transcription factors which control the super-enhancer network as well as their key target genes (b,c). I was commissioned by an authoritative journal of the disease to review these features and their clinical implications for esophageal squamous cancer (d).

- a. Jiang YY, **Lin DC\***, Mayakonda A, Hazawa M, Ding LW, Chien WW, Xu L, Chen Y, Xiao JF, Senapedis W, Baloglu E, Kanojia D, Shang L, Xu X, Yang H, Tyner JW, Wang MR, Koeffler HP. *Targeting super-enhancer-associated oncogenes in oesophageal squamous cell carcinoma*. **Gut**. 2017 Aug;66(8):1358-1368. PMCID: PMC5912916
- b. Jiang Y, Jiang YY, Xie JJ, Mayakonda A, Hazawa M, Chen L, Xiao JF, Li CQ, Huang ML, Ding LW, Sun QY, Xu L, Kanojia D, Jeitany M, Deng JW, Liao LD, Soukiasian HJ, Berman BP, Hao JJ, Xu LY, Li EM, Wang MR, Bi XG, **Lin DC\***, Koeffler HP. *Co-activation of super-enhancer-driven CCAT1 by TP63 and SOX2 promotes squamous cancer progression*. **Nat Commun**. 2018 Sep 6;9(1):3619. PMCID: PMC6127298
- c. Li LY, Yang Q, Jiang YY, Yang W, Jiang Y, Li X, Hazawa M, Zhou B, Huang GW, Xu XE, Gery S, Zhang Y, Ding LW, Ho AS, Zumsteg ZS, Wang MR, Fullwood MJ, Freedland SJ, Meltzer SJ, Xu LY, Li EM, Koeffler HP, **Lin DC\***. Interplay and cooperation between SREBF1 and master transcription factors regulate lipid metabolism and tumor-promoting pathways in squamous cancer. **Nat Commun**. 2021 Jul 16;12(1):4362. PMCID:PMC8285542
- d. **Lin DC\***, Wang MR, Koeffler HP. *Genomic and Epigenomic Aberrations in Esophageal Squamous Cell Carcinoma and Implications for Patients*. **Gastroenterology**. 2018 Jan;154(2):374-389. PMCID: PMC5951382

**IV. Establishing the functional relevance of epigenomic aberrations in cancer.** I have led a number of investigations which illustrated the biological relevance cancer-specific transcription factors, such as HNF4A (a) and BCL6 (b). I have also supervised a pan-cancer epigenomic project which revealed the extensive plasticity of Polycomb target genes with important functional significance (c). Recently, I directed a study using single-cell RNA-seq profiling of nonmalignant and tumor samples of 11 SCC patients (d).

- a. Pan J, Silva TC, Gull N, Yang Q, Plummer JT, Chen S, Daigo K, Hamakubo T, Gery S, Ding LW, Jiang YY, Hu S, Xu LY, Li EM, Ding Y, Klempner SJ, Gayther SA, Berman BP, Koeffler HP, **Lin DC\***. *Lineage-specific epigenomic and genomic activation of oncogene HNF4A promotes gastrointestinal adenocarcinomas*. **Cancer Res**. 2020 Jul 1;80(13):2722-2736 PMC Journal In Process
- b. Xu L, Chen Y, Dutra-Clarke M, Mayakonda A, Hazawa M, Savinoff SE, Doan N, Said JW, Yong WH, Watkins A, Yang H, Ding LW, Jiang YY, Tyner JW, Ching J, Kovalik JP, Madan V, Chan SL, Mischen M, Breunig JJ, **Lin DC\***, Koeffler HP. *BCL6 promotes glioma and serves as a therapeutic target*. **Proc Natl Acad Sci U S A**. 2017 Apr 11;114(15):3981-3986. PMCID: PMC5393201
- c. Y Zheng, G Huang, T Silva, Q Yang, Y Jiang, HP Koeffler, **Lin DC\***, BP Berman. *A pan-cancer analysis*

of CpG Island gene regulation reveals extensive plasticity within Polycomb target genes. **Nat Commun.** 2021 Apr 30;12(1):2485. PMCID: PMC8087678

- d. Dinh HQ, Pan F, Wang G, Huang QF, Olingy CE, Wu Z, Wang S, Xu X, Xu XE, He J, Yang Q, Orsulic S, Haro M, Li L, Huang G, Breunig J, Koeffler HP, Hedrick C, Xu L, **Lin DC\***, Li EM. *Integrated single-cell transcriptome analysis reveals heterogeneity of esophageal squamous cell carcinoma microenvironment.* **Nat Commun.** 2021 Dec 17;12(1):7335. doi: 10.1038/s41467-021-27599-5. PMCID: PMC8683407

V. Development of computational tools and databases for the analyses of cancer sequencing data. I have supervised the development of a bioinformatic software, *Maftools*, for comprehensive analysis of somatic variants in cancer (a). This software package is ranked top 1% most downloaded tools in the Bioconductor and has been cited over 3,444 times since its publication in 2018. I have also conceived and designed a mathematical method to reconstruct gene regulatory networks from DNA methylation and transcriptomes from cancer patient samples (b). I have also contributed to the development of methods and bioinformatic framework to analyze other sequencing data (c-d).

- a. Mayakonda A, **Lin DC\***, Assenov Y, Plass C, Koeffler HP. *Maftools: efficient and comprehensive analysis of somatic variants in cancer.* **Genome Res.** 2018 Nov;28(11):1747-1756. PMCID: PMC6211645
- b. Silva TC, Coetzee SG, Gull N, Yao L, Hazelett DJ, Noushmehr H, **Lin DC\***, Berman BP. *ELMER v.2: An R/Bioconductor package to reconstruct gene regulatory networks from DNA methylation and transcriptome profiles.* **Bioinformatics.** 2019 Jun 1;35(11):1974-1977. PMCID:PMC6546131
- c. Huang M, Wang Y, Yang M, Yan J, Yang H, Zhuang W, Xu Y, Koeffler HP, **Lin DC\***, Chen X. *dbInDel: a database of enhancer-associated insertion and deletion variants by analysis of H3K27Ac ChIP-Seq.* **Bioinformatics.** 2020 Mar 1;36(5):1649-1651. PMCID:PMC7703781
- d. **Lin DC\***. *Large-scale genomic analyses reveal alterations and mechanisms underlying clonal evolution and immune evasion in esophageal cancer.* **Nat Commun.** 2023 Feb 17; 14:893. PMCID: PMC9938131

Complete List of published work in my bibliography (112 publications; h-index=53; citation= 13,574)

<https://www.ncbi.nlm.nih.gov/myncbi/de-chen.lin.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: Qin, Chao

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

POSITION TITLE: Research 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)	END DATE MM/YYYY	FIELD OF STUDY
Shandong Agricultural University, Taian, Shandong	BAGR	06/2014	Veterinary Medicine
China Agricultural University, Beijing	PHD	06/2019	Basic Veterinary Medicine

### A. Personal Statement

I earned my Ph.D. from China Agricultural University and commenced my postdoctoral fellowship at USC in 2019. Subsequently, I advanced to the position of Research Associate in 2022, and further to Research Assistant Professor in 2024. My research primarily revolves around investigating virus-host interactions and metabolic reprogramming in viral infections, as well as in cancer cell proliferation. My research findings are as follows: (1) We discovered that SARS-CoV-2 activates a pyrimidine synthetic enzyme to increase nucleotide synthesis and block inflammatory response. A de novo synthesized inhibitor targeting this enzyme sufficiently impedes SARS-CoV-2 replication both in cells and in vivo. (2) We demonstrated that CTP synthase 1 dampens interferon induction via deamidating interferon regulatory factor 3 (IRF3). (3) My research revealed that a pyrimidine enzyme activates pentose phosphate pathway and serine synthesis pathway to promote tumor growth through regulating activities of the rate-limiting enzymes in these two pathways. In addition, I am also keen on leveraging mass spectrometry to deepen our comprehension of fundamental biological processes, including metabolism and immune defense mechanisms.

### B. Positions, Scientific Appointments and Honors

#### Research and Professional Experience

06/2024 – Research Assistant Professor, University of Southern California, Los Angeles, CA  
07/2022 – 05/2024 Research Associate, University of Southern California, Los Angeles, CA  
08/2019 – 06/2022 Postdoctoral Fellow, University of Southern California, Los Angeles, CA

#### Honors

The American Society of Microbiology (ASM), Member

The Purine and Pyrimidine Society, Member

The American Society for Cell Biology (ASCB), Member

Post-doctoral trainee awards in 2023 annual research day of Herman Ostrow School of Dentistry of USC

Excellent Student Award, China Agricultural University, Beijing, China.

Excellent graduates of Shandong Agricultural University, Shandong, China.

Excellent Student Award, Shandong Agricultural University, Shandong, China.

### C. Contribution to Science

#### 1. Explore the regulation of innate immunity and immune evasion by herpes virus.

Innate immunity is the first line of defense in response to invading pathogens. We uncovered Bclaf1, a cellular protein, as a novel regulator of the interferon (IFN) signaling pathway through enhancing the IFN-induced

transcription of antiviral genes. We observed that Bclaf1 promotes the phosphorylation of STAT1 and STAT2 in response to IFN. Additionally, it facilitates the binding of IFN-stimulated gene factor 3 (ISGF3) to IFN-stimulated response elements (ISRE), a crucial step for efficient gene transcription. Notably, the alphaherpesviral kinase, US3, can degrade Bclaf1 in a phosphorylation-dependent manner. This degradation is vital for the ability of alphaherpesviruses to block the expression of antiviral proteins.

(1). **Qin C**, Zhang R, Lang Y, Shao A, Xu A, Feng W, Han J, Wang M, He W, Yu C, Tang J. (2019). Bclaf1 critically regulates the type I interferon response and is degraded by alphaherpesvirus US3. **PLoS Pathogen**. 15, e1007559.

## 2. Characterize the crosstalk of nucleotide synthetic enzymes and innate immunity during viral infection.

Host immune defense and cellular metabolism are two fundamental processes that shape the pathogenesis of viral infection. We discovered that SARS-CoV-2 deploys Nsp9 to activate carbamoyl-phosphate synthetase, aspartate transcarbamoylase, and dihydroorotase (CAD) that catalyzes the rate-limiting steps of the de novo pyrimidine synthesis. Activated CAD not only fuels de novo nucleotide synthesis but also deamidates RelA. While RelA deamidation shuts down NF- $\kappa$ B activation and subsequent inflammatory response, it up-regulates key glycolytic enzymes to promote aerobic glycolysis that provides metabolites for de novo nucleotide synthesis. A newly synthesized small-molecule inhibitor of CAD restores antiviral inflammatory response and depletes the pyrimidine pool, thus effectively impeding SARS-CoV-2 replication.

Another study revealed that CTP synthase 1 (CTPS1), one of the rate-limiting enzymes of pyrimidine synthesis, dampens interferon induction via deamidating interferon regulatory factor 3 (IRF3). Mechanistically, CTPS1 interacts with and deamidates IRF3. CTPS1-mediated deamidation of IRF3 deprives its ability to bind promoters containing IRF3-responsive elements, thus muting IFN induction. Employing CTPS1 conditional knockout and IRF3 deamidated or deamidation-resistant knockin mice, we demonstrated that CTPS1-mediated IRF3 deamidation operates to restrict IFN induction in response to viral infection. This work uncovers a mechanism by which a metabolic enzyme restricts interferon response via deamidation, expanding the functional repertoire of metabolic enzymes in biology.

(2). **Qin C**, Rao Y, Yuan H, Wang T, Zhao J, Espinosa B, Liu Y, Zhang S, Savas AC, Liu Q, Zarinfar M, Rice S, Henley J, Comai L, Graham NA, Chen C, Zhang C and Feng P. (2022). SARS-CoV-2 Couples Evasion of Inflammatory Response to Activated Nucleotide Synthesis. **PNAS**. 119(26):e2122897119

(3). Rao Y<sup>#</sup>, **Qin C**<sup>#</sup>, Savas AC, Liu Q, Feng S, Zhu W, Jiang T, Graham N, and Feng P. (2024). Pyrimidine synthesis enzyme CTP synthetase 1 suppresses antiviral interferon induction by deamidating IRF3. **Immunity**. *In Press*. (<sup>#</sup>co-first author)

(4). **Qin C**, Feng S, Feng P. (2023). STEerING PI4P for innate immune activation. **Immunity**. 14;56(3):463-465.

(5). **Qin C**, Xie T, Yeh WW, Savas AC, Feng P. Metabolic Enzymes in Viral Infection and Host Innate Immunity. **Viruses** 2024, 16(1), 35. (co-corresponding author)

## 3. Examine the role of protein deamidation in cancer cell proliferation.

Uncontrolled proliferation is a hallmark of all cancer cells and requires extraordinary metabolic activity. Understanding how cancer cells rewire cellular metabolism will elucidate molecular events underpinning cancer cell biology, exposing new molecules that can be targeted for antitumor therapy. Carbamoyl-phosphate synthetase, aspartate transcarbamoylase and dihydroorotase (CAD) is the rate-limiting enzyme of de novo pyrimidine synthesis pathway. The established role of CAD in promoting cancer cell proliferation is by facilitating the pyrimidine synthesis pathway. Here, we found that CAD-mediated protein deamidation can reprogram cell metabolism and contribute to cancer cell proliferation. CAD depletion blocked the carbon flux from glycolysis to the pentose phosphate pathway (PPP) and serine synthesis pathway (SSP). Biochemical analyses demonstrate that CAD interacts with and deamidates glucose-6-phosphate dehydrogenase (G6PD) and phosphoglycerate dehydrogenase (PHGDH), which are rate-limiting enzymes of the PPP and SSP, respectively. Deamidated G6PD and PHGDH demonstrate elevated oligomerization and enzymatic activity. Interestingly, the glutaminase domain of CAD is sufficient to deamidate G6PD and PHGDH, thereby promoting nucleotide synthesis and cancer cell proliferation.



(6) **Qin C**, An Z, Feng S, Fu W, Savas AC, Xie T, Brenner C, Saito T, and Feng P. (2024) A pyrimidine synthetic enzyme promotes nucleotide synthesis via activating rate-limiting enzymes of the pentose phosphate pathway and serine synthesis pathway. **Molecular Cell**. In revision.

**Complete List of Published Work in MyBibliography:**

<https://www.ncbi.nlm.nih.gov/myncbi/chao.qin.1/bibliography/public/>

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

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

\*Name of Individual: **Casey Chen**  
Commons ID: CCCHEN

**Other Support – Project/Proposal**

\*Title: **Explore roles of HSV-1 in Alzheimer's disease using mouse models**

\*Major Goals: To investigate how aging and herpesvirus infection interact in normal and AD model mouse, and how aging and herpesvirus infection (as an environmental factor) may synergize to induce neurodegeneration

\*Status of Support: Active

Project Number: R01 AG070904-01A1

Name of PD/PI: Pinghui Feng

\*Source of Support: NIH/NIA

\*Primary Place of Performance: Univ. Southern California

Project/Proposal Start and End Date: 07/01/21-06/30/26

\* Total Award Amount (including Indirect Costs): \$3,990,000

\*Title: **A Miniaturized and High-frequency Acoustic Imaging System for Oral Health and Diseases of the Head and Neck**

\*Major Goals: Create a miniaturized high frequency transducer to diagnose periodontal disease via ultrasound and photoacoustic imaging.

\*Status of Support: Active

Project Number: R01 DE031307

Name of PD/PI: Jesse Jokerst

\*Source of Support: NIH- NIDCR

\*Primary Place of Performance: UCSD; VisualSonics; Univ. Southern California

Project/Proposal Start and End Date: 06/01/22 – 05/31/27

\* Total Award Amount (including Indirect Costs): \$3,425,956

\*Title: **A Chairside Diagnostic to Reduce the Need for Endodontic Retreatment**

\*Major Goals: Identifying study subjects, conducting clinical examinations, collecting samples, detecting *E. faecalis*, developing a protocol for quantitative PCR analysis of *E. faecalis*, and validating the rapid diagnostic test.

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

Name of Individual: Casey Chen  
Commons ID: CCCHEN

\*Status of Support: Active

Project Number: 1R21DE035344

Name of PD/PI: Jesse Jokerst

\*Source of Support: NIH- NIDCR

\*Primary Place of Performance: UNIVERSITY OF CALIFORNIA, SAN DIEGO

Project/Proposal Start and End Date: 07/01/25 – 06/30/27

\* Total Award Amount (including Indirect Costs): \$443,490.00

**Pending**

None

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

\*Signature: \_\_\_\_\_



Date: 10/13/2025

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

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

\*Name of Individual: Feng, Pinghui  
Commons ID: PHFENG

**Other Support – Project/Proposal**

**ACTIVE**

**\*Title: Explore roles of HSV-1 in Alzheimer's disease using mouse models**

\*Major Goals: To investigate how aging and herpesvirus infection interact in normal and AD model mouse, and how aging and herpesvirus infection (as an environmental factor) may synergize to induce neurodegeneration.

\*Status of Support: Active

Project Number: R01 AG070904-01A1

Name of PD/PI: Feng

\*Source of Support: NIH/NIA

\*Primary Place of Performance: University of Southern California

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

\* Total Award Amount (including Indirect Costs): \$3,990,000

**\*Title: Targeting IKKepsilon-mediated nucleotide synthesis in KSHV-associated lymphoma.**

Major Goals: To determine the mechanism by which IKKepsilon fuels lymphoma cell proliferation via activating nucleotide synthesis In KSHV infection.

\*Status of Support: Active

Project Number: R01CA285192

Name of PD/PI: Feng, Pinghui

\*Source of Support: NIH/NCI

\*Primary Place of Performance: University of Southern California

Project/Proposal Start and End Date: 07/01/2023 – 06/30/2028.

\* Total Award Amount (including Indirect Costs): \$1,897,481

**\*Title: Targeting the UL37 deamidase to impede HSV-1 infection.**

Major Goals: To develop small molecules that inhibit UL37 deamidase to combat HSV-1 infection.

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

Name of Individual: FENG, Pinghui  
Commons ID: PHFENG

\*Status of Support: Support: Active

Project Number: 1R21AI180537

Name of PD/PI: Feng, Pinghui

\*Source of Support: NIH

\*Primary Place of Performance: University of Southern California

\* Total Award Amount (including Indirect Costs): \$456,375

Project/Proposal Start and End Date: 05/14/2024-03/31/2026.

**\*Title: Regulated cGAS activation in HSV-1-associated neuropathogenesis.**

\*Major goals: To delineate the molecular mechanism by which cGAS is activated by HSV-1 infection via identifying cGAS ligand and characterizing the CAD-mediated deamidation of cGAS using AD mouse models.

\*Status of Support: Active

Project Number: 1R01AG088625-01A1

Name of PD/PI: Feng

\*Source of Support: NIH/NIA

\*Primary Place of Performance: University of Southern California

Project/Proposal Start and End Date: 09/15/2025-08/31/2029

\* Total Award Amount (including Indirect Costs): \$3,354,022

**\*Title: Explore a key nucleotide synthesis enzyme to develop a broad-spectrum antiviral therapy.**

\*Major goals: To characterize the viral immune evasion mechanisms by which HSV-1, IAV, and SARS-CoV-2 to couple immune evasion to activated nucleotide synthesis via CAD and test the proof-of-principle to develop a broad spectrum antiviral therapy via targeting CAD.

\*Status of Support: Pending

Project Number: 1R01AI184716-01A1

Name of PD/PI: Feng

\*Source of Support: NIH/NIAID

\*Primary Place of Performance: University of Southern California

Project/Proposal Start and End Date: 08/21/2025-07/31/2030

\* Total Award Amount (including Indirect Costs): \$3,969,535

### **Pending**

**\*Title: Harness the dual roles of a kinase to develop an antitumor immunotherapy.**

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

Name of Individual: FENG, Pinghui  
Commons ID: PHFENG

\*Major goals: To develop a small molecule based immunotherapy to treat breast cancer.

\*Status of Support: Pending

Project Number: 1R01 000000-00

Name of PD/PI: Feng

\*Source of Support: NIH

\*Primary Place of Performance: University of Southern California

Project/Proposal Start and End Date: 07/01/2026-06/30/2031

\* Total Award Amount (including Indirect Costs): \$4,141,700.00

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



\*Signature: \_\_\_\_\_

Date: 10/10/2025

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

UEI\*: G88KLJR3KYT5

Budget Type\*: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: University of Southern California

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.	Mirali		Pandya		PD/PI	125,000.00	7.29			75,927.00	24,069.00	99,996.00
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons:		File Name:		Total Senior/Key Person							99,996.00	

B. Other Personnel							
Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
Total Number Other Personnel						Total Other Personnel	
Total Salary, Wages and Fringe Benefits (A+B)							99,996.00

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

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

UEI\*: G88KLJR3KYT5

Budget Type\*: ☒ Project ☐ Subaward/Consortium

Organization: University of Southern California

Start Date\*: 07-01-2026

End Date\*: 06-30-2027

Budget Period: 1

**C. Equipment Description**

List items and dollar amount for each item exceeding \$5,000

**Equipment Item****Funds Requested (\$)\*****Total funds requested for all equipment listed in the attached file****Total Equipment****Additional Equipment:** File Name:**D. Travel****Funds Requested (\$)\***

1. Domestic Travel Costs ( Incl. Canada, Mexico, and U.S. Possessions)

2. Foreign Travel Costs

**Total Travel Cost****E. Participant/Trainee Support Costs****Funds Requested (\$)\***

1. Tuition/Fees/Health Insurance

2. Stipends

3. Travel

4. Subsistence

5. Other:

**Number of Participants/Trainees****Total Participant Trainee Support Costs**

RESEARCH &amp; RELATED Budget (C-E) (Funds Requested)



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

UEI\*: G88KLJR3KYT5

Budget Type\*: ☒ Project ☐ Subaward/Consortium

Organization: University of Southern California

Start Date\*: 07-01-2026

End Date\*: 06-30-2027

Budget Period: 1

<b>F. Other Direct Costs</b>	<b>Funds Requested (\$)*</b>
1. Materials and Supplies	25,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
<b>Total Other Direct Costs</b>	<b>25,000.00</b>

<b>G. Direct Costs</b>	<b>Funds Requested (\$)*</b>
<b>Total Direct Costs (A thru F)</b>	<b>124,996.00</b>

H. Indirect Costs			
Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
1 . Research : On Campus	8	124,996.00	10,000.00
Total Indirect Costs			10,000.00
Cognizant Federal Agency	DHHS, Jeanette Lu, 415-437-7820		
(Agency Name, POC Name, and POC Phone Number)			

<b>I. Total Direct and Indirect Costs</b>	<b>Funds Requested (\$)*</b>
<b>Total Direct and Indirect Institutional Costs (G + H)</b>	<b>134,996.00</b>

<b>J. Fee</b>	<b>Funds Requested (\$)*</b>

<b>K. Total Costs and Fee</b>	<b>Funds Requested (\$)*</b>
	<b>134,996.00</b>

<b>L. Budget Justification*</b>	<b>File Name:</b>
	Budget_Justification_K081052469827.pdf

RESEARCH &amp; RELATED Budget {F-K} (Funds Requested)

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

UEI\*: G88KLJR3KYT5

Budget Type\*: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: University of Southern California

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.	Mirali		Pandya		PD/PI	125,000.00	7.29			75,927.00	24,069.00	99,996.00
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons:		File Name:		Total Senior/Key Person							99,996.00	

B. Other Personnel							
Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
Total Number Other Personnel						Total Other Personnel	
Total Salary, Wages and Fringe Benefits (A+B)							99,996.00

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

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

UEI\*: G88KLJR3KYT5  
Budget Type\*: ☒ Project ☐ Subaward/Consortium  
Organization: University of Southern California

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

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

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

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

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

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

UEI\*: G88KLJR3KYT5

Budget Type\*: ☒ Project ☐ Subaward/Consortium

Organization: University of Southern California

Start Date\*: 07-01-2027

End Date\*: 06-30-2028

Budget Period: 2

<b>F. Other Direct Costs</b>	<b>Funds Requested (\$)*</b>
1. Materials and Supplies	25,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
<b>Total Other Direct Costs</b>	<b>25,000.00</b>

<b>G. Direct Costs</b>	<b>Funds Requested (\$)*</b>
<b>Total Direct Costs (A thru F)</b>	<b>124,996.00</b>

H. Indirect Costs			
Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
1 . Research : On Campus	8	124,996.00	10,000.00
Total Indirect Costs			10,000.00
Cognizant Federal Agency	DHHS, Jeanette Lu, 415-437-7820		
(Agency Name, POC Name, and POC Phone Number)			

<b>I. Total Direct and Indirect Costs</b>	<b>Funds Requested (\$)*</b>
<b>Total Direct and Indirect Institutional Costs (G + H)</b>	<b>134,996.00</b>

<b>J. Fee</b>	<b>Funds Requested (\$)*</b>

<b>K. Total Costs and Fee</b>	<b>Funds Requested (\$)*</b>
	<b>134,996.00</b>

<b>L. Budget Justification*</b>	<b>File Name:</b>
	Budget_Justification_K081052469827.pdf

RESEARCH &amp; RELATED Budget {F-K} (Funds Requested)

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

UEI\*: G88KLJR3KYT5

Budget Type\*: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: University of Southern California

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.	Mirali		Pandya		PD/PI	125,000.00	7.29			75,927.00	24,069.00	99,996.00
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons:		File Name:		Total Senior/Key Person							99,996.00	

B. Other Personnel							
Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
Total Number Other Personnel						Total Other Personnel	
Total Salary, Wages and Fringe Benefits (A+B)							99,996.00

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

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

UEI\*: G88KLJR3KYT5

Budget Type\*: ☒ Project ☐ Subaward/Consortium

Organization: University of Southern California

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	
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Total Equipment
-----------------

Additional Equipment: File Name:

**D. Travel**

Funds Requested (\$)\*

1. Domestic Travel Costs ( Incl. Canada, Mexico, and U.S. Possessions)

2. Foreign Travel Costs

Total Travel Cost
-------------------

**E. Participant/Trainee Support Costs**

Funds Requested (\$)\*

1. Tuition/Fees/Health Insurance

2. Stipends

3. Travel

4. Subsistence

5. Other:

Number of Participants/Trainees
---------------------------------

Total Participant Trainee Support Costs
---

RESEARCH &amp; RELATED Budget (C-E) (Funds Requested)

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

UEI\*: G88KLJR3KYT5

Budget Type\*: ☒ Project ☐ Subaward/Consortium

Organization: University of Southern California

Start Date\*: 07-01-2028

End Date\*: 06-30-2029

Budget Period: 3

<b>F. Other Direct Costs</b>	<b>Funds Requested (\$)*</b>
1. Materials and Supplies	25,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
<b>Total Other Direct Costs</b>	<b>25,000.00</b>

<b>G. Direct Costs</b>	<b>Funds Requested (\$)*</b>
<b>Total Direct Costs (A thru F)</b>	<b>124,996.00</b>

H. Indirect Costs			
Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
1 . Research : On Campus	8	124,996.00	10,000.00
Total Indirect Costs			10,000.00
Cognizant Federal Agency	DHHS, Jeanette Lu, 415-437-7820		
(Agency Name, POC Name, and POC Phone Number)			

<b>I. Total Direct and Indirect Costs</b>	<b>Funds Requested (\$)*</b>
<b>Total Direct and Indirect Institutional Costs (G + H)</b>	<b>134,996.00</b>

<b>J. Fee</b>	<b>Funds Requested (\$)*</b>

<b>K. Total Costs and Fee</b>	<b>Funds Requested (\$)*</b>
	<b>134,996.00</b>

<b>L. Budget Justification*</b>	<b>File Name:</b>
	Budget_Justification_K081052469827.pdf

RESEARCH &amp; RELATED Budget {F-K} (Funds Requested)

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

UEI\*: G88KLJR3KYT5  
Budget Type\*: ☒ Project ☐ Subaward/Consortium  
Enter name of Organization: University of Southern California

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 .	Mirali		Pandya		PD/PI	125,000.00	7.29			75,927.00	24,069.00	99,996.00
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons:		File Name:									Total Senior/Key Person	99,996.00

B. Other Personnel							
Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
Total Number Other Personnel						Total Other Personnel	
						Total Salary, Wages and Fringe Benefits (A+B)	99,996.00

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



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

UEI\*: G88KLJR3KYT5

Budget Type\*: ☒ Project ☐ Subaward/Consortium

Organization: University of Southern California

Start Date\*: 07-01-2029

End Date\*: 06-30-2030

Budget Period: 4

**C. Equipment Description**

List items and dollar amount for each item exceeding \$5,000

**Equipment Item****Funds Requested (\$)\*****Total funds requested for all equipment listed in the attached file****Total Equipment****Additional Equipment:** File Name:**D. Travel****Funds Requested (\$)\***

1. Domestic Travel Costs ( Incl. Canada, Mexico, and U.S. Possessions)

2. Foreign Travel Costs

**Total Travel Cost****E. Participant/Trainee Support Costs****Funds Requested (\$)\***

1. Tuition/Fees/Health Insurance

2. Stipends

3. Travel

4. Subsistence

5. Other:

**Number of Participants/Trainees****Total Participant Trainee Support Costs**

RESEARCH &amp; RELATED Budget (C-E) (Funds Requested)

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

UEI\*: G88KLJR3KYT5

Budget Type\*: ☒ Project ☐ Subaward/Consortium

Organization: University of Southern California

Start Date\*: 07-01-2029

End Date\*: 06-30-2030

Budget Period: 4

<b>F. Other Direct Costs</b>	<b>Funds Requested (\$)*</b>
1. Materials and Supplies	25,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
<b>Total Other Direct Costs</b>	<b>25,000.00</b>

<b>G. Direct Costs</b>	<b>Funds Requested (\$)*</b>
<b>Total Direct Costs (A thru F)</b>	<b>124,996.00</b>

H. Indirect Costs			
Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
1 . Research : On Campus	8	124,996.00	10,000.00
Total Indirect Costs			10,000.00
Cognizant Federal Agency	DHHS, Jeanette Lu, 415-437-7820		
(Agency Name, POC Name, and POC Phone Number)			

<b>I. Total Direct and Indirect Costs</b>	<b>Funds Requested (\$)*</b>
<b>Total Direct and Indirect Institutional Costs (G + H)</b>	<b>134,996.00</b>

<b>J. Fee</b>	<b>Funds Requested (\$)*</b>

<b>K. Total Costs and Fee</b>	<b>Funds Requested (\$)*</b>
	<b>134,996.00</b>

<b>L. Budget Justification*</b>	<b>File Name:</b>
	Budget_Justification_K081052469827.pdf

RESEARCH &amp; RELATED Budget {F-K} (Funds Requested)

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

UEI\*: G88KLJR3KYT5

Budget Type\*: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: University of Southern California

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.	Mirali		Pandya		PD/PI	125,000.00	7.29			75,927.00	24,069.00	99,996.00
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons:		File Name:		Total Senior/Key Person							99,996.00	

B. Other Personnel							
Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
Total Number Other Personnel						Total Other Personnel	
Total Salary, Wages and Fringe Benefits (A+B)							99,996.00

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

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

UEI\*: G88KLJR3KYT5  
Budget Type\*: ☒ Project ☐ Subaward/Consortium  
Organization: University of Southern California

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

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

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

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

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

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

UEI\*: G88KLJR3KYT5

Budget Type\*: ☒ Project ☐ Subaward/Consortium

Organization: University of Southern California

Start Date\*: 07-01-2030

End Date\*: 06-30-2031

Budget Period: 5

<b>F. Other Direct Costs</b>	<b>Funds Requested (\$)*</b>
1. Materials and Supplies	25,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
<b>Total Other Direct Costs</b>	<b>25,000.00</b>

<b>G. Direct Costs</b>	<b>Funds Requested (\$)*</b>
<b>Total Direct Costs (A thru F)</b>	<b>124,996.00</b>

H. Indirect Costs			
Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
1 . Research : On Campus	8	124,996.00	10,000.00
Total Indirect Costs			10,000.00
Cognizant Federal Agency	DHHS, Jeanette Lu, 415-437-7820		
(Agency Name, POC Name, and POC Phone Number)			

<b>I. Total Direct and Indirect Costs</b>	<b>Funds Requested (\$)*</b>
<b>Total Direct and Indirect Institutional Costs (G + H)</b>	<b>134,996.00</b>

<b>J. Fee</b>	<b>Funds Requested (\$)*</b>

<b>K. Total Costs and Fee</b>	<b>Funds Requested (\$)*</b>
	<b>134,996.00</b>

<b>L. Budget Justification*</b>	<b>File Name:</b>
	Budget_Justification_K081052469827.pdf

RESEARCH &amp; RELATED Budget {F-K} (Funds Requested)

## **JUSTIFICATION OF BUDGET**

### **Personnel Justification**

#### **Key Personnel**

##### **Mirali Pandya, DDS, PhD, Principal Investigator**

Dr. Pandya will devote 9.0 calendar months (75% effort) annually to this project. She will be responsible for the overall direction, design, coordination, and execution of all proposed Specific Aims. The K08 program requires a minimum commitment of 75% effort; however, salary support is capped at \$100,000 per year. As Dr. Pandya's institutional salary exceeds this cap, approximately 19% of her salary will be cost-shared by the School to ensure she contributes the full 75% effort required by the program.

##### **Casey Chen, Ph.D., Mentor**

Dr. Chen will provide mentorship in study design, experimental strategy, and data interpretation. He will meet regularly with Dr. Pandya and the mentoring team to ensure continued progress toward the research and career development objectives.

##### **Pinghui Feng, Ph.D., Mentor**

Dr. Feng will provide mentorship in molecular and cellular mechanisms of infection and immune regulation. He will work closely with Dr. Pandya and Dr. Chen to guide scientific development and ensure integration of training and research components.

#### **Fringe Benefits:**

Fringe benefits for faculty and research staff are calculated at 31.70%, based on the current institutional rate.

### **Materials and Supplies**

A total of \$90,000 (\$18,000 per year for Years 1–5) is requested to cover laboratory consumables and supplies necessary for the proposed studies. These include cell culture reagents, molecular biology kits, antibodies, ELISA kits, disposable plasticware, and other consumables directly related to the proposed experiments.

#### **Other Expenses**

##### **Software and Training Courses**

Funds totaling \$10,000 (\$2,000 per year for Years 1–5) are requested to support software licenses and registration fees for specialized courses and online training programs essential to the research and career development components of this K08. Examples include bioinformatics or metabolomics data analysis courses and software such as GraphPad Prism, MetaboAnalyst, or Ingenuity Pathway Analysis (IPA). These resources are critical for data analysis, interpretation, and professional development in alignment with the candidate's training plan.

##### **Publication Costs**

A total of \$8,000 is requested to support publication fees, including open-access charges and color figure costs for manuscripts resulting from this research.

##### **Travel**

Travel funds are requested for Dr. Pandya to attend two scientific meetings each year on the course of the award period to present findings and engage in professional development. Estimated costs include airfare, lodging, registration, per diem, and ground transportation approximately \$5,000 in Year 1 and \$3,000 in Years 2–5.

##### **Indirect Costs**

Per the K08 Program Announcement (PAR), Indirect Costs (Facilities & Administrative [F&A] Costs) are reimbursed at 8% of Modified Total Direct Costs (MTDC). MTDC excludes equipment, tuition remission, and the portion of each subaward exceeding \$25,000.

**RESEARCH & RELATED BUDGET - Cumulative Budget**

	Totals (\$)	
Section A, Senior/Key Person		499,980.00
Section B, Other Personnel		
Total Number Other Personnel		
Total Salary, Wages and Fringe Benefits (A+B)		499,980.00
Section C, Equipment		
Section D, Travel		
1. Domestic		
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		125,000.00
1. Materials and Supplies	125,000.00	
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1		
9. Other 2		
10. Other 3		
11. Other 4		
12. Other 5		
13. Other 6		
14. Other 7		
15. Other 8		
16. Other 9		
17. Other 10		
Section G, Direct Costs (A thru F)		624,980.00
Section H, Indirect Costs		50,000.00
Section I, Total Direct and Indirect Costs (G + H)		674,980.00
Section J, Fee		
Section K, Total Costs and Fee (I + J)		674,980.00



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

Are vertebrate animals euthanized? ☐ Yes ☐ No

If "Yes" to euthanasia

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

☐ Yes ☐ No

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

.....

**2. \*Program Income Section**

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

☐ Yes ☒ No

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

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

### 3. Human Embryonic Stem Cells Section

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

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: [http://grants.nih.gov/stem\\_cells/registry/current.htm](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

<b>Introduction</b>	
1. Introduction to Application (for Resubmission and Revision applications)	
<b>Candidate Section</b>	
2. Candidate Information and Goals for Career Development	Career_Development_and_Training_Plan1052469794.pdf
<b>Research Plan Section</b>	
3. Specific Aims	Specific_Aims1052469793.pdf
4. Research Strategy*	Research_Strategy1052469952.pdf
5. Progress Report Publication List (for Renewal applications)	
6. Training in the Responsible Conduct of Research	Training_in_responsible_Research1052469795.pdf
<b>Other Candidate Information Section</b>	
7. Candidate's Plan to Provide Mentoring	
<b>Mentor, Co-Mentor, Consultant, Collaborators Section</b>	
8. Plans and Statements of Mentor and Co-Mentor(s)	Mentor_LOS1052469796.pdf
9. Letters of Support from Collaborators, Contributors, and Consultants	LOS1052469806.pdf
<b>Environment and Institutional Commitment to Candidate Section</b>	
10. Description of Institutional Environment	USC_institutional_Enviornment1052469798.pdf
11. Institutional Commitment to Candidate's Research Career Development	Letter_of_Support_Dean_Chai_K081052469799.pdf
12. Description of Candidate's Contribution to Program Goals	
<b>Other Research Plan Section</b>	
13. Vertebrate Animals	
14. Select Agent Research	
15. Consortium/Contractual Arrangements	
16. Resource Sharing	Resource_Sharing_Plan_K081052469783.pdf
17. Other Plan(s)	DMS_Plan1052469784.pdf
18. Authentication of Key Biological and/or Chemical Resources	Authentication_of_Key_Biologicals1052469785.pdf
<b>Appendix</b>	
19. Appendix	

## PHS 398 Career Development Award Supplemental Form

Citizenship\*:

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

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

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

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

## Career Development and Training Plan for K08 Application

### A. Candidate's Background

As a dedicated clinician-scientist, I am committed to transforming and advancing the field of Periodontology through clinical and translational research. My research career has been built on my undergraduate dental degree, earned in 2012, and deep research methodologies perfected at a tissue culture lab in NUS Singapore. In addition, I earned a PhD in Oral Biology at Texas A&M College of Dentistry under the able guidance of Dr. Thomas Diekwisch, an innovator and a visionary, where my dissertation invigorated the research community to gain a new fresh perspective on enamel formation and post translational modifications in amelogenin protein while helping shed new investigative clarity on the temporo-spatial, mineralogic, spectroscopic and proteomic analysis of tooth enamel development. In my opinion, my pathbreaking research, coupled with my 9 first-authored publications and 11 co-authored papers in high-impact peer-reviewed journals, can fundamentally and constructively contribute to dental research.

I would also like to highlight that, following my PhD, I completed a three-year residency in Periodontics at Texas A&M College of Dentistry, where I gained extensive clinical experience in diagnosing and managing periodontal diseases, including surgical interventions and non-surgical therapies. For my master's thesis, I was convinced that the theoretical underpinnings had to be supported and corroborated with real-world impact. Consequently, I chose to conduct a pilot clinical study with the goal of understanding the importance of visualization in periodontal surgery, and the effect of any residual calculus on the transformation in the microbiome and clinical parameters of patients before and after periodontal surgery. For analysis, I collaborated with an industrial company that facilitated the analysis of GCF fluid collected from the patients at various timepoints before and after surgery and compared the variations in the microbiome based on the effect of a simple visualization enhancement tool. Such an exhaustive exercise sharpened my skills as a periodontist in patient recruitment, sample collection including gingival tissues, gingival crevicular fluid and saliva, and ethical considerations in human subjects research, as well as protection of patient's privacy.

My persistent and indefatigable pursuits to conduct research and provide cost-effective innovative solutions to the patient population took me to join USC as a faculty in 2024. I chose Ostrow School of Dentistry because of its excellence in teaching, research and patient care. I was also seeking a supportive environment to explore my pathway as a clinician-scientist. I have been fortunate to be mentored by accomplished researchers and forward-looking scientists Dr. Casey Chen and Dr. Pinghui Feng. They provide complementary expertise in clinical and basic sciences. After a few months participating in various activities in their labs, I was drawn to explore the relationship between metabolism and periodontitis. We collected and analyzed gingival tissues utilizing mass spectrometry from patients diagnosed with health and periodontitis. Interestingly, the tissue samples from periodontitis demonstrated downregulation of mitochondrial complex I genes and subsequent succinate accumulation compared to healthy samples. This critical discovery, derived from a small cohort of patient samples, has ignited my foundational interest in succinate's dual role as a biomarker and a mechanistic driver in periodontitis progression.

My PhD proficiency in proteomics and creating novel *in vitro* cell culture models positions me for a very strong foundation in researching metabolomics aspects, integrating multi-omics data, and utilization of single-cell RNA sequencing (scRNA-seq) to infer cell-specific pathways. With the ongoing guidance and inspiration provided by my mentors, I am focused on examining the intricate metabolic immune relations in Periodontal disease. In conclusion, by undergoing focused training, the K08 funding will help me transition from being a mentored researcher to an independent investigator who can oversee NIH-funded studies on the pathophysiology of periodontitis.

### B. Career Goals and Objectives

My long-term professional objective is to be an independent, R01-funded researcher with expertise in translational periodontal studies while focusing on how immune pathways and metabolites interact in chronic oral disorders. As one of the main causes of tooth loss in the United States, periodontitis affects more than 50% of individuals[3]. Studies have established that systemic diseases including diabetes and cardiovascular

disease are also associated with periodontal disease[6, 8]. The vast majority of currently offered treatments are non-specific, and current diagnostics rely on clinical indicators that only identify disease after considerable tissue damage. By identifying succinate, a TCA cycle metabolite that is upregulated in a dysbiotic oral environment, and investigating it as a non-invasive diagnostic and mechanistic target for early intervention, my research vision will allow us to overcome the current knowledge gaps.

By focusing on my three interrelated goals formulated by expansion of my preliminary data from human subjects, the K08 supports my goals to (1) validate succinate as a biomarker through integrated proteomics and metabolomics (2) mechanistically explore how downregulation in mitochondrial complex I genes leads to succinate accumulation and inflammation using in vitro coculture models with human oral keratinocytes and periodontopathogens like *P. gingivalis* and *A. actinomycetemcomitans* (3) elucidate succinate-associated inflammatory pathways via scRNA-seq by analyzing gingival tissues from human patients and provide functional validation for Aim 1 and Aim 2.

Executing these goals will allow me to generate a research path and preliminary data for the next grants, such as an R01 grant to investigate treatments that target succinate. In addition, my expertise to convert human-derived correlates into tractable models will be strengthened by the K08 training, and will promote innovation in medicine and periodontal disease diagnosis. These funding opportunities will decisively position me to mentor the upcoming generation of clinician-scientists and support NIH/NIDCR initiatives related to precision medicine and oral health disparities.

### **C. Career Development and Training Activities During the Award Period**

The five-year K08 period will feature a comprehensive, milestone-driven plan integrating hands-on laboratory training, bioinformatics proficiency, as well as professional development. My research activities are tailored to the three aims, leveraging the complementary expertise of my primary mentors: Dr. Casey Chen, a dual-trained periodontist with over 30 years of experience in microbiology who has mentored over a total of 72 trainees since 1992, including 7 postdoctoral fellows, 18 doctoral students, 7 DDS students, 16 residents, and 23 undergraduate students, and Dr. Pinghui Feng, a PhD virologist whose NIDCR, NCI, and NIAID-funded research on protein deamidation links metabolic enzymes to immune responses and who has mentored more than 9 PhD students and 14 post-docs through his career so far. A Career Advisory Committee (CAC), comprising Drs. Chen and Feng plus one other experts in meta-omics and sc-RNAseq, Dr. Dechen Lin and collaborator Dr. Chao Qin, will meet once every three months to evaluate the progress, adjust any research plans and troubleshoot any issues while ensuring timely milestones.

#### **Year 1: Foundational Training and Aim 1 Initiation**

In my first year, I will focus on Aim 1 and patient subject recruitment. Under Dr. Chen's guidance and with our approved IRB, I will work on refining current protocols for collecting, processing and storing patient samples of gingival tissue, GCF, and saliva from healthy and periodontitis cohorts, drawing on his expertise in microbial markers and clinical therapy. Weekly meetings with Dr. Chen will cover study design, including ethical recruitment and stratification by disease severity. I will also initiate working with Dr. Chao who has an expertise in metabolomics and proteomics to learn targeted techniques like LC-MS/MS for succinate quantification and mass spectrometry for proteomics integration. Additionally, I plan to attend the online National Cancer Institute offered "Introduction to Bioinformatics" summer workshop to gain introduction to bioinformatics tools, computing systems and software such as R and Python for pathway mapping. Milestones: Begin recruitment of the initial patient cohort, and submit an abstract to the International Association for Dental Research (IADR) meeting.

#### **Year 2: Continue Patient Recruitment for Aim 1, In Vitro Modeling and Aim 2 Execution**

Building on my foundational skills, Year 2 will emphasize on continuing patient recruitment for Aim 1 and mechanistic studies for Aim 2, which focus on understanding the mechanism behind mitochondrial complex I downregulation by using ex vivo models. With my prior experience in establishing cell culture models from my PhD and Dr. Chen and Dr. Feng will provide guidance and mentorship in establishing ex vivo coculture

models, leveraging their combined research experience on virulence determinants of periodontal pathogens. I also gathered preliminary data by culturing HOK/NOK cells with pathogenic bacteria like *P. gingivalis* or *A. actinomycetemcomitans* for Aim 2. The plan is to further refine my skillset and get continued guidance on monitoring MOI and assessing gene expression via RT-PCR and protein quantification with western blotting for complex I subunits. To integrate the metabolic insights, I will meet biweekly with my mentors to explore how succinate accumulation might be influenced by downregulation of mitochondrial complex I genes. This will inform correlations between succinate levels and inflammatory markers like CRP, IL-1 $\beta$ , IL-6, TNF $\alpha$ . Dr. Chen's lab resources, including anaerobic chambers and microbial strain libraries, will facilitate this. Dr. Feng will help mentor interventions like siRNA knockdowns and alternative use of inhibitors, linking complex I dysfunction to succinate buildup through metabolic enzyme pathways. I will participate in a hands-on training with Dr. Feng's post-doctoral researchers with expertise on mitochondrial assays. I also plan to continue to advance my bioinformatics training through self-paced tutorials on R for statistical correlations. Milestones: Generate preliminary *ex vivo* data and present at the American Academy of Periodontology (AAP) annual meeting.

### **Year 3: Advanced Omics and Aim 3 Advancement**

Year 3 will focus on the accomplishment of Aim 2 by concluding any remaining *in vitro* studies and initiate scRNA-seq analysis for Aim 3. Dr. Feng and Dr. Chao will oversee tissue dissociation and library preparation using 10x Genomics. We will attempt to stratify samples by succinate levels from Aim 1 and perform clustering/differential expression analyses to identify any cell-specific pathways. Focus will also be on establishing an *ex vivo* model to downregulate succinate via succinate addition or SDH inhibition will allow us to establish a causal relationship between inflammation and succinate. Interpreting metabolic-immune connections, such as how succinate affects inflammatory gene expression, will require Dr. Feng's assistance. Data integration across goals will be facilitated by biweekly joint meetings with both mentors. A plan to enroll in relevant Single-Cell Genomics courses offered online by Bioinformatics Training and Education Program (BTEP) at NCI/NIH and use Sanger lab's scRNAseq resources and online course offering will be made. Dr. Feng also has a vast ShRNA library that will be readily available to me for the experiments. Milestones: Complete scRNAseq on initial selected samples, complete studies for Aim 2 and submit a first-author manuscript on Aim 1 findings.

### **Years 4-5: Integration and Independence**

In the last two years of the K08 grant, I aim to synthesize data across aims, validating cross-correlations and completion of any pending experiments. Mentorship will transition to consultative, with quarterly reviews focusing on grant writing. Professional development will include attending the NIDCR K Awardees Workshop and submitting an R01 proposal in Year 5, building on the K08 data.

Additional activities span across all years: Annual responsible conduct of research training, bi-annual manuscript/grant writing workshops, and other relevant seminars. I will aim for 1-2 publications per year starting year 2, targeting high impact peer reviewed journals. Additional research training will occur through attendance at a joint weekly research lab meeting. I will regularly present my work at Dr. Chen's and Dr. Feng's joint lab meetings, and will formally present my work yearly at the AADOCR/ IADR meetings/ AAP meetings. I will also attend seminars offered periodically by USC on grant and manuscript writing and ethics in biomedical research.

Other responsibilities during the period of this award include attending half a day every week in the faculty practice clinic, and dedicating one day per week for teaching and supervising patient care in the predoctoral and post-graduate clinics. In addition, for recruiting patients for this K08, I will be coordinating with the Advanced Graduate periodontics clinic patients, gathering consents and collecting samples. This would allow me to dedicate 75% of my time to focus exclusively on the proposed K08 research.

## K08 Grant: “Probing the Role of a Succinate-Related Metabolic Reprogram in Periodontal Pathogenesis”

### Specific Aims:

Periodontitis is a chronic inflammatory disease of the oral cavity that is known to contribute to systemic inflammation and is associated with metabolic syndromes[6-8]. Current evidence from recent literature implicates metabolic reprogramming as a key driver of chronic inflammation[9, 10]. Among different metabolites, succinate, a metabolite with a significant role in tricarboxylic acid (TCA) cycle, has certainly gained attention as pro-inflammatory signal[11, 12] and mitochondrial dysfunction marker[13]. In this study, we have obtained preliminary data from gingival tissue collected during periodontal surgery of healthy and periodontitis patients. Our data demonstrates that a significant increase in the succinate levels correlates with simultaneous reduction in levels of mitochondrial complex I proteins NDUFA12, NDUFB1, NDUFS8, protein regulating alternative pathway of complement system CFH, and protein regulating TCA cycle metabolic influx FAH, all key enzymes involved in succinate metabolism. Furthermore, succinate accumulation may actively suppress oxidative phosphorylation[14], disrupt immune homeostasis[15], and reinforce chronic inflammation[16]. These observations suggest a molecular link between the downregulation of mitochondrial complex I, succinate accumulation, and immune regulation during periodontitis. Based on our preliminary data, we hypothesize that impaired mitochondrial electron transport induces succinate accumulation, thereby provoking inflammation to fuel periodontitis.

**Aim 1: Establish and validate relationship between succinate levels and mitochondrial dysregulation in a larger cohort of periodontitis patients and explore the potential of succinate as a biomarker.** Working hypothesis: Succinate levels in gingival tissue, gingival crevicular fluid (GCF), and saliva are elevated in periodontal disease patients compared to healthy controls and correlate with disease severity and progression. Leveraging periodontal biological samples, including gingival tissue, GCF, and saliva, from healthy individuals and patients with Stage III and IV Grade B periodontitis, we will perform metabolomics and proteomics analyses to identify the succinate-related metabolic signatures and explore succinate as a biomarker for periodontitis. Our findings from this aim will validate the succinate's sensitivity and specificity as a potential non-invasive biomarker for periodontitis.

**Aim 2: Investigate the role of downregulation of mitochondrial oxidative phosphorylation enzymes in driving succinate accumulation and inflammatory response in periodontitis.** Working hypothesis: In oral keratinocytes cocultured with keystone periodontal pathogens, microbial infection induced succinate accumulation in human oral keratinocytes cultured *ex vivo*, providing a model system to interrogate the underlying molecular mechanism. Capitalizing this model system, we will employ *Porphyromonas gingivalis* or *Aggregatibacter actinomycetemcomitans* to infect human oral keratinocytes to dissect the causal roles of inhibition of mitochondrial complex I components in succinate accumulation. Inhibition of mitochondrial complex I components can be achieved via genetic depletion and pharmacological or natural inhibitors like dimethyl malonate and itaconate. With this aim, we will unveil a molecular link between complex I downregulation and succinate buildup, guiding future therapeutics targeting SDH to treat periodontal diseases.

**Aim 3: Determine the contribution of elevated succinate levels to periodontal inflammation.** Working hypothesis: Elevated succinate levels in periodontitis correlate with upregulated inflammatory gene expression. To test this hypothesis, we will perform single-cell RNA-seq (ScRNA-seq) to identify key genes and pathways thereof whose expression are significantly altered by accumulated succinate. Leveraging the *ex vivo* cultured HOK system, we will manipulate SDH and succinate to monitor inflammatory gene expression. Guided by the single-cell RNAseq data, we will also interrogate the roles of succinate in immune cell infiltrated into the periodontal tissue. Findings from this work will elucidate the global impact of succinate in inflammation and periodontal diseases, broadening the research horizon of my research into multi-omics analysis integrated for periodontal studies.

The scientific objective of the K08 is to define the role of succinate as a biomarker and pathogenic driver in periodontitis progression, with the vision that it may be useful to uncover host-microbe interactions in *ex vivo* models of disease and ultimately improve options for diagnostics and next-generation therapeutic strategies. This portfolio of research and training will enable me to master and develop techniques and tools to translate potential correlates of periodontal disease from human studies into tractable systems to probe mechanisms of periodontal pathogenesis.



## B. Significance

**1. Periodontitis in Oral and Systemic Health:** Periodontal disease is a chronic inflammatory disease of the periodontium and nearly 50% of the people over the age of 35 are diagnosed with it[3]. Periodontitis is a leading cause of tooth loss[17] along with dental caries, thereby causing a negative impact on the life as well as economy. According to the 2017 periodontal world workshop, periodontitis can be classified into a stage and grade based on the severity of the disease[18]. Periodontitis is characterized by the progressive destruction of gingival tissue, periodontal ligament and alveolar bone, and currently the only way to identify and diagnose periodontal disease progression is by clinical and radiographic examination. Key pathogens implicated in causing periodontitis like *Porphyromonas Gingivalis*[19] has also been related to cardiovascular diseases[20, 21], diabetes[22], Alzheimer's[23], rheumatoid arthritis[24] and many other systemic conditions[25]. Unfortunately, due to the chronic nature of periodontal disease, it is identified at a point where there has already been a major destruction of the periodontal tissues. Due to this major drawback, finding noninvasive biomarkers of to assess severity and progress of periodontal disease is very crucial.

**2. Succinate in Inflammation and Periodontitis:** Role of succinate in periodontal disease has recently been under study[2, 26]. Succinate is a tricarboxylic acid (TCA) cycle metabolite that has been identified as one of the key players in metabolic reprogramming of the tissue in periodontal pathogenesis. Under inflammatory condition, changes in cellular metabolism can promote glycolysis and induce the accumulation of TCA cycle intermediates, such as succinate. Indeed, hypoxia, oxidative stress, or microbial assault alters the metabolism of TCA enzymes, leading to the accumulation of succinate[27]. Succinate functions as a signaling molecule in periodontitis by binding to succinate receptor 1, a G-protein-coupled receptor[1]. Succinate is known to alter gene expression via epigenetic regulation by inhibiting  $\alpha$ -ketoglutarate-dependent dioxygenases, including histone and DNA demethylases, which leads to hypermethylation and subsequent gene silencing[28]. However, the translation of succinate biology into clinical applications for periodontitis is still constrained by a few knowledge gaps, notwithstanding these discoveries.

The first significant gap is that although gingival tissues from periodontitis patients have higher succinate levels, its potential as a biomarker for the severity and course of the disease has not yet been investigated. Most studies so far have focused on isolated metabolites that have limited data as it does not take into account the complex nature of periodontal disease. Our approach would be correlating succinate across gingival tissue, gingival crevicular fluid, and saliva which are some of the non-invasive resources ideal for routine screening which takes the interplay between host response and oral microbiome into account. This gap hinders the development of diagnostic techniques that could detect subclinical inflammation or predict treatment responses which may be especially helpful in underserved populations where access to advanced imaging and professional care is limited.

The second gap pertains to an incomplete elucidation of the molecular causes of succinate buildup in periodontal cells, which our pilot data from human patient points to the possible role of mitochondrial dysfunction. *P. gingivalis* and other periodontal pathogens likely caused the mitochondrial reprogramming, which includes downregulating complex I enzymes like NDUFS8, NDUFB1 and other electron transport chain components. This hinders oxidative phosphorylation, thereby favoring succinate accumulation through the inhibition or reversal of succinate dehydrogenase. However, there are no clear causal connections found in periodontitis models between succinate buildup and complex I downregulation. I will establish an ex vivo system that could break down these pathways and pinpoint intervention sites utilizing pertinent cell types, such as human oral keratinocytes (HOK) co-cultured with pathogens. Further genetic and biochemical manipulation of metabolic enzymes will provide mechanistic insights into the causal roles of these components in periodontal inflammatory diseases.

Third gap is that there is very minimal knowledge about the relationship between increased succinate and inflammatory gene expression at the cellular level. Although bulk RNA sequencing studies have been published related to periodontitis[29, 30], it obscures the periodontium's biological heterogeneity, where various cell population such as fibroblasts and epithelial cells may respond differently. Our latest pubmed search results show that there are no published studies till date that have used scRNA-seq focusing on examining succinate in periodontitis[31-33], and this technique may be able to uncover some cell-specific mechanisms such SUCNR1-mediated signaling in macrophages or osteoclast precursors. By addressing this issue, we could find new inflammatory networks and connect systemic comorbidities to local succinate accumulation effects.

## C. Innovation

My K08 study recommends comparing healthy tissue from gingivitis or periodontal health to diseased gingival tissue during periodontitis to examine the function of succinate buildup and related signaling pathways. This study's novelty is found in two major points: 1) Using multi-omics techniques that combine transcriptomic, proteomic, and metabolomic data to identify important succinate-related signaling nodes, which would reveal interactions between inflammatory, microbial dysbiosis, and bone resorption pathways that have not been thoroughly studied though they have been separately explored up to this point. These discoveries will help us better understand how succinate contributes to immunological dysregulation and tissue damage in periodontitis, opening the door to more focused functional research and innovative treatments. 2) Using the large cohort of human data to explore a new biomarker and use integrated multi-omics framework to identify distinct succinate-driven signs of periodontitis, setting them apart from other inflammatory conditions and emphasizing on any future potential treatments, like receptor antagonists to reduce inflammation and stop the loss of alveolar bone.

## D. Approach

My approach is structured around three specific aims, with methods designed to ensure rigor, reproducibility, and feasibility within my 5-year K08 timeline. Years 1 and 2 will focus on patient recruitment, sample collection, and initial omics analyses for Aims 1 and 2. Years 3 and 4 will emphasize mechanistic *ex vivo* studies and scRNAseq for Aims 2 and 3. Year 5 will focus on integrating data across aims, perform validation experiments, and follow-on R grants. Manuscripts will be prepared and submitted to peer reviewed journals through year 2 to year 5. All studies will adhere to NIH guidelines for rigor and reproducibility, including authentication of key biological and chemical resources for our cell lines, antibodies via validation certificates, blinded sample processing and data analysis, and maintenance of HIPPA privacy laws and protection of patient data. Experiments will include minimum  $n=3$  biological replicates, with power calculations based on pilot data assuming  $\alpha=0.05$  and 80% power using G\*Power software. Data management will use secure encrypted platforms for any clinical data and NIH repositories for omics data sharing. Preliminary data from my pilot mass spectrometry (LC-MS) study of gingival tissues from 4 healthy/gingivitis and 5 periodontitis patients demonstrated a significant succinate elevation ( $p<0.01$ ) and downregulation of mitochondrial complex I genes, confirming feasibility and providing baseline variability for power estimates.

## Preliminary data

**Patient recruitment:** Patient recruitment for the pilot study was conducted in the Advanced Graduate Periodontics clinic at the Herman Ostrow School of Dentistry at USC. Tissue samples were collected from nine subjects with a periodontal diagnosis provided by the Periodontics resident as per the AAP guidelines[18]: For the diseased group, three patients diagnosed with generalized periodontitis stage III, grade B, one patient with generalized periodontitis with stage II, grade B, and one with generalized periodontitis stage III, grade C. Four subjects diagnosed with gingivitis were included in the healthy group. Gingival tissues were collected during osseous surgery/open flap debridement for the diseased group or crown lengthening surgery for the healthy controls. The tissues from the periodontitis patients were collected from the sites with 5-9 mm of PPD, BOP (+), and 5-10 mm of clinical attachment loss (CAL). The tissues from subjects with gingivitis were collected from the sites with 2-4 mm PPD, BOP (+/-), and  $\leq 1$  mm gingival recession, and no CAL due to periodontitis.

**Cell and bacteria culture:** Normal human oral keratinocytes (NOK), from [Dr.Münger's](#) lab at Tufts University were cultured with keratinocyte SFM culture media (1X) (Gibco 17005042). Human papillomavirus 16-keratinocytes, (HOK16B cells)[34], from Dr. Ren Sun's lab at UCLA. HOK cells were cultured with KBM™ Keratinocyte Basal Medium (LONZA, CC-3104) with KGM® Gold Keratinocyte Growth Medium SingleQuots® Supplements and Growth Factors (LONZA, 00192152). Both cell lines were cultured in 6-well plates with one plate for control and one plate for the experimental group until they reached ~80% confluency. Simultaneously, *Aggregatibacter actinomycetemcomitans* (Aa) strain D7S-1 was routinely cultured in a modified trypticase soy broth (mTSB) containing 3% trypticase soy broth and 0.6% yeast extract or on mTSB with 1.5% agar [Becton Dickinson and Company, Franklin Lakes, NJ, USA] and incubated in an atmosphere supplemented with 5% CO<sub>2</sub> at 37 °C in a humidified incubator. Aa cells were collected from an overnight liquid culture, washed with sterile 1x PBS, and the optical density of the culture was adjusted to 1.0 in PBS. For the Aa infected group, 10<sup>5</sup> CFU/ml Aa were added to each well and allowed to coculture for 48 hours. At the end of the experiment, cells were washed with ammonium nitrate and harvested for metabolite analysis by mass spectrometry.

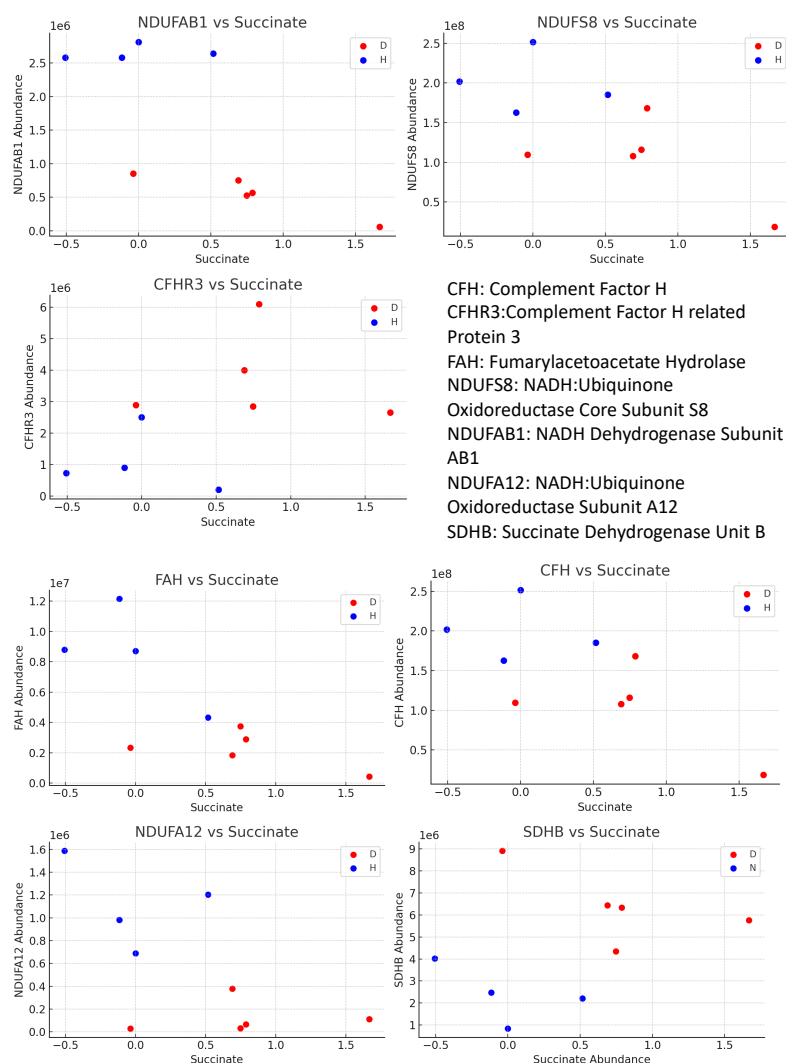
**Sample collection:** For human tissue samples, gingival tissues obtained from the patients during periodontal surgery were washed with cold PBS, snap-frozen with liquid nitrogen, and stored at -80 °C until further use. The tissues were homogenized using Zirconia/Silica Beads and beads beater (Bio-Spec Products Inc.) and used for quantitative analysis of metabolites, proteins, and mRNA (transcriptome). For the cell culture, after freezing with 80% methanol at -80 °C overnight, cell scraper was used to collect the cells for further analysis.

**Metabolite profiling analysis:** For the tissues, the metabolites from the homogenized healthy and diseased tissues (~25 mg) were extracted twice with 80% methanol at -80 °C and vacuum dried. The protein pellet was resuspended in RIPA lysis buffer for normalization, and the protein concentration was determined using the BCA assay. Equivalent amounts of extracts were used for the LC-MS run. Dried metabolites were re-suspended in water and analyzed on a Q-Exactive Plus hybrid quadrupole-Orbitrap mass spectrometer coupled to Vanquish UHPLC system (Thermo Fisher) as described in the several publications from Dr. Feng's group[35-37].



metabolites increased in diseased gingival tissues, succinate of the TCA cycle was significantly increased in periodontitis tissues compared to that in healthy gingival tissues (**Fig 1**). We also note an increase in other TCA cycle related metabolites like the  $\alpha$ -Ketoglutarate which is immediately upstream of the succinate in the TCA cycle, aconitate which is an intermediate between the citrate and isocitrate and flavin adenine dinucleotide (FAD) which is a cofactor for succinate dehydrogenase and required for succinate oxidation. To further explore the molecular mechanism underlying the metabolic changes, we performed a proteomics analysis aiming to identify altered metabolic enzymes whose expression is high enough to MS-based quantification (**Fig 2**). In the volcano plot, succinate-related genes such as GRN (progranulin) and PYHIN1 (a DNA sensor in the PYHIN family), that have been linked to succinate-associated pathways like inflammation[39, 40], and immune response are activated in the diseased state suggesting that succinate drives their expression, potentially amplifying disease processes. The overall plot reveals a bias toward upregulation in succinate-related genes, potentially implying metabolic reprogramming favors pro-inflammatory processes in periodontitis, while significant downregulation of mitochondrial genes like NDUFA12 that support succinate's role in impairing energy metabolism. Surprisingly, our MS analysis showed that succinate dehydrogenase subunit B (SDHB), the catalytic subunit of the SDH complex, was significantly higher in periodontitis gingival tissues than that of healthy gingival tissues. The increase in SDHB likely reflects a feedback upregulation resulting from succinate accumulation. Mitochondrial complex I proteins such as NDUFA12, NDUFS8 and NDUFAB1 demonstrated a decrease in periodontitis tissues compared to healthy gingival tissues. When correlation analysis was performed between succinate and the levels of the complex I components, we observed an inverse correlation between succinate and these metabolic proteins/enzymes (**Fig 3A**). There is also a direct correlation noted between increase in the complement pathway related proteins CFHR3 and decrease in CFH indicating that CFHR3, that may be acting as a competitor for CFH and amplify alternative pathway activation, potentially leading to inflammatory exacerbation in periodontitis. FAH is inversely related to succinate increase in diseased samples which could point to a disruption in the tyrosine catabolism leading to a toxic metabolite buildup. Together, these results suggest that the reduction in mitochondrial complex I components may contribute to the succinate accumulation.

Further, a co-cultured cell model with periodontal pathogenic bacteria was developed to allow a controlled system for performing additional mechanistic studies and establish a causal validation based on using gene knockdowns and inhibitors. The samples were analyzed by mass spectrometry to compare the level of succinate between the control and Aa-infected group. Our metabolite analysis reveals a significant difference in the succinate levels between mock- and Aa-infected oral keratinocytes (**Fig 4**) with succinate accumulation noted in the Aa-infected group. A significant increase was also observed in some of the other TCA cycle metabolites such as  $\text{NAD}^+$ , citrate/isocitrate and  $\alpha$ -ketoglutarate ( $\alpha$ -KG) in the Aa-infected group, suggesting the overall inhibition of mitochondrial oxidative phosphorylation that may lead to metabolite accumulation, which has to be determined by seahorse assay and further analysis.



**Figure 3. Correlation analysis between metabolite and proteins.** Correlation between proteins with functions implicated in biological processes relevant to succinate with inverse correlation between levels of succinate and mitochondrial complex I genes including FAH and CFH. Direct positive correlation noted between succinate and succinate related proteins SDHB and CFHR3.

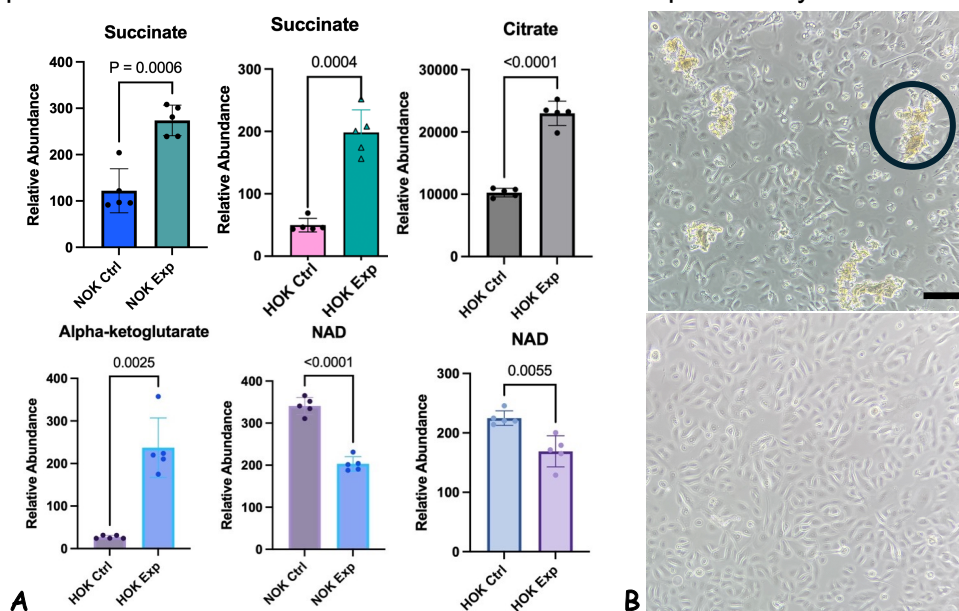


## Specific Aim 1: Perform metabolomics and proteomics analyses with a larger cohort of periodontitis patients to explore the biological significance and potential of succinate as a biomarker

**Hypothesis:** Succinate accumulation, integrated with proteomic profiling, will correlate with periodontitis severity and progression, serving as a novel non-invasive biomarker.

**Patient recruitment and sample collection:** We plan to enroll 30 healthy/gingivitis controls with no active periodontitis, probing depth <3 mm, no bleeding on probing and 75 periodontitis patients (stages II-IV per 2017 AAP world workshop classification, confirmed by full-mouth radiographs and clinical exam) from our Advanced Graduate Periodontics clinic, with IRB approval and informed consent. Inclusion criteria: age 18 and above, non-smokers, no systemic antibiotics in the past 3 months, scheduled for a crown lengthening surgery for healthy/gingivitis patients and for an osseous/open flap debridement surgery for periodontitis patients; exclusion criteria: pregnancy, uncontrolled diabetes (HbA1c >7%), or other inflammatory diseases and diseases affecting bone metabolism. Tissue samples will be collected during the surgical procedure appointment, GCF will be collected via Periopaper strips from the most severely affected sites, 30s collection per site, along with unstimulated whole saliva 5 mL via passive drool prior to the surgery. Tissue samples will be snap-frozen in liquid nitrogen immediately and stored at -80°C until processed further for mass spectrometry, qPCR and western blot. Clinical data will be monitored via changes in pocket depth, attachment loss, and bleeding on probing. These samples will be processed for proteomics and metabolomics as mentioned in the preliminary data.

For metabolites, we will systematically analyze those of the central carbon metabolism, including the glycolysis, pentose phosphate pathway (PPP), serine synthesis pathway (SSP), nucleotide synthesis, and TCA cycle. Emphasis will be placed on the metabolites of the TCA cycle from pyruvate and glutamine, two entry portals via glucose and glutamine. In parallel, gingival tissues will be processed to perform proteomics analysis with the focus to quantitatively determine the abundance of central carbon metabolic enzymes that catalyze reactions of the aforementioned pathways. Given that the metabolic enzymes are among the most abundant proteins in cells, we expected that most, if not all, enzymes will be quantified by



**Figure 4. Metabolomics results from cell culture experiment** (A) Quantitative comparison between the control (Mock) and experimental group (*Aa* infected) for metabolites such as succinate and other TCA cycle metabolites like NAD, citrate/isocitrate and Alpha ketoglutarate in NOK and HOK cells. (B) NOK cells cultured with (top) and without (bottom) *A. actinomycetemcomitans* (marked with a circle) bacteria. Scale bar - 100 μm

tandem MS analysis. With abundance of metabolites and metabolic enzymes, we will perform correlation analysis. Considering succinate being the most significantly elevated metabolite in periodontitis tissue, we will first expand the correlation analysis to all enzymes/proteins that were altered in periodontitis tissue based on our preliminary data, focusing primarily on the mitochondrial electron transport chain like NDUFS8 and NDUFB1, TCA cycle intermediates, while the inflammatory signaling proteins such as C-Reactive protein (CRP), NF-κB, HIF-1α, IL-1, and IL-6, oxidative stress markers including ROS-related enzymes, and osteoclastogenesis factors may need to be quantified using western blot, ELISA, or multiplexed protein arrays (to save the precious patient samples) as their detection level may be too low for mass-spec. Alternatively, real-time PCR or RNAseq will reveal their mRNA expression levels (see Aim 3). Initial analysis has identified additional enzymes in these pathways including SDHB of the TCA cycle/complex II, and NDUFS8 of complex I. We reason that the analysis with a larger cohort will improve the statistical significance of these correlations and more paired correlation with significance will be identified. Because fumarate is a byproduct of succinate metabolism and is among the most altered metabolites in diseased tissues, we will also perform initial analysis using fumarate, and a few other

metabolites such as  $\alpha$ -KG, OAA, aspartate and malate levels, as they are very important to the TCA cycle as well. The initial screen will further focus on pathways relevant to succinate metabolism and signaling, expand to all proteins identified in our proteomics dataset, integrate with metabolomics from gingival tissue, gingival crevicular fluid, and saliva to establish succinate as a potential non-invasive biomarker for disease severity and progression.

**Anticipated Results:** We expect 1) Correlations between succinate and other TCA cycle metabolites, mitochondrial complex I and complex II proteins, and inflammatory proteins will be much more apparent and significant between healthy and diseased gingival tissues from non-periodontitis and periodontitis patients. 2) The correlation between the level of succinate accumulation and disease severity will help us establish succinate as a non-invasive and useful biomarker for periodontal disease.

**Pitfalls and Alternatives:** Although we do not anticipate any major setbacks for this aim, some of the potential delays could be: 1) Gingival tissues from patients with gingivitis may exhibit variation compared to those from periodontally healthy individuals. Therefore, we will also analyze the tissues separately from periodontal health and gingivitis and increase the number of study subjects as needed. 2) Low metabolite yield from the samples could be a potential issue. If this situation arises, we will optimize extraction with methanol-chloroform protocols.

**Specific Aim 2: Investigate the role of downregulated mitochondrial complex I components of oxidative phosphorylation in driving succinate accumulation.**

**Hypothesis:** Downregulation of mitochondrial complex I impairs electron transport that leads to disruption of TCA cycle and succinate accumulation, which may underpin periodontitis pathogenesis.

**Ex vivo Model Setup:** Two cell lines mentioned in the preliminary data HOK cell and NOK cells will be cultured in an incubator at 37°C, 5% CO<sub>2</sub>. For the Aa infected group, cells will be co-cultured with live *P. gingivalis* or *A. actinomycetemcomitans* from Dr. Chen's lab at multiplicity of infection (MOI) 10:1 or 100:1 for 48 hours similar to the preliminary *in vitro* model to mimic bacterial infection, while confirming bacterial viability by CFU. To simulate downregulation of mitochondrial complex I enzymes using shRNA targeting NDUFS8, NDUFB1, and NDUFA12, based on the preliminary data, cells will be transfected using Lipofectamine RNAiMAX, achieving >70% knockdown confirmed by qPCR. The Feng laboratory has a large shRNA library that is readily available to silence human genes. To ensure that observed changes in succinate levels are due to complex I downregulation rather than non-specific cellular stress, non-targeting scramble shRNA and a minimum of two pairs of shRNA targeting the same transcript will be used rule out off-target effects, while uninfected cells with no bacterial co-culture will act as baselines for evaluating infection-specific responses. Rotenone (Sigma-Aldrich, 1–10  $\mu$ M), a complex I inhibitor that binds to the ubiquinone site of complex I and blocks electron transfer from NADH, causing ROS production, and mimicking inflammatory pathology, will be added to untreated cells to copy the knockdown effects and confirm the succinate buildup, investigate underlying mechanisms and validate causality. Succinate and TCA metabolites will be quantified by LC-MS as noted in the preliminary data section. Mitochondrial function will be assessed via Seahorse XF Analyzer for oxygen consumption rate (OCR, basal/maximal respiration) and extracellular acidification rate (ECAR, glycolysis). Inflammatory proteins will be quantified via qPCR (SYBR Green, primers for CRP, IL-6, TNF- $\alpha$ , IL-1 $\beta$ ; normalized to GAPDH) and ELISA (R&D Systems kit). All experiments will have n=3 biological replicates.

**Anticipated Results:** 1) We anticipate a successful shRNA-mediated knockdown of mitochondrial complex I in oral keratinocytes cells co-cultured with *P. gingivalis* or *A. actinomycetemcomitans*, achieving a >70% reduction in the gene expression. This should lead to impaired mitochondrial complex I activity, reduced oxygen consumption rate and significant succinate accumulation, mirroring our preliminary data from periodontitis patients and thereby establishing a successful *in vitro* model. 2) The metabolic reprogramming is expected to increase inflammatory responses, which would be evident by upregulated proinflammatory cytokines like IL-6, TNF- $\alpha$ , IL-1 $\beta$  very commonly seen in periodontitis patients which will be analyzed via qPCR and ELISA. 3) We also anticipate Rotenone phenocopying *in vivo* effects to confirm complex I's causal role in reverse electron transport and ROS production. The rescue experiments with dimethyl malonate should successfully mitigate any succinate buildup and downregulate inflammation, validating therapeutic potential for targeting SDH in periodontitis models.

**Pitfalls and Alternatives:** For the possible pitfalls, we may see variable bacterial adhesion in different groups and to overcome this potential issue, we will standardize MOI via OD600 as done for the preliminary data. 1)

Transient knockdown of complex I components may be toxic to cells and inhibits cell proliferation, our chemical inhibitors will offer acute intervention without altering basal cellular physiology. Alternatively, we will generate inducible knockdown via Tet-inducible system to overcome the toxicity issue. 3) There may also be potential non-specific shRNA effects, and although we do not anticipate this as Dr. Feng's lab has successfully established protocols and experience in these assays, we propose to use CRISPR-Cas9 as backup which will use guide RNA targeting of the same proposed genes.

**Specific Aim 3: Determine the contribution of elevated succinate levels to periodontal inflammation and use our *ex vivo* model to validate pathways related to succinate contributing to inflammation**

**Hypothesis:** Elevated succinate correlates with cell-specific activation of inflammatory pathways in periodontal tissues, resolvable at single-cell resolution.

**Sample Preparation and scRNAseq:** Gingival tissues from 10 periodontitis patients (stages III-IV, succinate-stratified based on Aim 1 LC-MS data) and 3 control samples will be dissociated into single cells with enzymatic digestion using collagenase/dispase and by further following the sample preparation protocol from USC's Molecular Genomic Core at the Norris Comprehensive Cancer center where the samples will be submitted for the scRNAseq. The aim will be to get a yield of >80% viability for each sample, which will be confirmed using Trypan blue. Our goal will be to have approximately 50,000 reads per cell, which would potentially give us around 150M reads for each sample providing sufficient depth to successfully detect low abundance inflammatory cytokines in the samples. This aim will focus on using the high and low abundance succinate samples and correlating them with the inflammatory genes such as CRP, IL-1, IL-6, NFKB1 and TNF $\alpha$  in different cell types including fibroblast, keratinocytes, macrophages, and neutrophils. To functionally validate our hypothesis, the *ex vivo* HOK and NOK coculture system from Aim 2 will be treated with exogenous succinate for 24-48 hours after determining an appropriate dose which will be optimized by cell viability assay. Further, to test our rescue *ex vivo* model, our plan is to use a succinate dehydrogenase (SDH/complex II) inhibitor dimethyl malonate (1 mM, Sigma-Aldrich). SDH acts as a competitive analog to succinate and blocks the succinate oxidation to fumarate, thereby inhibiting the TCA cycle flux. This experiment will help us probe succinate-dependent signaling and will test whether preventing succinate metabolism either exacerbates or reduced accumulation and downstream inflammatory response in knockdown or infected cells. Meanwhile, we will also express exogenous SDH (all subunits via lentivirus transduction) to determine whether excessive SDH can blunt the succinate accumulation in Aa-infected keratinocytes. If so, proteomics and metabolomics analyses will be performed on these cells to parse the signaling events downstream of succinate accumulation. This information will prove very useful when designing therapeutic modulation points for future research. Career development plan is to get proficient in bioinformatics with NIH seminars and trainings which would further help in pathway mapping by integrating proteomics and metabolomics data from patients across all aims. For the scRNAseq data, bioinformatics as a tool will reveal cell-specific inflammatory pathways driven by succinate, enabling an integration of multi-omics to uncover therapeutic targets for future grants.

**Anticipated Results:** 1) ScRNAseq will identify succinate-high clusters with upregulated inflammatory genes such as CRP, IL-1 $\beta$ , TNF $\alpha$ , IL-6 in macrophage cell population and HIF1 $\alpha$  and NFKB $\beta$  in the keratinocytes/fibroblasts. 2) HOK manipulations by succinate addition or SDH inhibition will increase pro inflammatory cytokine expression confirming succinate's causal pro-inflammatory role. Immune cell assays will show enhanced migration/cytokine release in succinate-treated macrophages and neutrophils, with spatial transcriptomics localizing these to clinically inflamed and deep periodontal pockets, synergizing with Aims 1-2 to validate succinate as a driver of chronic inflammation and a potential therapeutic target.

**Pitfalls and Alternatives:** To ensure the success, we have enlisted Dr. Dechen Lin and his team to assist the scRNAseq experiments. We may encounter low cell viability, we will improve cell viability via rapid tissue procurement and processing via automated single cell suspension preparation, efficient cell isolation and preservation. Additionally, bulk RNAseq is routinely performed in Dr. Feng's lab via a long-standing collaboration with UCLA. We may also isolate immune cells from human patients and use *ex vivo* model to study the inflammatory mechanism further, but this is likely a path that will be explored in a follow up R grant as it is currently beyond the scope of this K08 award.



I have taken multiple sessions of Responsible conduct in research during my PhD and residency training. I will fulfill NIH requirements for training in the Responsible Conduct of Research (RCR) through a blend of online modules and in-person sessions at my institution. This training is available through the Keck School of Medicine's INTD 500: Ethics and Accountability in Biomedical Research course. The course consists of five one-hour lecture sessions and five one-hour small group discussion sessions. I will take this course every year as well as my mentors will incorporate RCR principles into regular lab meetings, reinforcing ethical standards across all research endeavors.

October 6, 2025

To the K08 Committee,

We are pleased to provide support as co-mentors for Dr. Mirali Pandya's application for the K08 Mentored Clinical Scientist Research Career Development Award. We met with Dr. Pandya before she completed her Periodontics residency training at Texas A&M College of Dentistry. We were impressed with Mirali's accomplishments and potential for an academic career. Since joining us as a junior faculty member, Mirali has been everything we expected. We are fully committed to her further scientific development.

Mirali received her PhD and completed her Periodontics residency with a Master's in Oral Biology from Texas A&M College of Dentistry. Her PhD focused on investigating the mechanism underlying tooth enamel formation and the role of amelogenin fragments in organizing enamel prism structure. In addition, she developed a Clathrin mouse model deposited in the MMRRC repository and a novel 3D systems to culture ameloblast cells using a combination of multiple growth factors and scaffolds. Mirali has published nine first-author papers in high-impact-factor journals and eleven co-author papers, including a JOVE protocol for the bioreactor cell culture. After obtaining her PhD, Mirali completed a residency in Periodontics at Texas A&M College of Dentistry, where she excelled in clinical care of periodontal diseases while continuing research on understanding the microbiome before and after surgical intervention in a periodontitis patient.

Since joining as a Clinical Assistant Professor at USC in 2024, Mirali has flourished, become an American board-certified Periodontist, manages an NIH-funded clinical project, teaches, and serves as a research mentor for students in both the predoctoral and postgraduate programs. She participates in weekly joint lab meetings (including members from Infection and Immunity and the Center for Craniofacial Molecular Biology) that present diverse topics in cancer biology, bacteriology, virology, and periodontal disease. She draws from her prior experiences and the research expertise at the Ostrow School of Dentistry to identify her research direction. Gradually and under our mentorship, Mirali develops an interest in applying an omics approach to explore the mechanism of periodontal disease pathogenesis.

Periodontitis is an inflammatory disease associated with systemic inflammation. Evidence further implies metabolic reprogramming in immune cells as a key driver of periodontitis. The current research plan hypothesizes that succinate accumulation contributes to periodontitis via an impairment of mitochondrial electron transport and immune regulation. Strong preliminary data from the analysis of clinical samples of periodontal health and disease support the hypothesis. The hypothesis is also supported by Mirali's preliminary data from the *in vitro* experiments of co-culturing oral keratinocytes with periodontal pathogen *Aggregatibacter actinomycetemcomitans*. The research plan outlines an important goal to define succinate's role as a biomarker and mechanistic driver in periodontitis progression. Despite the burden of periodontal disease, very little is known about the role of metabolites in periodontal inflammation. The advances in understanding succinate's signaling via pathways like the succinate-SUCNR1 axis have recently reached the periodontitis field. Further insight into the host-microbe relationship in this context will not just elucidate the pathogenesis of disease but also help to build more biologically relevant models to help inform the direction and design of future human studies. Our laboratories and clinical programs at the Ostrow School of Dentistry provide an excellent environment to support the research plan and to expand Mirali's scientific expertise as a clinician-scientist.

Mirali has numerous attributes that bode well for her future success, such as intelligence, dedication, honesty, clarity, and a winning personality. She has an exceptional ability to work and learn from others. She interacts with faculty, staff, postdoctoral research associates, PhD students, predoctoral students, and residents. She is the point person for several ongoing research projects, including the pilot study for this research plan. She is currently guiding five residents for their Master's thesis project and is supervising three dental students in research. She does this while also maintaining her clinical skills in our faculty practice half a day a week. She attends weekly clinical, surgical, and treatment planning seminars in the Advanced Periodontics Program. She engages with her clinical mentors and many others in the periodontology department who are also particularly interested in metabolic aspects of oral diseases.

We have demonstrated accomplishments in mentorship. Collectively, we have mentored more than 100 trainees since 1992, including postdoctoral fellows, doctoral students, DDS students, residents in postgraduate programs,



and undergraduate students. Many of our trainees have become successful practicing clinicians, faculty, research scientists, and leaders of their fields. Multiple grants from NIH support our research. The expertise and strength of our labs lie within our cross-disciplinary approaches to understand the microbial basis of periodontal heterogeneity that underlies clinical disease and treatment outcomes. Our expertise includes clinical periodontics, periodontal disease pathogenesis, microbial genomics and metagenomics, innate immunity, infection, Kaposi's sarcoma-associated herpesvirus, and herpes simplex viruses. In particular, Feng's lab has the expertise in multi-omics and immune functions, and will be critical to train Mirali in this new area. We are confident that Mirali will thrive under our guidance.

Mirali has outlined a clear path for career development during this phase of her training. She has chosen coursework to specifically gain more experience independently in omics techniques and computational analyses critical to the aims of her proposal. Moreover, she will take advantage of the resources and courses available at USC to transition from K to R regarding grantsmanship and leadership. She has previously completed training requirements for human subjects research and the responsible conduct of research and will continue to update them. She has also outlined annual national conferences, such as IADR, where she can share her work and establish new collaborations. In addition, Mirali will benefit from the cutting-edge scientific research hub at USC, including mass spectrometry cores, genomics facilities, bioinformatics support, BL2 labs, and experience with human samples and multi-omics. Given that Mirali focuses on succinate's role in periodontal pathogenesis, we believe a five-year period of mentored career development K08, is appropriate.

As mentors, we fully commit to Mirali's development as a clinician-scientist. As department chairs, we will modify Mirali's profile by reducing Mirali's teaching and other commitments to 25% or less to devote time to research and training. We will provide the necessary support, including materials and supplies, laboratory space, technician support, access to cutting-edge technology, and career advice. We will meet jointly with Mirali weekly to review her progress. As Mirali gains further experience, the frequency of these meetings will decrease, but at a minimum, once a month. We will provide an annual evaluation of Mirali's progress as required in the annual progress report by NIH. We anticipate multiple publications from the research plan, with one annually in Years 2, 3, and 4. We expect Mirali to prepare to submit an R01 in Year 5 with the support of USC's grant writing seminars and K to R transition courses. Mirali will be encouraged to submit applications for other grant mechanisms to the NIH or other funding opportunities should they arise. Thus, Mirali will become more independent in her last two years of this project, relying on us for mainly advice and guidance as opposed to direct oversight. Ultimately, we expect Mirali to chart her course upon transitioning to an independent NIH-funded clinician-scientist.

We anticipate that managing a collaborative project will be challenging, as it will require Mirali to master two disciplines. However, we have confidence in Mirali's growth potential. This award would be pivotal in Mirali's career as she transitions towards independence. We support her application with enthusiasm.

Sincerely,



Casey Chen, BDS, PhD, DDS

Co-Chair, Department of Endodontics & Periodontics

Professor and Associate Dean of Applied Biomedical & Clinical Sciences



Pinghui Feng, PhD

Professor and Chair, Infection and Immunity



**USC** University of  
Southern California

Herman Ostrow School  
of Dentistry of **USC**

**Dear Mirali,**

I am pleased to confirm my enthusiastic participation as a member of your Advisory Committee for your K08 proposal entitled “*Probing the Role of a Succinate-Related Metabolic Reprogram in Periodontal Pathogenesis.*” After reviewing your preliminary data and our discussion, I am convinced that elevated succinate levels play a pivotal role in driving periodontal inflammation by modulating mitochondrial function and immune cell responses, thereby exacerbating tissue destruction and potentially linking local oral disease to systemic conditions such as diabetes and cardiovascular disease. Your proposal addresses an important and timely area of investigation with clear potential to elucidate novel mechanisms underlying periodontal disease and its systemic sequelae.

By way of background, I am an Assistant Professor at the Herman Ostrow School of Dentistry and serve as the Associate Director at the USC Head and Neck Cancer Center within the Keck School of Medicine. My laboratory at the USC Center for Craniofacial Molecular Biology focuses on cancer genomics, transcriptional regulation, and the tumor microenvironment, with a particular emphasis on head and neck and gastrointestinal cancers. A central component of my research involves dissecting immune–tumor interactions through advanced single-cell genomics and spatial transcriptomic approaches to resolve cellular heterogeneity and define immune-regulatory networks within diseased tissues.

I will bring to your Advisory Committee my expertise in applying single-cell RNA sequencing (scRNA-seq) and associated computational frameworks to map diverse immune and stromal populations, uncover transcriptional programs driving disease progression, and identify cell–cell interaction networks that shape inflammatory processes. This expertise will be directly relevant to your proposed studies and will complement the metabolomics and periodontal microbiology expertise of your other mentors. I will be available to provide input on experimental design, data

**University of Southern California**

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analysis strategies, and interpretation of single-cell datasets to help delineate succinate-driven immune reprogramming in periodontal pathogenesis.

I look forward to contributing to your training and research plans through regular meetings, discussions, and collaborative interactions. I am confident that your project will make significant contributions to the field and that our complementary expertise will strengthen the success of this innovative endeavor.

Sincerely,

*Dechen Lin*

De-Chen Lin, Ph.D.

Assistant Professor, Center for Craniofacial Molecular Biology, Norris Comprehensive Cancer Center

Associate Director, USC Head and Neck Center

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[sites.usc.edu/linlab/](https://sites.usc.edu/linlab/)    [ccmb.usc.edu/faculty/dechen-lin-phd/](https://ccmb.usc.edu/faculty/dechen-lin-phd/)

# Herman Ostrow School of Dentistry of USC

## Section of Infection and Immunity

Dear Mirali,

I am pleased to accept your invitation to serve as a collaborator for your NIH K08 Mentored Clinical Scientist Research Career Development Award application titled “**Probing the Role of a Succinate-Related Metabolic Reprogram in Periodontal Pathogenesis**”. With my research background and expertise with mass spectroscopy, I can certainly provide collaborative and technical support with the design, execution, and interpretation of mass spectrometry-based proteomic and metabolomic analyses of your gingival tissue samples, gingival crevicular fluid, and saliva samples. Specifically, I can provide guidance with optimization of sample preparation and LC-MS workflows, help with quantitative analysis of metabolites such as succinate and related TCA intermediates, proteomic profiling of inflammatory and mitochondrial pathways and with data quality control, normalization, and bioinformatics support for pathway integration as well as any troubleshooting.

I look forward to productive collaborations with you and your advisory committee on this exciting project.

Sincerely,



Dr. Chao Qin, PhD  
Research Assistant Professor of Dentistry  
Section of Infection and Immunity,  
Herman Ostrow School of Dentistry of USC

### **Scientific Research Environment:**

The Herman Ostrow School of Dentistry and the Keck School of Medicine at the University of Southern California (USC) provide an outstanding environment for biomedical research. Both Schools actively foster collaboration between basic scientists and clinicians, creating a stimulating and interactive research community.

The Ostrow School of Dentistry houses investigators with expertise in oral biology, oral microbiology, craniofacial development, viral infections, and single-cell analysis. Dr. Casey Chen is a Professor who studies oral microbial infection and biofilm ecology, while Dr. Pinghui Feng leads the Section of Infection and Immunity, focusing on the mechanisms by which human herpesviruses evade immune responses. Collaborators within the Center for Craniofacial Molecular Biology (CCMB), including Drs. Dechen Lin, Jianfu Jeff Chen, Jian Xu, and Yang Chai, provide further opportunities for scientific exchange and synergy.

Collaborations extend across USC and beyond. Within USC, partnerships with experts in medicinal chemistry, protein-nucleic acid interactions, endothelial biology, and mass spectrometry strengthen research capabilities. Externally, collaborations with the Cleveland Clinic Foundation, UCLA, and other institutions expand translational opportunities. Together, these resources ensure access to rich intellectual rapport, interdisciplinary collaborations, and strong institutional support critical for the success of independent research.

Access to mentors is readily available due to the office spaces in close proximity which is conducive for daily interactions.

# Herman Ostrow School of Dentistry of USC

## OFFICE OF THE DEAN

Yang Chai, DDS, PhD  
Dean

*G. Donald and Marian James Montgomery Professor of Dentistry  
University Professor of Dentistry, Stem Cell Biology and Regenerative Medicine, and  
Otolaryngology-Head & Neck Surgery*

October 9, 2025

To the K08 committee:

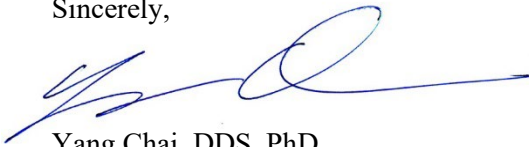
I write this letter in strong support of Dr. Mirali Pandya's application for the NIH K08 Mentored Clinical Scientist Research Career Development Award. As the Dean of Herman Ostrow School of Dentistry at the University of Southern California, I am committed to developing outstanding faculty. Mirali fits the profile of a clinician-scientist and a priority in our faculty development program. The K08 program provides crucial support for her career development, and I have confidence that the Herman Ostrow School of Dentistry will offer an excellent, nurturing environment to broaden Mirali's career.

Mirali joined the Herman Ostrow School of Dentistry of USC in July 2024 as an Assistant Professor of Clinical Dentistry with the Department of Endodontics and Periodontics. As a dual-degree junior faculty member, she is active in teaching, research, and patient care. I am impressed by the scope of her activities, which include leading multiple research projects, mentoring student research, teaching in predoctoral and post-graduate programs, and delivering patient care in our faculty practice. I am very excited that her research on "Probing the Role of a Succinate-Related Metabolic Reprogram in Periodontal Pathogenesis," which is innovative and timely, addresses critical gaps in the knowledge about the role of metabolites in microbial dysbiosis and periodontitis. I am convinced this project will contribute significantly to scientific knowledge and clinical practice and advance Dr. Pandya's career as a clinician-scientist.

The Herman Ostrow School of Dentistry is fully committed to supporting Dr. Pandya's development as an independent investigator, providing her with 75% protected time for research over the five-year award period, access to state-of-the-art laboratory facilities, mentorship from senior faculty, and administrative support. In addition, the University of Southern California has a strong track record of nurturing early-career researchers and providing robust infrastructure, including core facilities for proteomics and metabolomics, biostatistics support, and collaborative research centers. Furthermore, Mirali will benefit from our comprehensive career development programs, which include grant writing seminars, workshops, and mentorship programs.

In conclusion, Dr. Mirali Pandya possesses the intellect, drive, and institutional backing necessary to succeed in this award and emerge as a leader in Periodontics. I enthusiastically endorse her K08 application.

Sincerely,



Yang Chai, DDS, PhD  
Dean

G. Donald and Marian James Montgomery Professor of Dentistry  
University Professor of Dentistry, Stem Cell Biology and Regenerative Medicine, and Otolaryngology-Head & Neck Surgery

University of Southern California

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PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001

Expiration Date: 12/31/2027

Use of Human Specimens and/or Data

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

☒ Yes ☐ No

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

Are Human Subjects Involved

☒ Yes ☐ No

Is the Project Exempt from Federal regulations?

☐ Yes ☒ No

Exemption Number

☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8

Other Requested Information

Human Subject Studies

Study#	Study Title	Clinical Trial?
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The form does not have any study records

Delayed Onset Studies

Delayed Onset Study#	Study Title	Anticipated Clinical Trial?	Justification
1	Probing the Role of a Succinate-Related Metabolic Reprogram in Periodontal Pathogenesis	No	<a href="#">Delayed_Onset_Study_K08105246979_2.pdf</a>

## **NIH Human Subjects Delayed Onset Study.**

### **Study Title**

Probing the Role of a Succinate-Related Metabolic Reprogram in Periodontal Pathogenesis

### **1. Study Overview**

This study involves recruiting adult subjects who are undergoing routine dental care at the Ostrow School of Dentistry at USC. The aim is to assess biomarkers related to succinate metabolism in periodontitis through the analysis of discarded gingival tissue obtained during surgical procedures.

### **2. Inclusion of Human Subjects**

Yes – Human subjects will be involved in this study.

No clinical trial – This is \*not\* a clinical trial.

### **3. Protection of Human Subjects**

#### **A. Recruitment and Consent Process**

Subjects will be recruited from patients receiving routine care at USC's dental clinics, which treat over 4,000 new patients annually. Recruitment will use intake forms, and participation is entirely voluntary.

Informed written consent will be obtained from all participants. The consent form:

- Describes procedures and potential risks
- Includes contact information for the research team and IRB
- States participation will not affect dental care
- Discloses use of dental records, images, and future access to specimens
- Explains the Certificate of Confidentiality protecting participant identity

Each participant receives a copy of the signed consent form, and the originals are stored securely.

#### **B. Minimizing Risk**

The gingival tissues collected will be obtained from the discarded tissues in routine surgical procedures. Risks are minimal and primarily associated with routine clinical examination.

#### **C. Privacy and Confidentiality**

All protected health information will be securely maintained through:

- Locked physical storage
- Secure servers with password protection
- Use of unique subject IDs and blinding of data analysts
- Restricted access to only essential study personnel

No data will be shared outside the research team without appropriate authorization.

#### D. Adverse Events

No vulnerable populations will be included. Dr. Chen and I will respond to any adverse events promptly and in accordance with USC IRB policies.

### **4. Potential Benefits**

There are no direct benefits to participants. However, study findings may improve diagnostic and therapeutic strategies for periodontitis, which will be beneficial to future patients.

### **5. Importance of Knowledge to Be Gained**

This research may have a significant impact on the understanding of periodontitis pathogenesis, especially regarding metabolic deficiencies, and may identify new biomarkers for diagnosis and risk stratification.

### **6. Data and Safety Monitoring Plan**

A formal Data and Safety Monitoring Plan is not required, as this study is not a clinical trial.

## **Subjects**

### **Inclusion criteria:**

Adults (>18 yrs old) of two subject groups:

1. Diseased (N=50): Subjects diagnosed with stage III-IV chronic periodontitis. The diseased sample sites should have  $\geq 5$  mm PPD and  $\geq 2$  mm clinical attachment loss, bleeding on probing, and radiographic evidence of alveolar bone loss.
2. Periodontally Healthy (N=25): Subjects diagnosed with periodontal health or healthy but reduced periodontium with or without gingivitis. The healthy sample sites will have pocket depth  $\leq 4$  mm and  $\leq 1$  mm clinical attachment loss without bleeding on probing.

### **Exclusion criteria:**

- Subjects taking antibiotics within the past two months
- Subjects with a medical condition (e.g., diabetes) or taking medications (e.g., cyclosporine) that may affect the characteristics of the subgingival bacteria
- Pregnant or lactating women
- Patients taking anticoagulants
- Patients requiring antibiotic prophylaxis (e.g., for prevention of infective endocarditis) before dental procedures

### **Justification of exclusion:**

"Chronic periodontitis" is a common periodontal disease found almost exclusively in adults. Subjects younger than 18 years old with significant periodontal destruction are affected by "aggressive periodontitis," which is a rare form of periodontal disease distinct from chronic periodontitis, and therefore are not the appropriate subjects for this study.

Antibiotics will affect the composition of the bacteria in saliva and plaque. Subjects with certain medical conditions will have altered immune responses to viral and bacterial infections. Hormonal changes associated with pregnancy or lactation may affect the host's immune response to infections.

## Resource Sharing Plan

We will adhere to the NIH Grants Policy on Sharing of Unique Research Resources including the “Sharing of Biomedical Research Resources: Principles and Guidelines for Recipients of NIH Grants and Contracts” issued in December 1999: [http://www.ott.nih.gov/policy/rt\\_guide\\_final.html](http://www.ott.nih.gov/policy/rt_guide_final.html).

All ‘model organisms’ generated by this project will be distributed freely or be deposited into an NIH supported repository/stock center, making them available to the non-profit research community, either before or immediately after publication. Generally, such model organisms will also be provided to for-profit investigators under non-exclusive license agreements negotiated by our institution’s technology transfer office.

If we assume primary responsibility for distributing the newly generated model organisms, we will request in a timely fashion. In addition, we will provide relevant protocols and published genetic and phenotypic data upon request. Material transfers will be made with no more restrictive terms than in the Simple Letter Agreement (SLA) or the Uniform Biological Materials Transfer Agreement (UBMTA) and without reach through requirements. For large data set of genome-wide analyses of RNA-seq, succinate-inflammatory proteins interactions and networks, we will strive to build a website so that we can post our new findings and share within the research community.

Should any intellectual property arise which requires a patent, we will ensure that the technology (materials and data) remains widely available to the research community in accordance with the NIH Principles and Guidelines document.

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

**Cell line identity and authentication:** Though not applicable to this study, the cell lines for research in the mentor Dr. Feng's laboratories including normal oral keratinocytes NOK and HOK16B cells are obtained from Dr. Karl Munger at Tufts University and Dr. Ren Sun at UCLA with authentication, and cultured following their instructions. All of the cell lines used in the proposed study are not listed in the Database of Cross-contaminated or Misidentified Cell Lines (ICLAC). These cell lines can be used for the proposed study.

To minimize potential genetic drift with cell passage, we will strictly follow good cell culture practice – for instance, by returning to early passage frozen stocks rather than passaging a cell line for extended periods. However, even with good practice, genetic drift can occur in some cell lines. Referenced by the ATCC's Cell Line Authentication standards (ASN-0002), the identity of the cell lines will be further authenticated by short tandem repeat (STR) DNA profiling analysis<sup>1</sup>, when new cell stocks are prepared, or periodically checked during passages, to check if any contaminants previously below the detection threshold have grown to be more evident. The cell lines will be tested to rule out mycoplasma contamination in our laboratory using the MycoAlert kit (Lonza) and at the USC/Norris Comprehensive Cancer Center Bioreagent & Cell Culture Core (<http://uscnorriscancer.usc.edu/Core/Bioreagent/Info.aspx>).

**Antibodies, bacteria, chemicals, and other reagents:** The bacteria used in this study, *Porphyromonas gingivalis* (ATCC 33277) and *Aggregatibacter actinomycetemcomitans* (Aa) (ATCC 29522), will be authenticated upon receipt from ATCC by confirming strain identity through 16S rRNA gene sequencing, colony morphology on selective media, and growth characteristics including antibiotic susceptibility profiles to ensure purity and absence of any other contaminants. Authentication will be repeated annually or if unexpected results occur, with records maintained per NIH guidelines for reproducibility. Since Dr. Casey Chen's lab already has Aa bacterial line purchased and authenticated, it may be used for experiments as well. All primary and secondary antibodies required for the proposed research will be mainly purchased from the providers such as *Sigma-Aldrich*, *Santa Cruz*, *Cell Signaling*, *Covance*, or *In-Vitrogen*. Antibodies will be profiled for use according to the published evidence in literatures, citation history, the quality validation data provided by vendors, user reviews and community ratings, and the antibody validation profile provided by *Antibodypedia* or *1DegreeBio*. According to standard validation criteria (supportive or non-supportive)<sup>2</sup>, we will further verify the antibody in our application using a range of biochemical/cell biological techniques, including but not limiting to Western blot and microscopy analyses. Antibody effectiveness will be determined by specific gene knockdown assay with appropriate controls included.

All chemicals and other reagents of analytical grade will be purchased from the vendors with the solid authentication of their chemical identity, quality, and concentration. We will independently verify their identity and purity (or concentration) before putting them to use by a quantitative comparison with a previously used reference standard. To minimize the batch-to-batch and supplier-to-supplier variability, we will use validated reagents of the same batch from the same vendor to assure good reproducibility. Upon receipt, all chemicals and other reagents will be properly labeled with the acquisition and expiration date. A reagent solution prepared in the laboratory will be labeled with the date of preparation, the concentration of active ingredients, an expiration date, and the initials of the personnel who prepared it. All researchers involved in handling specialty chemicals and other reagents will be trained and fully informed of quality requirements. The practice of pouring back unused portions of solutions into original reagent bottles and of inserting pipettes into the stock container of solvent will be strictly discouraged to prevent contamination. We will acquire and maintain reference standards of specialty chemicals and other reagents. A central register of these materials should be kept and updated to trace possible sources

of error in an analysis, including all official reference substances and reference preparations, non-official reference standards procured from various outside sources, as well as working standards prepared at our laboratory. Good laboratory practices will be followed to avoid the production of unreliable or erroneous data.

**Reference:**

- 1 Reid, Y., Storts, D., Riss, T. & Minor, L. in *Assay Guidance Manual* (eds G. S. Sittampalam *et al.*) (2004).
- 2 Bordeaux, J. *et al.* Antibody validation. *Biotechniques* **48**, 197-209, doi:10.2144/000113382 (2010).