OMB Number 4040-0001
Expiration Date 06/30/2016

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

c. Previous Grants.gov Tracking Number

2. DATE SUBMITTED 2015-04-16

Application Identifier P0508060

5. APPLICANT INFORMATION **Organizational DUNS*:** [redacted]

Legal Name*: The Regents of the University of California, San Francisco
Department: Research Management Services
Division: Office of Sponsored Research
Street1*: 3333 California Street
Street2: Suite 315
City*: San Francisco
County: San Francisco
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 941430962

Person to be contacted on matters involving this application
Prefix: First Name*: [redacted] Middle Name: [redacted] Last Name*: [redacted] Suffix:
Position/Title: Contracts and Grants Officer
Street1*: 3333 California Street
Street2: Suite 315
City*: San Francisco
County: San Francisco
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 941430962
Phone Number*: +[redacted] Fax Number: [redacted] Email: [redacted]

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 1946036493A6

7. TYPE OF APPLICANT* H: Public/State Controlled Institution of Higher Education

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

● New ❍ Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).

❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●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*
Defining the role of immune-neuronal crosstalk in dry eye disease

12. PROPOSED PROJECT
Start Date* 09/01/2015
Ending Date* 08/31/2020

13. CONGRESSIONAL DISTRICTS OF APPLICANT
CA-012

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. First Name*: Nancy Middle Name: A. Last Name*: McNamara Suffix: OD, PhD

Position/Title: Associate Professor
Organization Name*: The Regents of the University of California, San Francisco
Department: Ophthalmology
Division: School of Medicine
Street1*: [redacted]
Street2:
City*: San Francisco
County: San Francisco
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 94143-0412
Phone Number*: +1 415 476-8132 Fax Number: +1 415 476-0527 Email*: Nancy.McNamara@ucsf.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$2,243,059.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$2,243,059.00
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*: [redacted] Middle Name: [redacted] Last Name*: [redacted] Suffix:

Position/Title*: Contracts and Grants Officer
Organization Name*: The Regents of the University of California, San Francisco
Department: Research Management Services
Division: Office of Sponsored Research
Street1*: 3333 California Street
Street2: Suite 315
City*: San Francisco
County: San Francisco
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 941430962
Phone Number*: [redacted] Fax Number: [redacted] Email*: [redacted]

Signature of Authorized Representative*

[redacted]

Date Signed*

04/16/2015

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:McNamara_Knox_R01_CoverLetter_4_10_15_.pdf

424 R&R and PHS-398 Specific Table Of Contents

OMB Number: 4040-0010
Expiration Date: 06/30/2016

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:	The Regents of the University of California, San Francisco
Duns Number:	[redacted]
Street1*:	[redacted]
Street2:	[redacted]
City*:	San Francisco
County:	San Francisco
State*:	CA: California
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	941430412
Project/Performance Site Congressional District*:	CA-012

Project/Performance Site Location 1

❍ 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:	The Regents of the University of California, San Francisco
DUNS Number:	[redacted]
Street1*:	[redacted]
Street2:	[redacted]
City*:	San Francisco
County:	San Francisco
State*:	CA: California
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	941430512
Project/Performance Site Congressional District*:	CA-012

File Name

Additional Location(s)

Funding Opportunity Number: PA-13-302. Received Date: 2015-04-16T19:25:16.000-04:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ❍ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ❍ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6

If NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ❍ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ● Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number A3400-01

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **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? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Project_summary_R01_4_16_15_v2 pdf
8. Project Narrative*	Project_narrative_R01_4_16_15.pdf
9. Bibliography & References Cited	NerveR01_REFS_4_16_15.pdf
10.Facilities & Other Resources	Facilities_and_Other_Resources12_16_14_1_copy. df
11.Equipment	Equipment12_2014M_K_copy.pdf

Funding Opportunity Number: PA-13-302. Received Date: 2015-04-16T19:25:16.000-04:00

PROJECT SUMMARY

Aqueous-deficient dry eye is among the most common and debilitating clinical manifestations of systemic autoimmune diseases such as Sjögren's syndrome. It is well established that chronic inflammation represents the predominant driving force in dry eye disease, however, the causal mechanisms underlying disease initiation and progression are unclear. As such, there is no cure for dry eye and few treatment options are available for sufferers. A long overlooked component of aqueous-deficient dry eye in autoimmune diseases like SS, is disruption of the complex reflex network of corneal nerves that connect the sensory tissues with the secretory glands. The integrity of this neural network is essential for maintaining a healthy ocular surface and there is growing evidence that innervation itself is a negative modulator of inflammation. ***In this application we will use a mouse model of Sjögren's syndrome to define the cellular processes that lead to denervation of the cornea and lacrimal gland in dry eye, as well as identify mechanisms to restore innervation and reverse ocular surface damage in Sjögren's syndrome patients.*** We hypothesize that chronic, T cell-mediated inflammation alters innervation by inhibiting epithelial maintenance of nerves in the cornea and lacrimal gland, resulting in loss of tissue integrity and reduced tear secretion that perpetuate ocular surface disease and aqueous tear deficiency. To test this hypothesis we will*:* 1) Define the timing of denervation and corresponding pathological changes in the cornea, lacrimal gland and associated nerves during dry eye development; and 2) Determine the mechanisms by which T cell-mediated inflammation alters innervation of the cornea and lacrimal glands. To achieve these aims we will employ a combination of in *vivo* and *ex vivo* studies, as well as transcriptional profiling, in a well-characterized mouse model of Sjögren's syndrome. Outcomes here will define modulators of ocular organ innervation, identify potential biomarkers of disease progression, and reveal novel regenerative strategies for reversing dry eye disease.

PROJECT NARRATIVE

Aqueous-deficient dry eye in Sjögren's syndrome is highly recalcitrant to existing therapies. Neurological manifestations of Sjögren's syndrome have long been recognized, but how chronic inflammation affects the complex neural network that connects the ocular surface with the tear-secreting machinery of the lacrimal gland has not been defined. Here, we will determine the cellular processes that link chronic inflammation in Sjögren's syndrome to nerve loss in the cornea and lacrimal gland. Knowledge gained will be critical for achieving our long-term goal of developing mechanism-based, regenerative therapeutics for dry eye patients.

FACILITIES AND OTHER RESOURCES

University of California, San Francisco

McNamara

Laboratory: The current laboratory is located [redacted]. The McNamara space consists of an ~ 1500 square foot area with 6 workstations with desks, a fume hood, and a 800 square foot cell culture room. There are separate shared rooms for fluorescence microscopy and animal surgery. There is a shared cold room, equipment room and fully equipped histology room.

Animal: The UCSF Animal Care and Use Committee (IACUC) review animal use protocols annually. The UCSF Animal Care Facility provides complete support for the housing and maintenance of mice and rats, including a barrier facility and several procedure rooms. Within the barrier facility, one large room shared by multiple investigators will house 280 cages in ventilated racks assigned for use by the McNamara lab. In the animal facility, there are rooms designated for procedures including the non-invasive procedures outlined in this application.

Computer: The laboratory is equipped with five computers and a high-resolution laser printer. All lab members have access to the Proctor Departmental Server for Data storage. Shared equipment includes use of an imaging module supported by an NEI Vision Research Core Grant, located in the adjacent Department of Ophthalmology building.

Office: Office space for Dr. McNamara includes 400 sq. ft. immediately adjacent to the lab. Postdoctoral fellows, graduate students, and technicians have use of desk space in the main lab.

Administrative: The Francis I. Proctor Foundation provides administrative and accounting support. The University provides IT support and additional administrative support. In addition to this department-based support, UCSF provides centralized pre- and post-award administration for the preparation and administration of grants, and centralized human resources assistance.

Other: UCSF has several core facilities including mouse pathology, flow cytometry, and the laboratory for cell analysis. Additional facilities include a library, oligonucleotide and peptide sequencing facilities, and many core laboratories including sequencing, microarrays, proteomics and mouse transgenic and gene targeting cores. Microarray hybridization and imaging instruments are available for use at the UCSF Sandler Center Functional Genomics Core Facility at Mission Bay campus.

Scientific Environment: The Proctor Foundation is located on [redacted] There is also the Harry William Hind Library and the Elizabeth C. Proctor Library that provide facilities for journal clubs, seminars, guest lectures and weekly lab meetings. The Department of Ophthalmology is organized around faculty members with subspecialty skills and expertise. The department combines one of the nation's leading vision research programs with outstanding clinical care. The research and multicenter clinical facilities of the Department of Ophthalmology comprise the Beckman Vision Center that includes the Koret Vision Research Laboratory Building and the Vision Care and Research Unit (VCRU). The establishment of The Beckman Vision Center and the building of the Koret Vision Research Laboratory is the achievement of That Man May See, Inc., a foundation dedicated to vision research and care. Several laboratories adjacent to the McNamara lab work on topics directly relevant to their interests and provide excellent resources for the work funded by this proposal. Dr. McNamara's lab is highly interactive with the laboratories of Dr. Sarah Knox (as outlined in this proposal), and [redacted], a mouse geneticist and stem cell expert whose research interests include epithelial and stromal cell regeneration after injury. The research environment at UCSF, along with its collaborative culture, affords virtually unlimited opportunities for interdisciplinary research and education. This stimulating and diverse clinical and research environment will afford us a high rate of success for this project.

Knox

Laboratory: The main laboratory is in an open configuration contiguous with the laboratories of [redacted] The P.I. has dedicated laboratory space (1500 ft) consisting of 4 benches and associated laboratory and office support space assigned to her. There is [redacted] This space is in [redacted]

Animal: The animal facility at the UCSF is f[redacted], and is fully accredited with the American Association for Accreditation of Laboratory Animal Care (AAALAC). All experiments involving mice are reviewed by the UCSF Institutional Animal Care and Use Committee, and are subject to annual review. Within the barrier facility, one large room shared by multiple investigators will house 280 cages in ventilated racks assigned for use by the Knox lab. In the animal facility, there are rooms designated for procedures including the non-invasive procedures outlined in this application.

Computer: Our lab possesses two shared Dell laptop computers for general usage and has access to 2 Dell desktop computers designated for imaging purposes located in a computer room adjacent to the lab. A tablet PC is used for mouse colony maintenance, and the PI has an Apple desktop computer for her own use.

Office: Postdoctoral fellows, graduate students, and technicians have use of desk space in the main lab. An office of 150 ft^2 has been set aside for the use of the PI.

Administrative: The department of Cell and Tissue Biology has five full-time administrators shared between eleven faculty members. These provide post-award support in purchasing, accounting, as well as 15% of a personal administrative assistant. In addition to this department-based support, UCSF provides centralized pre-award administration for the preparation of grants, and centralized human resources assistance.

Other: Additional facilities include a library, oligonucleotide and peptide sequencing facilities, and many core laboratories including sequencing, microarrays, proteomics and mouse transgenic and gene targeting cores. Microarray hybridization and imaging instruments are available for use at the UCSF Sandler Center Functional Genomics Core Facility at Mission Bay campus. Two gamma-producing irradiators are on site at Parnassus and will be used with the support of the Radiation Office.

Scientific Environment: The laboratory is part of the Department of Cell and Tissue Biology http://ctb.ucsf.edu/ at University of California San Francisco School of Dentistry. The department brings together 15 cell, developmental and molecular biologists interested in cancer biology, mesenchymal tissues, stem cells, craniofacial development and mechanisms of pathogenesis. The department ([redacted]) provides an outstanding and collegial environment to perform the proposed work. Several colleagues in the department work on topics directly relevant to our labs interests, and will provide excellent resources in the work funded by this proposal. The laboratory of [redacted] is adjacent to the Knox Lab and post-docs within both labs are highly interactive. [redacted] is a mouse geneticist who works on the role of Ephrin signaling in craniofacial development and disease. The P.I. and [redacted] have established a collaboration exploring Ephrin signaling in the salivary gland. [redacted] works on mechanisms of signaling as they regulate cell movement and proliferation. The laboratory of [redacted] uses high-resolution confocal fluorescence microscopy combined with biochemistry and molecular biology to elucidate molecular mechanisms by which signaling controls cytoskeleton dynamics and regulates cell behavior. The laboratory of [redacted] focuses on TGF-β signaling in epithelial and mesenchymal differentiation, and in epithelial-mesenchymal transition.

In addition, our lab is a part of the Program in Craniofacial and Mesenchymal Biology http://cmb.ucsf.edu/, a program which brings together basic, translational and clinical researchers, who are interested in the development and function of the craniofacial complex and how deregulation of craniofacial development gives rise to craniofacial anomalies. The program brings together 33 researchers from different departments and graduate programs and provides a unique opportunity for interaction. Of these researchers, the laboratories of [redacted], with whom we share open lab space (see above) have research interests particularly similar to those of our lab, and as such are an outstanding resource. The Klein lab works on questions related to stem cell biology, with a particular focus on the morphogenesis and regeneration of the

mammalian tooth. In addition, [redacted] is a specialist in pediatric medical genetics that treats children with craniofacial and dental anomalies. [redacted] is a plastic surgeon that specializes in pediatric plastic and reconstructive surgery including the treatment of craniofacial anomalies such as craniosynostosis, whose laboratory focuses on skeletal muscle regeneration through the use of stem cells. We have significant interaction with these labs including a monthly seminar series and a work in progress seminar that meets every other week. This atmosphere is therefore ideal for the work we propose.

The Knox Lab is also affiliated with the Eli and Edyth Broad Center for Regeneration Medicine and Stem Cell Research (http://stemcell.ucsf.edu/). This program consists of 125 labs focused on understanding and developing treatment strategies for such conditions as heart disease, multiple sclerosis, Parkinson's disease, Lou Gehrig's disease, spinal cord injury and cancer. The Center encourages cross-pollination of ideas among scientists of different disciplines to promote discovery through collaboration. UCSF has a Clinical and Translational Science Institute (http://ctsi.ucsf.edu/) to support the translation of basic research into human therapies. The institute provides infrastructure, services, and training programs including clinical and translational science and early translational research.

Institutional Investment in the Success of the Investigator: The P.I. on this grant is an early stage investigator and received a tenure-track appointment as assistant professor, step III. This position includes partial salary support in the form of a state-of California FTE as well as a start-up package for expenses related to her research program. Teaching and administrative requirements are minimal with at least 80% protected research time.

UCSF has an extremely active faculty mentoring system in which faculty are paired with senior faculty career mentors to provide career guidance and support. Meetings take place frequently, with a minimum of twice yearly, with the goals of assisting faculty with career advancement, increasing productivity, and career satisfaction. Through this program, the P.I. is formally mentored by [redacted], a highly successful scientist in the fields of placenta and salivary gland biology and by [redacted], a top craniofacial physician/scientist in the field of tooth development. In addition, the P.I. receives constant mentorship from the chair of the CTB department, [redacted], as well as guidance in grant writing and manuscript preparation by [redacted], an eminent physician/scientist in breast cancer research and a senior member of the CTB.

UCSF also offers a number of career enrichment programs such as faculty development day from the Chancellor's council on faculty life. This program covers a variety of topics including advancement and promotion, grant writing, and management workshops. Other seminars related to these topics are offered throughout the year by the UCSF Academic Affairs office.

Biohazards: This work involves handling of human tissue that has not been screened for human pathogens. These are fresh tissue biopsies taken from adult and fetal humans and are essential for the identification of human stem/progenitor cells in this study. Specimens are manipulated under BSL2 conditions with the appropriate approvals from the UCSF IBC.

EQUIPMENT

University of California, San Francisco

McNamara

General: In the main lab space we have a tissue culture incubator, a biosafety laminar flow hood, water baths, electroblotting apparatus, gel dryer, power supplies, and UV light box for agarose gels, balances, a pH meter, a Forma ULT -80 freezer, microwave, Biorad UV/visible spectrophotometer, refrigerated and room temperature tabletop microcentrifuges, and a PCR thermocycler for genotyping. Shared equipment includes a Beckman ultracentrifuge, a Sorvall RC-5C centrifuge, a Leica Cryostat and a Fuji LAS imager.

Microscopy: The lab is equipped a Ti-E Nikon microscope with standardized inverted fluorescence, Hoffman modulation contrast and phase-contrast capabilities. It is equipped with 2 cameras, a Nikon DS-QiMc black and white camera and a Nikon Digital Sight DS-Fi1 color camera. The image capture system is run by NIS elements Advanced Research and Object Classifier software. There is also an inverted tissue culture microscope with fluorescence, 2 dissecting microscopes,

Molecular Biology and Biochemistry: For qPCR experiments there is an Applied Biosystems 7500 realtime PCR system, a nanodrop system for concentration measurements, and an Agilent bioanalyzer for RNA quality assessment

Core facility:
We have access to the Vision Morphology Core in the Department of Ophthalmology, which includes a mouse pathology core, an animal tissue IHC core, human morphology tissue core and a confocal microscopy core. Costs are covered by an Ophthalmology core grant.

Computers: Our lab has 4 iMac desk or laptop computers for general usage, a Dell computer for running NIS elements Advanced Research and Object Classifier software for our Ti-E microscope and a Dell desktop computer for Fuji LAS imaging purposes located in a microscope/imaging room adjacent to the lab. The PI has an iMac laptop computer for her own use.

Knox

Microscopy: The lab is equipped with four small dissection scopes, an inverted Axiovert M200 with digital camera for imaging tissue, one Zeiss Discovery V12 automated stereo microscope for fluorescence, bright field and dark field microscopy and one Cell Observer Spinning Disc with Yokogawa X1-A scan head. This scope is equipped with an MrC5 camera for whole mount color imaging, and an MrM camera for whole mount fluorescence imaging. The lab also is equipped with a Zeiss Imager Z2 automated upright microscope for viewing slides. This microscope is also equipped with an MrC5 camera for color imaging and an MrM camera for fluorescence. These are all located within a designated imaging room that adjoins our main lab. For confocal, we have access to the UCSF Stem Cell imaging core equipment http://stemcell.ucsf.edu/research/imaging-core, which is located in an adjacent building which is a five minute walk from our lab. This core provides access to two Leica SP5 upright confocal microscopes: one Leica SP5 inverted confocal microscope, and one Leica SP5 multiphoton microscope, as well as Velocity and Imaris image rendering and analysis software. These are all available to members of the institute for regenerative medicine for a subsidized hourly recharge fee (around $42/hour for most equipment). In addition, our department has a shared Nikon Ti with Yokogawa CSU-X1 Borealis spinning disk confocal that is available for our use at no charge.

Molecular Biology and Biochemistry: The lab is equipped with two CFX96 Touch™ Real-Time PCR Detection System (BioRad) and two 96 well Bio-Rad thermocyclers for PCR and other molecular biology applications. Our lab has a full complement of equipment for molecular cloning and western blotting as well as a GE imager for western blot development and quantitation. We have access to two shared Nano-drops for quantification of nucleic acids.

Histology: The lab is equipped with one Leica RM2235 microtome for cutting paraffin sections along with

associated water bath and slide warmers, and a Microm HM550 cryostat for cutting cryosections. These are both located in a shared histology space adjacent to our main laboratory. We have access to a shared paraffin embedding machine a short walk from our lab.

Computers: Our lab possesses two shared Dell laptop computers for general usage and has access to 2 Dell desktop computers designated for imaging purposes located in a computer room adjacent to the lab. A tablet PC is used for mouse colony maintenance, and the PI has an iMac desktop computer for her own use.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: Nancy Middle Name A. Last Name*: McNamara Suffix: OD, PhD

Position/Title*: Associate Professor
Organization Name*: The Regents of the University of California, San Francisco
Department: Ophthalmology
Division: School of Medicine
Street1*: [redacted]
Street2:
City*: San Francisco
County: San Francisco
State*: CA: California
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 941430412

Phone Number*: +1 415 476-8132 Fax Number: +1 415 476-0527 E-Mail*: Nancy.McNamara@ucsf.edu

Credential, e.g., agency login: [redacted]

Project Role*: PD/PI Other Project Role Category:

Degree Type: PhD Degree Year: 1996

	File Name
Attach Biographical Sketch*:	NIH_Biosketch_McNamara_4_16_15 pdf
Attach Current & Pending Support:	

Tracking Number: GRANT11891691
Funding Opportunity Number: PA-13-302 . Received Date: 2015-04-16T19:25:16.000-04:00

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Sarah Middle Name Monica Last Name*: Knox Suffix: Ph.D

Position/Title*:	Assistant Professor
Organization Name*:	The Regents of the University of California, San Francisco
Department:	Cell and Tissue Biology
Division:	School of Dentistry
Street1*:	[redacted]
Street2:	
City*:	San Francisco
County:	San Francisco
State*:	CA: California
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	941430512

Phone Number*: +1 415 502-0811 Fax Number: 415-476-1499 E-Mail*: sarah.knox@ucsf.edu

Credential, e.g., agency login: [redacted]

Project Role*: PD/PI Other Project Role Category:

Degree Type: PhD Degree Year: 2002

	File Name
Attach Biographical Sketch*:	Knox_biosketch_2015.pdf
Attach Current & Pending Support:	

Tracking Number: GRANT11891691 Funding Opportunity Number: PA-13-302 . Received Date: 2015-04-16T19:25:16.000-04:00

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Nancy A. McNamara	Associate Professor of Ophthamology and Anatomy University of California, San Francisco Associate Professor of Clinical Optometry University of California, Berkeley
eRA COMMONS USER NAME (credential, e.g., agency login) [redacted]	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Michigan State University, E. Lansing, MI		1985-1987	Biology
Michigan College of Optometry, Big Rapids, MI	O.D.	1987-1991	Optometry
University of California, Berkeley, CA		1991-1992	Cornea Residency
Mayo Graduate School, Rochester, MN		1995	Clinical Trials
University of California, Berkeley, CA	Ph.D.	1992-1996	Vision Science

A. Personal Statement

One of the most debilitating forms of dry eye results from autoimmune-mediated destruction of the lacrimal gland, with Sjögren's syndrome (SS) serving as a prime example. Little is known about the pathogenesis of aqueous-deficient dry eye and there is no cure. As an investigator for the Sjögren's International Collaborative Clinical Alliance (SICCA) and director of a dry eye specialty practice, I have come to appreciate the severe consequences of dry eye disease, as well as the unmet demand for targeted therapies that are effective and affordable. *The goal of our research is to determine how chronic inflammation provokes clinically recalcitrant dry eye and to discover novel approaches for therapeutic management.* As a clinician and cell biologist, I have studied the ocular surface and tear film for nearly two decades and the pathogenesis of dry eye disease for over seven years. My lab explores dry eye pathogenesis through interdependent investigations of its main components: chronic inflammation, reduced tear secretion, altered differentiation of epithelial progenitor cells and loss of innervation to the cornea and lacrimal gland. As damage to nerves innervating the cornea and lacrimal gland becomes recognized as a significant contributing factor to the pathogenesis of SS-associated dry eye, we have teamed up with Dr. Sarah Knox, a developmental biologist at UCSF with extensive expertise in characterizing neuronal-epithelial cell interactions in development and regeneration. Studies proposed in this application will highlight the strengths of each labs to define the cellular processes that provoke neuropathy in dry eye, as well as provide evidence to support a potential paradigm shift in our current understanding of dry eye pathogenesis in SS patients.

B. Positions and Honors

Positions and Employment

1987-1990 Ocular Research Assistant, Ferris State University, Big Rapids, MI
1989-1990 Ophthalmic Technician, Vanderbilt University Medical Center, Nashville, TN
1991-1992 Contact Lens/Cornea Resident, University of California, Berkeley, CA
1992-1996 Doctoral Candidate in Vision Science, University of California, Berkeley, CA
1992-1995 Staff Research Optometrist, California Pacific Medical Center, San Francisco, CA
1996-1998 Ciba Vision Corporation Postdoctoral Fellow
1995-2000 Research Optometrist, Morton D. Sarver Laboratory, University of California, Berkeley, CA
1996-1998 Assistant Research Biologist, University of California, Berkeley, CA
1998-2006 Associate Research Biologist, University of California, San Francisco, CA

2006-2011 Assistant Professor, University of California, San Francisco, CA
2011- Associate Professor, University of California, San Francisco, CA
2012- Associate Professor of Clinical Optometry, University of California, Berkeley

Other Experience and Professional Memberships

1985-91 Student Volunteer Optometrists Serving Humanity
1985-91 Omicron Delta Kappa Honorary Leadership Fraternity
1987-91 Beta Sigma Kappa International Optometric Honor Fraternity
1998-05 UCSF Postdoctoral Scholars Association
2005-06 American Thoracic Society
2006-07 North American Cystic Fibrosis Foundation
1991-present American Optometric Association
1991-present Association for Research in Vision and Ophthalmology
1997-present International Society for Contact Lens Research
2005-present American Society for Cell Biology
2008-present American Association for Cancer Research
2009-present International Ocular Surface Society
2009-2011 Consortium for Functional Glycomics, Participating Investigator
2007-2012 National Eye Institute, Anterior Eye Disease Study Section, reviewer
2012-present National Eye Institute, Translational and Training Grant Special Emphasis Panel, reviewer
2012-present National Eye Institute, Disease and Pathophysiology of the Vision System, standing member

Honors

1990-1991 Medivision Scholarship
1991 Beta Sigma Kappa Silver Medal Award
1991 George N. Holcomb Memorial Scholarship
1991 Michigan College of Optometry Academic Recognition Award
1992 Polymer Technology Travel Award
1992-1994 American Academy of Optometry Travel Fellowship
1992-1995 Morton D. Sarver Fellowship
1995-1996 National Institute of Health Training Grant
1995-1996 American Optometric Foundation Ezell Fellowship
1995-1997 International Society for Contact Lens Research Student Travel Grant
1998-2003 National Eye Institute, Mentored Clinical Scientist Award
2006 U.S. Patent - UC Case No.: B98-040-3
2007 Irvin M. and Beatrice Borish Award – American Academy of Optometry
2009 American Cancer Society Research Scholar
2009 Best Poster Award – American Academy of Ophthalmology
2012 Ezell Research Symposium – Women on the Front Lines of Ocular Surface Research -
2012 Ocular Surface: Application of Cutting Edge Research – American Academy of Optometry
2013 UC Berkeley Oxyopia – Invited speaker
2014 64th Annual UC Berkeley Alumni Program Reunion – Invited Speaker
2015 13th Mucins in Health and Disease Workshop – Robinson College Cambridge
2015 Allergan Foundation Research Award

C. Selected Peer-reviewed Publications

Most relevant to the current application (Selected from 58)

1. Li S, Nikulina K, DeVoss J, Wu A, Strauss EC, Anderson MS, **McNamara NA**: Small protein rich-protein 1B (SPRR1B) is a biomarker for squamous metaplasia in dry eye disease. *Invest Ophthalmol Vis Sci* 49:34-41, 2008. PMID: 18172072
2. Chen YT, Li S, Nikulina K, Porco T, **McNamara NA**: Immune profile of squamous metaplasia development in autoimmune regulator-deficient dry eye. *Mol Vis* 15:563-576, 2009. PMID: 19365590

3. Whitcher JP, Shiboski CH, Shiboski SC, Heidenreich AM, Kitagawa K, Zhang S, Hamann S, Larkin G, **McNamara NA**, Greenspan J, Daniels TE: A simplified quantitative method of assessing keratoconjunctivitis sicca from the Sjögren's Syndrome International Registry. *Am J Ophthalmol* 149:405-15, 2010. PMID: 20035924
4. Li S, Gallup N, Chen Y-T, **McNamara NA**: Molecular mechanisms of proinflammatory cytokine-mediated squamous metaplasia in human corneal epithelial cells. *Invest Ophthalmol Vis Sci* 51:2466-75, 2010. PMID: 20042643
5. Chen YT, Nikulina K, Lazarev S, Gallup M, **McNamara NA**: Interleukin-1 as a phenotypic immuno-modulator in keratinizing squamous metaplasia of the ocular surface in Sjögren's syndrome. *Am J Pathol* 177:1333-43. 2010. PMID: 20696775
6. Shiboski SC, Shiboski CH, Criswell L, Baer A, Challacombe S, Lanfranchi H, Schiødt M, Umehara H, Vivino F, Zhao Y, Dong Y, Greenspan D, Heidenreich AM, Helin P, Kirkham B, Kitagawa K, Larkin G, Li M, Lietman T, Lindegaard J, **McNamara NA**, Sack K, Shirlaw P, Sugai S, Vollenweider C, Whitcher J, Wu A, Zhang S, Zhang W, Greenspan J, Daniels T: New Classification Criteria for Sjögren's Syndrome: A data-driven expert-clinician consensus approach within the SICCA Cohort. *Arthritis Care Res* 64:475-87, 2012. PMID: 22563590
7. Chen YT, Lazarev S, Bahrami AF, Noble LB, Zhou D, Yadav M, **McNamara NA**: Interleukin-1 receptor signaling mediates the functional interplay between autoreactive CD4+ T cells and ocular resident cells during keratinizng squamous metaplasia in Sjögren's syndrome. *Lab Invest* 92:556-70, 2012. PMID: 22231738
8. Zhou D, Chen YT, Chen F, Gallup M, Vijmasi T, Bahrami AF, Noble L, Rooijen N, **McNamara NA**: Critical involvement of macrophage infiltration in the development of Sjögren's syndrome-associated dry eye. *Am J Pathol* 181:753-60, 2012. PMID: 22770665
9. Chen YT, Chen F, Vijmasi T, Gallup M, **McNamara NA:** Pax6 downregulation mediates abnormal lineage commitment of the ocular surface epithelium in aqueous-deficient dry eye disease. *PLoS One* 8:e77286, 2013. PMID 24143217
10. Vijmasi T, Chen F, Chen YT, Gallup M, **McNamara NA:** Topical administration of IL-1 receptor antagonist as a therapy for aqueous-deficient dry eye in autoimmune disease. *Mol Vis* 19:1957-65, 2013. PMID: 24068863
11. Zhou D, **McNamara NA**: Macrophages: important players in primary Sjögren's syndrome? *Expert Rev Clin Immunol* 10:513-20, 2014. PMID: 24646086
12. Chen YF, Zhou D, Metzger T, Gallup M, Jeanne M, Gould DB, Anderson MS, **McNamara NA**: Spontaneous development of autoimmune uveitis is CCR2 dependent. *Am J Pathol* 184:1695-705, Jun 2014. PMID: 24736166
13. Vijmasi T, Chen FY, Balasubbu S, Gallup M, McKown R, Laurie G, **McNamara NA**: Topical administration of lacritin is a novel therapy for aqueous-deficient dry eye disease. *Invest Ophthalmol Vis Sci* 55:5401-9, Jul 2014. PMID: 25034600
14. Zhang L, Gallup M, Zlock L, Chen F, Finkbeiner WE, **McNamara NA**: Pivotal role of MUC1 glycosylation by cigarette smoke in modulating disruption of airway adherens junctions in vitro. *J Pathol* 234:60-73, Sept 2014. PMID: 24838315
15. **McNamara NA**, Gallup M, Porco T: Establishing PAX6 as a biomarker to detect early loss of ocular phenotype in human patients with Sjögren's syndrome. *Invest Ophthalmol Vis Sci* 16;55(11):7079-84, Sept 2014. PMID: 25228544

D. Research Support

ACTIVE

[redacted]

[redacted]

RECENTLY COMPLETED

R01EY016203-01 (PI: N. McNamara) 09/01/06-08/30/14
NIH/NEI
Molecular Mechanisms of Squamous Metaplasia in Dry Eye
Define the molecular events that provoke the conversion of the ocular surface from a secretory epithelium to a dry and pathologically keratinized epithelium during chronic inflammation in aqueous-deficient dry eye disease using the autoimmune regulator-deficient mouse model and human patients with Sjögren's syndrome.
Role: PI

Research Project Grant (PI: N. McNamara) 02/01/2014-01/31/2015
That Man May See
Defining the Functional Link Between Inflammation and Corneal Neuropathy in Dry Eye Disease.
Role: PI

R01EY024327-01 (PI: Gordon Laurie)
NIH/NEI 04/01/2014-03/31/2015
Lacritin Regulated Ocular Surface Homeostasis
Explore the role of lacritin in maintaining homeostasis of the ocular surface.
Subcontract: $45,700 (direct cost)

R01EY021509-01 (PI: Jorge Alvarado) 04/01/12-03/31/15
NIH/NEI
Mechanisms of Aqueous Humor Homeostasis – Role of Monocytes.
Identify the monocyte subsets and cytokines that modulate the permeability and junctional integrity of Schlemm's canal endothelial cells.
Role: Consultation for in vitro studies involving macrophages.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Knox, Sarah, Monica	Assistant Professor
eRA COMMONS USER NAME (credential, e.g., agency login) [redacted]	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of New South Wales, Sydney, Australia	Ph.D.	April 2002	Biomedical Engineering
University of New South Wales, Sydney, Australia	B.S (Hon)	April 1998	Biochemistry
Post-Doctoral Fellow, Department of Molecular, Cell and Developmental Biology, Mount Sinai School of Medicine, New York, NY, USA		2002-2003	Cell Biology
Post-Doctoral Fellow, Center of Genetics, Cell Biology and Development, University of Minnesota, MN, USA		2003-2005	Developmental Biology
Post-Doctoral Fellow, Graduate School of Biomedical Engineering, University of NSW, Sydney, Australia		2005-2006	Biochemistry
Postdoctoral Visiting Fellow, Laboratory of Cell and Developmental Biology, NIDCR, NIH, Bethesda, MD, USA		2006-2011	Developmental Biology

A. Personal Statement

My labs research program is to investigate how neuronal-epithelial interactions control epithelial organ development and regeneration after injury and disease. Our work primarily utilizes the mouse and human fetal and adult salivary and lacrimal glands (mice and humans) to define the influence of the peripheral nervous system on developmental programs controlling salivary gland formation and to decipher the effects of radiation induced damage on organogenesis and stem cell mediated regeneration of epithelial tissues. Our current projects explore the relationship between nerves and progenitor/stem cells during homeostasis and after radiation- or immune cell-induced injury with the expectation that our discoveries will lead to new therapies for patients suffering organ dysfunction after radiotherapy or autoimmune disease. Our approach utilizes mouse genetics, high-resolution microscopy, flow cytometry, and gene expression and biochemical analysis to identify both physiological and genetic based mechanisms of regulation. These techniques along with our extensive expertize in lacrimal gland development and regeneration will allow the successful completion of this project.

B. Positions and Honors

Positions and Employment

2002 Post-Doctoral Associate, Molecular, Cell and Developmental Biology, Feb 2002-May 2003, Mount Sinai School of Medicine, New York, NY, USA. Supervisor: Dr. Robert Krauss

2003 Post-Doctoral Associate, Genetics, Cell Biology and Development, July 2003-July 2005, University of Minnesota, MN, USA. Supervisor: Dr. Scott Selleck

2005 Post-Doctoral Fellow, Graduate School of Biomedical Engineering, August 2005-May 2006, University of NSW, Sydney, Australia. Supervisor: Dr. John Whitelock

2006 Senior Postdoctoral Visiting Fellow, Matrix and Morphogenesis Unit, Laboratory of Cell and Developmental Biology. NIDCR, NIH, USA. Supervisor: Dr. Matthew Hoffman

2012 Assistant Professor, Department of Cell and Tissue Biology, University of California, San Francisco, San Francisco, CA, USA

Honors

1995 CRC Cardiac Technology Summer Scholarship (UNSW)
1996 School of Pharmacology and Physiology Summer Scholarship (UNSW)
1998 Pacific Dunlop Postgraduate Scholarship ($30000)
1998 Australian Postgraduate Award (APA)
2005 Australian Post-Doctoral Industrial Fellowship (APDI)
2005 UNSW Faculty of Engineering Research Grant ($20000)
2008 NIH Pathway to Independence Award (K99/R00)
2008 Poster Award at Oral Biology Conference, Rochester, NY
2008 Pathway to Independence Award (K99/R00)

Other Experience and Professional Memberships

Memberships

1999-05 Matrix Biology Society of Australia and New Zealand
2005-15 Society for Neuroscience
2011-14 Society for Developmental Biology
2011-15 American Association for Dental Research

Professional Activities

2004-08 Proteoglycans Gordon Research Conference, Post-doctoral coordinator
2011 7th International Conference on Proteoglycans, Discussion Leader
2012 Proteoglycan Gordon Research Seminar, Chair
2012 Society for Craniofacial Genetics, Organizing committee member
2012-14 Salivary Gland Group, Junior Director
2014-15 Salivary Gland Group, Vice President
2013- UCSF Program in Craniofacial Biology Steering Committee member
2015 Proteoglycans Gordon Conference, Co-Chair

C. Selected Peer-reviewed Publications

Original Research Papers

2001 **Knox, S. M,** Melrose, J., and Whitelock, J. Electrophoretic, Biosensor and bioactivity analysis of perlecans of different cellular origins. *Proteomics* 1 (12): 1534-41. PMID: 11747213

2002 **Knox, S. M.,** Merry, C., Stringer, S., Melrose, J., and Whitelock, J.M. Not all perlecans are created equal: interactions with fibroblast growth factor (FGF) 2 and FGF receptors. *J. Biol. Chem.* 277 (17): 14657-65. PMID: 11847221

2002 Melrose, J., Smith, S., **Knox, S. M.,** and Whitelock, J. Perlecan, the multidomain HS-proteoglycan of basement membranes is a prominent pericellular component of ovine hypertrophic vertebral growth plate and cartilaginous endplate chondrocytes. *Histochem. Cell Biol.* 118 (4): 269-80. PMID: 12376823

2003 Kang, S., Feinlab, J., **Knox, S. M.,** Ketteringham, M., and Krauss, R. Promyogenic members of the Ig and cadherin families associate to positively regulate differentiation. *Proc. Natl. Acad. Sci. USA* 100(7): 3989-94. PMCID: PMC153035

2005 **Knox, S. M.,** Fosang, A., Last, K., Melrose, J., and Whitelock, J.M. Perlecan from epithelial cells is a hybrid heparin/chondroitin/keratin sulfate proteoglycan. *FEBS Lett* 579 (22): 5019-23. PMID:16129435

2006 Melrose, J., Roughley, P., **Knox, S. M**., Smith, S., Lord, M. and Whitelock J. The structure, location, and function of perlecan, a prominent pericellular proteoglycan of fetal, postnatal, and mature hyaline cartilages*. J. Biol. Chem*. 281(48): 36905-14. PMID: 16984910

2006 Kirkpatrick, C., **Knox, S. M*.,** Staaz, W., Fox, B., Lercher, D., and Selleck, S. The function of a Drosophila glypican does not depend entirely on heparan sulfate modification. *Dev. Biol.* 300(2): 570-82. PMID: 17055473

2007 **Knox, S.M**, Ge, H., Ren, Y., Dimitroff, B., Howe, K., Arsham, A., Easterday, M., Neufeld, T., O'Connor, M., and Scott B. Selleck. Mechanisms of TSC-mediated Control of Synapse Assembly and Axon Guidance. *PLoS ONE*. 2:e375. PMCID:PMC1847706

2007 Patel, V.N., **Knox, S.M**., Likar, K. M., Lathrop, C.A., Hossain, R., Eftekhari, S., Elkins, M., Vlodasky, I., Whitelock, J. M., and Hoffman, M. P. Heparanase cleavage of heparan sulfate regulates FGF10

function during submandibular gland branching morphogenesis, *Development* 134:4177-86. PMID: 17959718

2008 Whitelock, J., Ma, JL., Davies, N., Nielsen, N., Chuang, C., Rees, M., Iozzo, RV., **Knox, S**., Lord, M. *J* Recombinant heparan sulfate for use in tissue engineering applications. *Chem Tech Biotech.* (83(4):496-504.

2009 Chuang, C. Melrose, J., **Knox, S**., Iozzo, R., and Whitelock. J. The role of heparan sulfate on chondrocyte perlecan - to proliferate or not? *Int J Clin Exp Pathol.* 90(2):A102-103

2010 **Knox, S.M**., Lombaert, I., Reed, X., Vitale-Cross, L., Gutkind, J.S., and Hoffman, MP. Parasympathetic innervation maintains epithelial progenitor cells during salivary organogenesis, *Science* 329 (5999): 1645 – 1647. PMID 20929848

2013 **Knox, S.M.,** Lombaert, I.M., Haddox, C.L., Abrams, S.R., Cotrim, A., Wilson, A.J., and Hoffman, M.P. Parasympathetic stimulation improves epithelial regeneration. *Nature Comm.* 4:1494. DOI: 10.1038. PMID:23422662

2014 Finley, J., Farmer, D., Emmerson, E., Cruz Pacheco, N., and **Knox, S.M**[#]. Manipulating the murine lacrimal gland. *J. Visual Exp*. (in press).

2014 Nedvetsky, P., Emmerson, E., Finley, J., Ettinger, A., Curz Pacheco, N., Prochazka, J., Haddox, C., Northrup, E., Hodges, C., Mostov, K.E., Hoffman, M.P., and **Knox, S.M**[#]. Parasympathetic innervation regulates tubulogenesis in the developing salivary gland. *Developmental Cell.* 30, 449–462

2015 Knosp, W.M., **Knox, S.M***, Lombaert, I.M, Haddox, C.L., Patel, V.N and Hoffman, M.P. Submandibular parasympathetic gangliogenesis requires Sprouty-dependent Wnt signals from epithelial progenitors *Developmental Cell* 32(6):667-77

* co-first author

#corresponding author

Non-experimental papers

2006 Di Giusto, D.,**Knox, S. M**., Lai, Y., Tyrelle, G., Aung, M., and King, G. Multitasking by Multivalents Circular Aptamers. *Chembiochem*. 7(3):535-44

2006 **Knox, S. M.** and Whitelock, JM. Perlecan: how does one molecule do so many things? *CMLS* 63(21): 2435-45

2008 **Knox, SM**., and Hoffman, MP. *Saliva Diagnostics.* Ed. Wong, D. Book Chapter: Salivary Gland Development and Regeneration.

2010 Lombaert, I., **Knox, S.M**., and Hoffman, MP. Salivary gland progenitor cell biology provides a rationale for therapeutic salivary gland regeneration, *Oral Diseases* 17(5):445-9

2011 Knosp, WM., **Knox, S.M**., and Hoffman, MP. Salivary Gland Organogenesis. *WIRES Developmental Biology*. DOI: 10.1002/wdev.4.

2013 Klein, O., Bush, J., **Knox, S.M**., Jheon, A., Cordero, D.R, and Richtsmeier, J.T. The Society of Craniofacial Genetics and Developmental Biology 35th Annual Meeting. *Am J Med Genet Part A* 9999:1–15

2015 Mattingly, A., Finley, J.K, and **Knox, S.M**[#]. Salivary Gland Development and Disease. *WIRES Developmental Biology (accepted).*

D. Research Support

Ongoing Research Support

R01 DE024188-01 (Knox) 04/01/2014 – 03/31/2019

NIH

Neuronal regulation of salivary stem cells

The major goals of this proposal are to: 1) Define the mechanisms by which parasympathetic nerves regulate progenitor cell fate, 2) Determine the contribution of salivary progenitor cells and neuronal signaling to adult homeostasis and repair and 3) Define epithelial progenitor cells in the human salivary gland.

Pending

[redacted]

[redacted]

Completed Research Support

That Man May See (McNamara/Knox) 02/01/2014 – 01/31/2015
UCSF
Defining the functional link between inflammation and corneal neuropathy in dry eye disease
In this project we aim to (1) define the timing of neuronal and immune cell changes relative to each other during disease progression; (2) determining the extent to which dampening the immune response modulates corneal sensory innervation during dry eye development and identifying the specific neuromodulators that are restored in response to anti-inflammatory therapy.

R00 DE018969-03 (Knox) 01/24/2011 – 12/31/2014
NIH
Neuronal-epithelial interactions that regulate mouse salivary gland development
The major goal of this project is to investigate how the parasympathetic nervous system regulates submandibular gland development.

Resource Allocation Program (Knox) 02/01/2013 – 01/31/2014
UCSF
Parasympathetic innervation and the role of neuropeptide-Y in lacrimal gland development.
The major goals of this project are to: 1) Define the timing of innervation of the LG and the expression profile of NPY and its receptors (Npyr1,2,3,4 and 5) during embryonic and post-natal development; and 2) Assess NPY-mediated epithelial and neuronal morphogenesis using an ex vivo LG culture system

RFA-DE-12-005 / R21 (Knox) 07/01/2012 – 06/30/2014
NIH
Salivary gland repair and regeneration via Schwann cell-nerve interactions
The major goals of this project are to 1) determine the impact of gamma radiation on Schwann cell-neuronal cell interactions, 2) to identify the effects of Neuregulin-1 on Schwann cell and neuronal survival, nerve function, and epithelial regeneration after irradiation, and 3) to determine the ability of Schwann cells to restore salivary function after irradiation.

5K99DE018969 Knox (PI) 12/01/2008-11/04/2011
Neuronal-epithelial interactions that regulate mouse salivary gland development
Pathway to Independence Award: to investigate how the parasympathetic nervous system regulates submandibular gland development
Role: PI

Early Career Research Award Knox (PI) 07/01/2005-07/01/2006
Proteoglycan Proteomics
UNSW Faculty of Engineering Research Award: to develop platform methodology to identify proteoglycan biomarkers for the early detection of osteoarthritis
Role: PI

OMB Number: 4040-0001
Expiration Date: 06/30/2016

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Regents of the University of California, San Francisco

Start Date*: 09-01-2015 **End Date*:** 08-31-2016 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Nancy	A.	McNamara		PD/PI	[redacted]	[redacted]			[redacted]	[redacted]	[redacted]
2 .	Dr.	Sarah	Monica	Knox		Co-PD/PI	[redacted]	[redacted]			[redacted]	[redacted]	[redacted]

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
2	Post Doctoral Associates	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Assistant	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
3	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]

Total Salary, Wages and Fringe Benefits (A+B) **219,249.00**

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

Funding Opportunity Number: PA-13-302 . Received Date: 2015-04-16T19:25:16.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, San Francisco

Start Date*: 09-01-2015 **End Date*:** 08-31-2016 **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)	4,000.00
2. Foreign Travel Costs	
Total Travel Cost	**4,000.00**

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

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

Tracking Number: GRANT11891691 Funding Opportunity Number: PA-13-302 . Received Date: 2015-04-16T19:25:16.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, San Francisco

Start Date*: 09-01-2015 **End Date*:** 08-31-2016 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	44,880.00
2. Publication Costs	3,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Data Network Fee, CCDSS-Basic	2,348.00
9 . Other	640.00
Total Other Direct Costs	**50,868.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**274,117.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Cost (MTDC)	58.50	274,117.00	160,358.00
		Total Indirect Costs	**160,358.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, Jeanette Lu, 415-437-7820

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**434,475.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: Budget_Justification_NEI_RO14_16_2015_.pdf (Only attach one file.)

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

OMB Number: 4040-0001
Expiration Date: 06/30/2016

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: The Regents of the University of California, San Francisco

Start Date*: 09-01-2016 **End Date*:** 08-31-2017 **Budget Period:** 2

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Nancy	A.	McNamara		PD/PI	[redacted]	[redacted]			[redacted]	[redacted]	[redacted]0
2 .	Dr.	Sarah	Monica	Knox		Co-PD/PI	[redacted]	[redacted]			[redacted]	[redacted]	[redacted]

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
2	Post Doctoral Associates	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Assistant	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
3	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]

Total Salary, Wages and Fringe Benefits (A+B) **220,510.00**

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

Funding Opportunity Number: PA-13-302 . Received Date: 2015-04-16T19:25:16.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, San Francisco

Start Date*: 09-01-2016 **End Date*:** 08-31-2017 **Budget Period: 2**

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

Additional Equipment: File Name:

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

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

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

Tracking Number: GRANT11891691 Funding Opportunity Number: PA-13-302 . Received Date: 2015-04-16T19:25:16.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, San Francisco

Start Date*: 09-01-2016 **End Date*:** 08-31-2017 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	44,880.00
2. Publication Costs	3,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Data Network Fee, CCDSS-Basic	2,520.00
9 . Other	640.00
Total Other Direct Costs	**51,040.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**275,550.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Cost (MTDC)	58.50	275,550.00	161,197.00
		Total Indirect Costs	**161,197.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, Jeanette Lu, 415-437-7820

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**436,747.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: Budget_Justification_NEI_RO14_16_2015_.pdf (Only attach one file.)

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

OMB Number: 4040-0001
Expiration Date: 06/30/2016

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Regents of the University of California, San Francisco

Start Date*: 09-01-2017 **End Date*:** 08-31-2018 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Nancy	A.	McNamara		PD/PI	[redacted]	[redacted]			[redacted]	[redacted]	[redacted]
2 .	Dr.	Sarah	Monica	Knox		Co-PD/PI	[redacted]	[redacted]			[redacted]	[redacted]	[redacted]

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
2	Post Doctoral Associates	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Assistant	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
3	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]

Total Salary, Wages and Fringe Benefits (A+B) **227,425.00**

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

Funding Opportunity Number: PA-13-302 . Received Date: 2015-04-16T19:25:16.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, San Francisco

Start Date*: 09-01-2017 **End Date*:** 08-31-2018 **Budget Period: 3**

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

Additional Equipment: File Name:

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

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

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

Tracking Number: GRANT11891691 Funding Opportunity Number: PA-13-302 . Received Date: 2015-04-16T19:25:16.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, San Francisco

Start Date*: 09-01-2017 **End Date*:** 08-31-2018 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	44,880.00
2. Publication Costs	3,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Data Network Fee, CCDSS-Basic	2,650.00
9 . Other	640.00
Total Other Direct Costs	**51,170.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**282,595.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Cost (MTDC)	58.50	282,595.00	165,318.00
		Total Indirect Costs	**165,318.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Jeanette Lu, 415-437-7820

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**447,913.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: Budget_Justification_NEI_RO14_16_2015_.pdf (Only attach one file.)

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

OMB Number: 4040-0001
Expiration Date: 06/30/2016

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Regents of the University of California, San Francisco

Start Date*: 09-01-2018 **End Date*:** 08-31-2019 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Nancy	A.	McNamara		PD/PI	[redacted]	[redacted]			[redacted]	[redacted]	[redacted]
2 .	Dr.	Sarah	Monica	Knox		Co-PD/PI	[redacted]	[redacted]			[redacted]	[redacted]	[redacted]

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
2	Post Doctoral Associates	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Assistant	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
3	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]

Total Salary, Wages and Fringe Benefits (A+B) **234,051.00**

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

Funding Opportunity Number: PA-13-302 . Received Date: 2015-04-16T19:25:16.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, San Francisco

Start Date*: 09-01-2018 **End Date*:** 08-31-2019 **Budget Period: 4**

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

Additional Equipment: File Name:

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

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

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

Tracking Number: GRANT11891691 Funding Opportunity Number: PA-13-302 . Received Date: 2015-04-16T19:25:16.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, San Francisco

Start Date*: 09-01-2018 **End Date*:** 08-31-2019 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	44,880.00
2. Publication Costs	3,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Data Network Fee, CCDSS-Basic	2,671.00
9 . Other	640.00
Total Other Direct Costs	**51,191.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**289,242.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Cost (MTDC)	58.50	289,242.00	169,207.00
		Total Indirect Costs	**169,207.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Jeanette Lu, 415-437-7820

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**458,449.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: Budget_Justification_NEI_RO14_16_2015_.pdf (Only attach one file.)

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

OMB Number: 4040-0001
Expiration Date: 06/30/2016

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Regents of the University of California, San Francisco

Start Date*: 09-01-2019 **End Date*:** 08-31-2020 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Nancy	A.	McNamara		PD/PI	[redacted]	[redacted]			[redacted]	[redacted]	[redacted]
2 .	Dr.	Sarah	Monica	Knox		Co-PD/PI	[redacted]	[redacted]			[redacted]	[redacted]	[redacted]

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
2	Post Doctoral Associates	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Assistant	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
3	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]

Total Salary, Wages and Fringe Benefits (A+B) **238,484.00**

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

Funding Opportunity Number: PA-13-302 . Received Date: 2015-04-16T19:25:16.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, San Francisco

Start Date*: 09-01-2019 **End Date*:** 08-31-2020 **Budget Period: 5**

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

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

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

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

Tracking Number: GRANT11891691 Funding Opportunity Number: PA-13-302 . Received Date: 2015-04-16T19:25:16.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, San Francisco

Start Date*: 09-01-2019 **End Date*:** 08-31-2020 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	44,880.00
2. Publication Costs	3,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Data Network Fee, CCDSS-Basic	2,671.00
9 . Other	640.00
Total Other Direct Costs	**51,191.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**293,675.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Cost (MTDC)	58.50	293,675.00	171,800.00
		Total Indirect Costs	**171,800.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Jeanette Lu, 415-437-7820

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**465,475.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: Budget_Justification_NEI_RO14_16_2015_.pdf (Only attach one file.)

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

BUDGET JUSTIFICATION

Personnel Justification

A.Senior/Key Personnel

Nancy McNamara, Ph.D., Principal Investigator (effort, Years 1-5, salary support requested). Dr. McNamara will provide scientific oversight for the project. She has nearly 10 years of experience in the area of dry eye research with a major focus on autoimmune-mediated dry eye in Sjögren's syndrome using both human subjects and the autoimmune regulator (*Aire*)-deficient mouse as models. She will be responsible for all aspects of the corneal studies, outlined in Aims 1 and 2 and will provide all the wild type and *Aire*-deficient mice for in vivo and ex vivo analyses. She will design, plan, and carry out the proposed research, including the analysis and interpretation of results. She will supervise and mentor postdoctoral scholar, , and Staff Research Associate, , and will work with Co-PI, Dr. Knox, to prepare progress reports and manuscripts for publication. She will coordinate meetings to discuss experiments with Dr. Knox and members of her team on a weekly or daily basis as needed.

Sarah Knox, Ph.D., Principal Investigator (effort, Years 1-5, salary support requested). Dr. Knox will provide scientific oversight for the studies of lacrimal gland innervation in Aims 1 and 2. Specifically, she will design, plan, and carry out the proposed research, analyze the results, supervise and mentor postdoctoral scholar, , and the writing and editing of manuscripts. Dr. Knox has extensive expertise in the area of epithelial-neuronal interactions using ex vivo and in vivo mouse models, as well as high-resolution microscopy. She has significant expertise in gene expression analysis. Note: Per Dr. Knox's UCSF appointment she has total protected research time of 80%.

B. Other Personnel

Post-Doctoral Fellow (effort, Years 1-5, salary support requested).

., **Post-Doctoral fellow** (effort, Years 1-5, salary support requested).

Staff Research Associate II (effort, Years 1-5, salary support requested).

C. Equipment:

N/A

D. Travel

Funds are requested for the Co-PIs and one postdoc for travel to the annual ARVO or Gordon Conference. We estimate expenses to be **$4000/yr**.

F. Other Direct Costs

Materials and Supplies

-Immunohistochemistry/Immunofluorescence supplies: These supplies will be used in Aims 1 and 2 to assess proteins involved in pathological changes during ADDE development and include slides, OCT, paraffin, stains, fixatives and secondary antibodies. We estimates these costs to be **$2000**

-Western blot supplies: These include buffers, gels, ECL membranes and secondary antibodies: We estimate these costs to be **$1500.**

-Antibodies and antagonist (myelin protein zero, CD-4, IFN gamma, Semaphorins, Anakinra): 4 antibodies @$500each = $2000, Anakinra 100 mg/$260. Total = **$2260**

-qPCR supplies- kits for DNA/RNA isolation, reverse transcription, 96-well plates and Sybr Green **$5000**

-RNAseq and Ingenuity Pathway Analysis costs ($750 per sample x 12 samples) = **$9000**

-Mouse culture media with Laminin 3D matrix and growth factors: media ($250/2 weeks) x 3 months= $1500; glass bottom organ culture plates @ $370/box x 2 boxes = $740; growth factors including recombinant mouse IFN gamma IL1 beta ($350/vial, 4 vials) = $1400; laminin 3D matrix@ $690 per vial/2 vials = $1380. Total = **$5020**

-Recombinant proteins: Sema7a-Fc (2x50µg @ $300ea) and neurturin (2x25 µg @$300ea) **= $1200**

-Inhibitors of neuromodulators and signalling pathways: siRNA, adeno- associated viral constructs, chemical inhibitors for Aims 1 and 2. We estimate these costs to be **$3500**

-Intracellular phosphoprotein array (Pathscan antibody array, Cell Signaling) = **$800**

-Animal care and housing for Aire Wt/KO used in Aims 1 and 2: Mice will be housed at UCSF's LARC facilities. Approximate housing expenses are 50 cages x $0.80/ cage/ day x365 = **$14,600/yr**

Total = $44,880

Publication Costs

We anticipate publishing 7 articles over the 5 year period of the grant and request **$3000/yr**

ADP Computer Services

Data Networking Recharges: The University has an approved campus recharge for data network recharges. Costs are calculated monthly per FTE and pro-rated by the support provided by this grant. **Total charges per year are $1,046, $1,117, $1,163, $1,184, $1,184.**

Computing and communication device support services (CCDSS): CCDSS provides integral support to campus voice and data technology functions. CCDSS includes software installation/updates, internet security, hardware setup/configuration, and centrally managed patching, storage and backup. The university charges these expenses to all funding sources based on a monthly recharge rate per FTE, consistent with the university's current methodology used for data network services. The recharge rates are provided for under our approved DS-2, will be computed in accordance with applicable OMB requirements, including 2 CFR Part 220 (formerly Circular A-21), and will be reviewed and adjusted annually. **Total charges per year are $1,302, $1,403, $1,487, $1,487, $1,487.**

Other Expenses

Genotyping costs for Aim 1 and 2, 40 litters *8 mice per litter * $2/mouse = **$640/yr**

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		[redacted]
Section B, Other Personnel		[redacted]
Total Number Other Personnel	15	
Total Salary, Wages and Fringe Benefits (A+B)		1,139,719.00
Section C, Equipment		
Section D, Travel		20,000.00
1. Domestic	20,000.00	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		255,460.00
1. Materials and Supplies	224,400.00	
2. Publication Costs	15,000.00	
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	12,860.00	
9. Other 2	3,200.00	
10. Other 3		
Section G, Direct Costs (A thru F)		1,415,179.00
Section H, Indirect Costs		827,880.00
Section I, Total Direct and Indirect Costs (G + H)		2,243,059.00
Section J, Fee		

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix:	Dr.
First Name*:	Nancy
Middle Name:	A.
Last Name*:	McNamara
Suffix:	OD, PhD

2. Human Subjects

Clinical Trial?	❍ No	❍ Yes
Agency-Defined Phase III Clinical Trial?*	❍ No	❍ Yes

3. Permission Statement*

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

● Yes ❍ No

4. Program Income*

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)*

PHS 398 Cover Page Supplement

5. Human Embryonic Stem Cells

Does the proposed project involve human embryonic stem cells?* ● No ❍ Yes

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

Cell Line(s): Specific stem cell line cannot be referenced at this time. One from the registry will be used.

6. Inventions and Patents (For renewal applications only)

Inventions and Patents*: ❍ Yes ❍ No

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

Previously Reported*: ❍ Yes ❍ No

7. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:

First Name*:

Middle Name:

Last Name*:

Suffix:

❑ Change of Grantee Institution

Name of former institution*:

PHS 398 Research Plan

Please attach applicable sections of the research plan, below.

OMB Number: 0925-0001

1. Introduction to Application (for RESUBMISSION or REVISION only)	
2. Specific Aims	NerveR01specific_Aims_4_16_15.pdf
3. Research Strategy*	NerveR01_FINAL_research.pdf
4. Progress Report Publication List	
Human Subjects Sections	
5. Protection of Human Subjects	
6. Inclusion of Women and Minorities	
7. Inclusion of Children	
Other Research Plan Sections	
8. Vertebrate Animals	Vertebrate_animals_RO1_16_2015.pdf
9. Select Agent Research	
10. Multiple PD/PI Leadership Plan	McNamara_Knox_Leadership_4_15_final.pdf
11. Consortium/Contractual Arrangements	
12. Letters of Support	
13. Resource Sharing Plan(s)	Model_Organism_Sharing_plan2.pdf
Appendix (if applicable)	
14. Appendix	

SPECIFIC AIMS

Dry eye is a multifactorial disease affecting millions of people worldwide including nearly 15% of Americans 50 years of age and older (1-3). Autoimmune diseases such as Sjögren's syndrome (SS) are among the many conditions in which dry eye manifests. In SS, infiltrating lymphocytes target the exocrine glands to provoke an aqueous-deficient dry eye, which is among the most common and debilitating clinical manifestations. Despite important insights in defining the immunopathologic conditions that are features of dry eye, the underlying mechanisms that cause and perpetuate the disease are not well defined. As such, there is no cure for aqueous-deficient dry eye (4) where current treatment options target symptoms rather than resolving disease. Thus, **it is essential to identify new regenerative therapies to restore ocular health in dry eye patients**.

An important yet often overlooked component of dry eye disease is altered innervation of the cornea and lacrimal gland (5-8). Neurological manifestations of SS have long been recognized and can have a substantial negative impact on the ability of target organs to contend with chronic pathological insult. While the pathogenic mechanisms responsible for most forms of neurological involvement in SS are unknown, IFN gamma (IFNγ) was recently shown to be absolutely required for the development for peripheral neuropathy in the autoimmune regulator gene (*Aire*)-deficient mouse model of SS (9). Yet, the mechanism by which IFNγ caused peripheral neuropathy, or if peripheral organs became denervated, was not described. In preliminary studies we found innervation and function of the cornea and lacrimal gland of *Aire*-deficient mice to be severely diminished, with altered expression of neuromodulators and basement membrane molecules necessary for innervation. We partially rescued innervation by ablating the interleukin receptor 1 (IL1R1), suggesting that IFNγ secreted by infiltrating CD4+ T cells, and local activation of IL1/IL1R1 signaling, cooperate to deplete the signals and structures required for maintaining innervation in the adult. Thus, we hypothesize that immune factors released in response to CD4+ T cell infiltration impair epithelial maintenance of nerves to the cornea and lacrimal gland resulting in loss of tissue integrity and reduced tear secretion that perpetuate ocular surface disease and aqueous tear deficiency in autoimmune-mediated dry eye. ***Here, we propose to define the cellular processes that lead to denervation of target organs in aqueous-deficient dry eye and to identify mechanisms to reinnervate the epithelium and reverse dry eye in SS patients.***

Specific Aim 1: Define the timing of denervation and corresponding pathological changes in the cornea, lacrimal gland and associated nerves during dry eye development. SS patients exhibit reduced innervation of the cornea and abnormalities in the epithelial basement membrane, which suggests a disruption in neuronal-tissue communication. Consistent with this, our preliminary data show that innervation is reduced, the basement membrane is disrupted and expression of axon guidance factors (e.g., semaphorin 7a) and neurotrophic factors (e.g., glial cell-derived neurotrophic factor) are altered in the inflamed corneas and lacrimal glands of *Aire*-deficient mice. However, it is not known when dry eye-mediated denervation begins (in mice or human) or if it leads to or results from pathological changes in the ocular tissues. In **Aim 1A** we will first determine when denervation of the cornea and lacrimal glands appears in relation to other pathological events (e.g., basement membrane disruption) during dry eye development in *Aire*-deficient mice. We will then define changes in the expression of neuromodulators and basement membrane proteins using transcriptomic profiling in conjunction with immunohistological and biochemical assays. In **Aim 1B**, we will confirm that immune-mediated changes in neuromodulators correspond to altered innervation by rescuing disease with anti-inflammatory therapies. *Outcomes will define the timing of denervation and indicate potential mechanisms by which denervation occurs.*

Specific Aim 2. Determine the mechanisms by which T cell-mediated inflammation alters innervation of the cornea and lacrimal glands. Based on preliminary data showing partial rescue of innervation and the basement membrane in *Aire* knockout (KO) mice deficient in IL1R1, and previous reports that IFNγ released from activated T-cells is an essential mediator of peripheral neuropathy in the *Aire*-deficient mouse, we will test the hypothesis that both IFNγ and IL1β cause corneal and lacrimal gland denervation by altering the production of basement membrane proteins and neuromodulators required for maintaining epithelial and neuronal function. In **Aim 2A**, we will determine if IFNγ and IL1β directly affect the growth and function of nerves and/or epithelial cells. Then, we will define signaling pathways activated by IFNγ and IL1β that alter the production of neuromodulators and basement membrane components, as well as axon growth/function. In **Aim 2B,** we will determine the extent to which manipulating neuromodulators and the downstream signaling molecules identified above can maintain or restore innervation of the inflamed cornea using *ex vivo* co-cultures and the *Aire* KO mouse. *Outcomes will elucidate the molecular events that provoke corneal denervation in aqueous-deficient dry eye and reveal novel regenerative strategies.*

A. SIGNIFICANCE

Autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus, ocular cicatricial pemphigoid, graft versus host disease and Sjögren's syndrome (SS), are among the many conditions in which aqueous tear deficiency manifests. Unfortunately, patients suffering from autoimmune disorders with symptoms of ocular dryness have a limited number of therapeutic options available. Diseased eyes are commonly treated with 'artificial tears' that temporarily alleviate symptoms or with topical steroids to reduce inflammation but where significant side effects limit long-term use. Furthermore, such therapies do not address tissue damage and do little to restore ocular function, which significantly reduces quality of life. **Consequently, there is substantial need to identify regenerative strategies for improving ocular function in those affected by dry eye disease.**

Tissue injury due to immune cell infiltration is a consistent feature of autoimmune-mediated, aqueous-deficient dry eye. However, an important yet often overlooked component of dry eye is altered innervation of the cornea and lacrimal gland (5-8). The neurological manifestations of Sjögren's syndrome have long been recognized and the trigeminal nerve is one of the most common cranial nerves affected. In a majority of SS patients, neurological manifestations are noted to precede sicca symptoms (10, 11) with up to 93% of patients diagnosed with SS after neuropathy symptoms appeared (12). While the pathogenic mechanisms responsible for most forms of neurological involvement in SS are unknown, there is growing evidence that innervation itself is a negative modulator of inflammation (13, 14) and a positive regulator of progenitor cell-mediated restoration of damaged tissues (15). However, despite the essential need for a functional nerve supply, **current medications and therapeutic strategies do not address the need to maintain and/or reinnervate the target organs affected by chronic inflammation in SS as a means to preserve tear secretion and ocular surface integrity.**

One potential regenerative strategy for reversing ocular dysfunction due to SS (and other autoimmune disorders) is resupplying the ocular tissues with sensory innervation. Yet, before we can develop therapies to resolve and/or cure dry eye, there is a critical need to define the cellular processes regulating epithelial-neuronal communication during homeostasis and the molecular events that link chronic inflammation to loss of innervation in the chronically inflamed dry eye. Over the past 8 years we have used the autoimmune regulator gene (*Aire*)-deficient mouse model of SS to define the immunopathological mechanisms that elicit the damaging consequences of chronic inflammation on the cornea and lacrimal gland. We have identified IFNγ -secreting, CD4+ T cells as the main immune effectors promoting dry eye development in *Aire* KO mice, which initiate and sustain local inflammation through the activation of IL1/IL1R1 signaling in resident cells of the ocular tissues (i.e., epithelial and resident immune cells) (16). Interestingly, IFNγ secreted by CD4+ T cells was also recently shown to be the primary cause of peripheral neuropathy in *Aire* KO mice, however, the mechanism by which IFNγ achieved this outcome was not studied (9). In preliminary data we provide clear evidence that T-cell mediated neuropathy of the cornea and lacrimal gland occurs in *Aire* KO mice and that this neurological manifestation is associated with altered expression of epithelial basement membrane proteins that facilitate neuronal-epithelial interactions and neuromodulators known to be involved in the maintenance and function of nerves during development. In support of a link between IFNγ and IL1/IL1R1 signaling, we also demonstrate that genetic ablation of IL1R1 partially restores innervation and the basement membrane, indicating these cytokines act together to regulate peripheral organ denervation. These findings suggest that IFNγ, in cooperation with local IL1/IL1R1 signaling, depletes signals and structures required for maintaining innervation in the adult. Here, **we propose to determine the cellular processes that lead to denervation of target organs in SS-associated dry eye and to define the molecular events that can be exploited to reinnervate the epithelium and reverse dry eye.** *Discoveries resulting from this work will enhance our understanding of dry eye pathogenesis and lead us closer to our long-term goal of developing mechanism-based, efficacious therapeutic modalities to treat dry eye.*

B. INNOVATION

1) Patients with SS exhibit extensive neuropathy including reduced innervation of the cornea and lacrimal gland (8, 17). However, current approaches for treating dry eye have so far failed to address the need to maintain or restore innervation of ocular structures as a novel therapeutic strategy. Thus, our search for new therapeutic targets and mechanistic approaches to maintain or restore innervation in the setting of chronic inflammation is highly novel.

2) We are innovative in our use of the *Aire*-deficient mouse model of SS to determine the mode of action by which denervation of the cornea and lacrimal gland occurs. *Aire*-deficient mice are a model of spontaneous, autoimmune-mediated, dacryoadenitis that closely mimics the clinical characteristics of SS. They have proven

to be an excellent model of immune-mediated, aqueous tear deficiency and peripheral neuropathy that occurs in human patients with SS. The *Aire*-deficient model differs from existing mouse models where dry eye is induced in wild-type (WT) mice through the use of anticholinergic medications and reduced ambient humidity, which does not permit examination of the natural course of nerve loss and disease development. Induced models of aqueous-deficient dry eye are also not optimal for addressing pathogenic features of the lacrimal gland that accompany, and likely initiate dry eye in SS.

3) We utilize innovative techniques to explore epithelial-neuronal interactions. We have developed an *ex vivo*, whole mount, cornea-trigeminal co-culture assay that can be used to simultaneously determine changes in tissue morphology and gene expression within tissue compartments (e.g. cornea vs. ganglion). Furthermore, we have developed an *ex vivo* culture system for the adult lacrimal gland. These *ex vivo* systems are a significant advantage over previous *ex vivo* studies where culture conditions destroyed the epithelial and neuronal architecture, thus preventing analysis of its components.

C. APPROACH

Innervation of the cornea and lacrimal gland is reduced in a mouse model of SS. Corneal and lacrimal epithelial homeostasis is dependent on innervation. In the cornea, sensory nerves from the trigeminal ganglion enter the corneal stroma and run centrally and anteriorly in a radial fashion toward the central cornea and give rise to branches that innervate the anterior and mid stroma. At the interface between the basement membrane (Bowman's layer) and the anterior stroma, the stromal nerves form a sub-epithelial nerve plexus and perforate Bowman's layer to form the subbasal epithelial nerve plexus. Thus, these sensory nerve fibers provide innervation to the basal epithelial cell layer and terminate within the superficial epithelial layers. Similarly, the secretory epithelium of the lacrimal gland receives the majority of its innervation from the parasympathetic branch of the autonomic nervous system (actively controls tear secretion), as well as from sympathetic nerves and sensory nerves from the trigeminal ganglion. Surgical ablation of parasympathetic innervation to the lacrimal gland (18) or sensory innervation of the cornea (19) results in tissue atrophy, providing strong evidence that epithelial homeostasis requires nerve input. Given that innervation is essential for the maintenance of epithelial health, we further analyzed innervation of the cornea and lacrimal gland in the *Aire* KO mouse using 3D high-resolution imaging. *Aire*-deficient mice spontaneously develop a CD4+ T cell-mediated autoimmune disease that targets multiple organs, including peripheral nerves, exocrine glands, and the eyes (**Fig.1** and (20-22)), and mimics pathologies found in patients with SS. In support of a recent report demonstrating a functional link between IFNγ-mediated inflammation and denervation (9), we found reduced nerve input and extensive infiltration of IFNγ-secreting CD4+ T cells in the adult corneas and lacrimal glands of *Aire* KO compared to wild type mice (WT; **Fig.1** and (20)) and denervation was concurrent with tissue damage

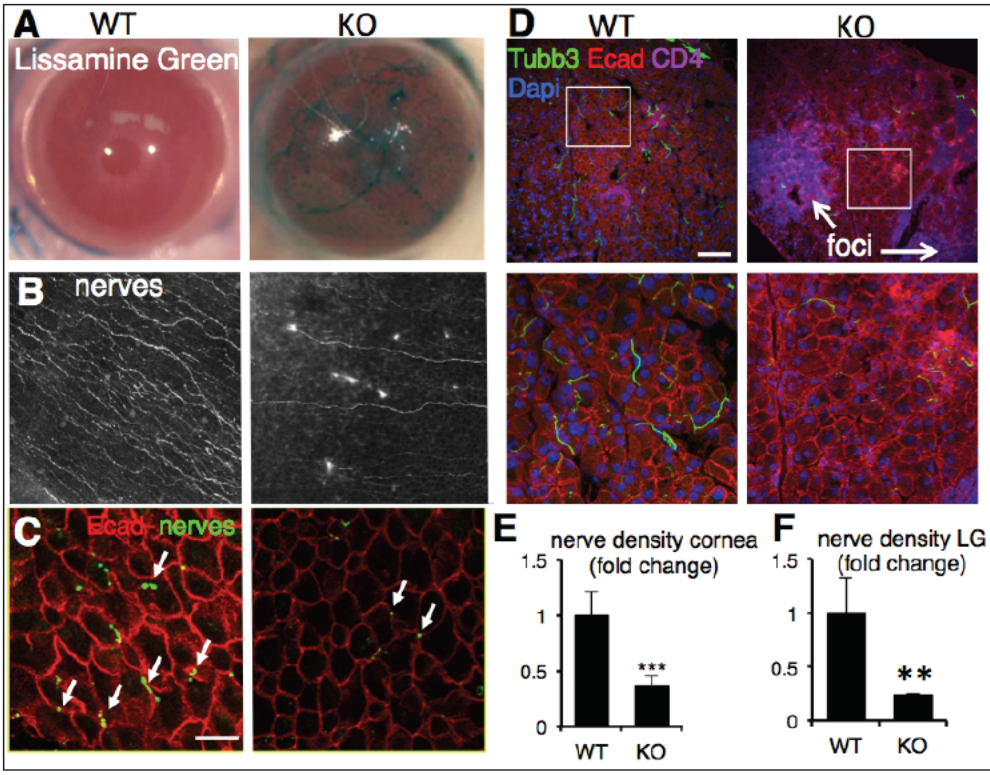


Figure 1: Innervation of the cornea and lacrimal gland (LG) is decreased in Aire KO. (A-C,E) Epithelial barrier is compromised in the Aire KO cornea as shown by lissamine green uptake (A) and innervation is significantly reduced (B,C and E). Scale bar = 10μm. (**D and F**) The LG from Aire KO mice shows extensive lymphocytic infiltration (CD4+, purple) and reduced innervation of the epithelium (Tubb3, green). Scale bar = 100μm. Lower panel images are higher magnification of boxes in upper panel. Innervation of the cornea (limbus to central cornea, sub-basal to suprabasal) and LG was evaluated in whole mounts using ImageJ/Fiji with particle analysis to determine nerve density over defined area. n = 3-4 mice per genotype (mice aged 10wks). Mean± SD; **P<0.01; ***P<0.001;

due to CD4+ T cells (**Fig. 1D** and ref (16, 21, 23)). *Now we will determine if denervation is a result of target organ or neuronal damage (**Specific Aim 1**) by defining the timing of denervation in relation to the appearance of epithelial and neuronal pathologies in the cornea and lacrimal gland.*

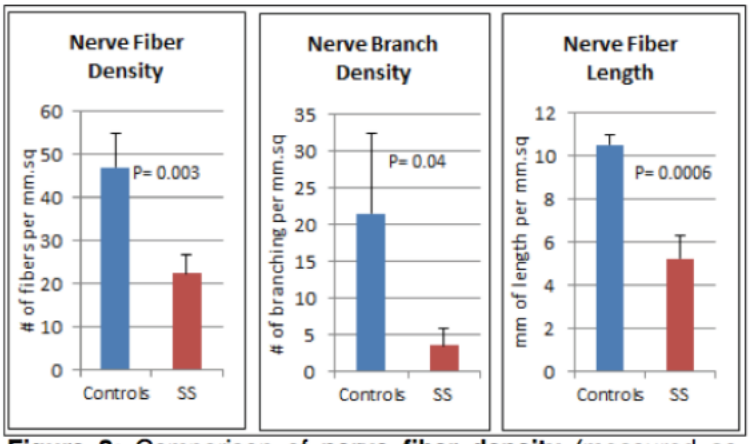


Figure 2: Comparison of **nerve fiber density** (measured as number of fibers per mm.sq. area of cornea), **nerve branch density** (measured as number of branch points per mm.sq. area) **and nerve fiber length** (measured as millimeters per mm.sq. area of cornea) in four patients with SS vs. four age-matched controls with no history of eye disease. Significant differences were observed in all parameters.

SS patients exhibit corneal neuropathies. Previous studies have demonstrated that neuropathies, including corneal neuropathy, occurs in patients with SS (5) (24). Loss of innervation in the *Aire* KO mouse cornea matched the results of our human studies where we used in vivo confocal microscopy (IVCM) to quantify changes in the corneal nerve supply in a small cohort of SS patients diagnosed with moderate to severe dry eye. Using IVCM we found significant decreases in corneal nerve fiber density, nerve branch density and nerve fiber length in SS patients compared to age-matched controls (**Fig. 2**). Estimated means and standard deviations for SS and controls were compared for each variable (using the Welch t-test) for each of three nerve parameters (see below) P<0.04. Thus, in a well-defined population of SS patients, using standardized IVCM measurements we noted compelling differences in corneal innervation.

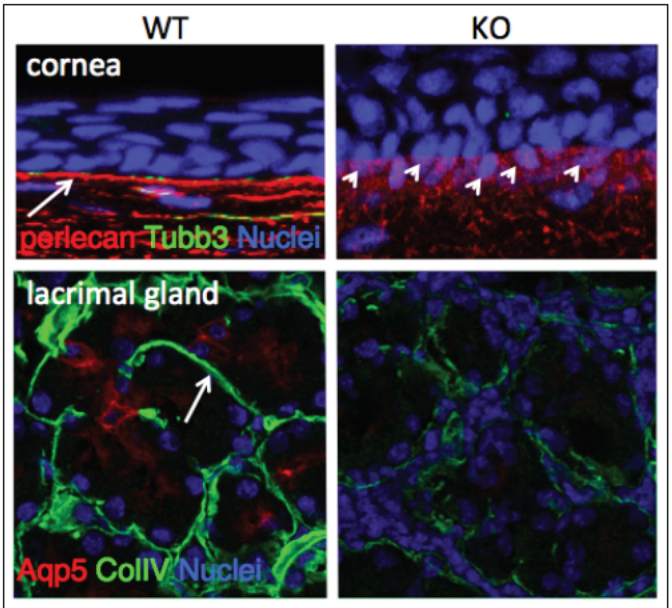


Figure 3. The basement membrane is reduced in the Aire KO cornea and lacrimal gland. WT and Aire KO immunostained for basement membrane using antibodies to perlecan (cornea, red) or collagen IV (ColIV, lacrimal gland, green) and nuclei (blue). Arrow indicates basement membrane. Arrowheads highlight epithelial nuclei aberrantly residing within the stroma of the cornea. Images of the cornea and lacrimal gland are 1μm confocal sections.

Corneal and lacrimal gland epithelial basement membrane morphology is altered in *Aire* KO mice. The basement membrane is essential for regulating epithelial cell differentiation, cell adhesion, and migration, as well as the function and repair of epithelial tissues (rev in (25) (26)). It is also necessary for maintaining innervation of the epithelium. In the cornea, sensory nerves provide innervation to the basal, suprabasal and superficial epithelial layers. In mice deficient in the basement membrane protein collagen XVIII (27) and in human diseases such as epithelial basement membrane dystrophy (28), innervation of the cornea is substantially reduced. Much less is known about the function of the basement membrane in the lacrimal gland. However, basement membrane molecules such as collagen IV are required for glandular morphogenesis (29) as well as for organ function (26). Furthermore, basement membrane surrounding secretory acini is disorganized in human patients with SS, which is thought to impair progenitor cell mediated repair of the tissue (30).

To determine whether changes in the basement membrane of cornea and lacrimal gland were associated with defects in innervation we immunostained for the basement membrane molecules perlecan (cornea) or collagen IV (lacrimal glands). Perlecan is the most prevalent heparan sulfate proteoglycan in the corneal basement membrane and is critical for normal basement membrane formation, as well as epithelial development and terminal differentiation (31). Compared to WT mice, the perlecan (+) basement membrane of the *Aire* KO cornea was thin and no longer separated the basal epithelial cell layer from the stroma, as evidenced by basal epithelial nuclei appearing within the stroma (**Fig. 3, arrowheads**). Similarly, expression of collagen IV is reduced in *Aire* KO lacrimal glands and the basement

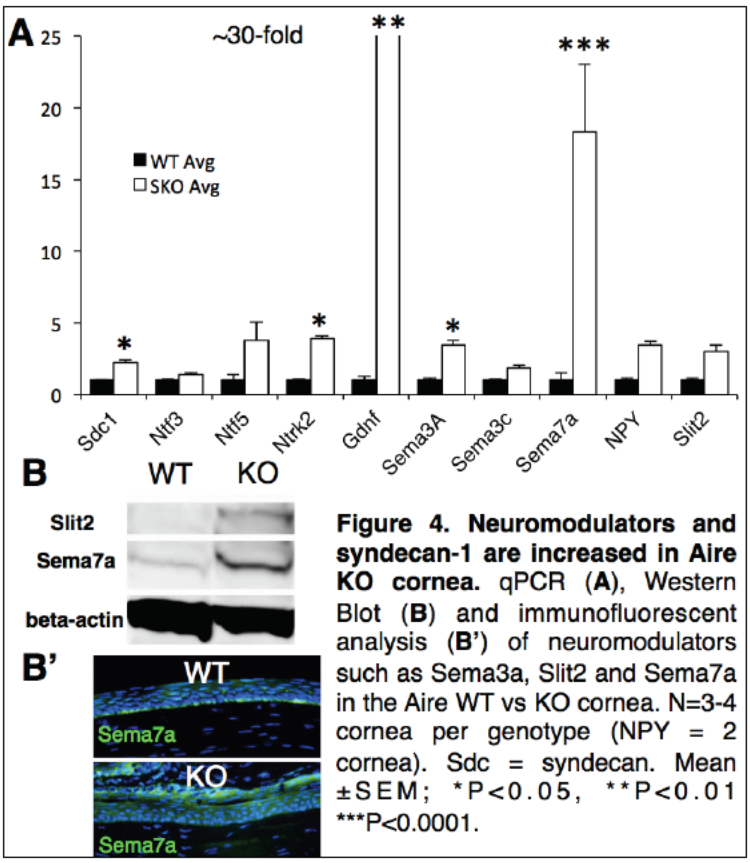


Figure 4. Neuromodulators and syndecan-1 are increased in Aire KO cornea. qPCR (**A**), Western Blot (**B**) and immunofluorescent analysis (**B'**) of neuromodulators such as Sema3a, Slit2 and Sema7a in the Aire WT vs KO cornea. N=3-4 cornea per genotype (NPY = 2 cornea). Sdc = syndecan. Mean ±SEM; *P<0.05, **P<0.01 ***P<0.0001.

membrane was also thin and irregular (**Fig. 3**). This result is consistent with studies in human SS glands where collagen IV was reduced and the basement membrane network surrounding acinar cells was highly disorganized (30, 32). We also noted that aquaporin 5 (AQP5) (**Fig. 3**, red) was severely reduced, suggesting epithelial cells were no longer polarized and/or functional. Based on these findings, *we hypothesize that altered expression, production and/or organization of basement membrane proteins in the chronically inflamed dry eye contributes to the loss of sensory reinnervation and corneal/lacrimal gland epithelial homeostasis.*

Neuromodulators are altered in the cornea and lacrimal gland of Aire KO mice. Innervation of the developing cornea by the trigeminal ganglion is dependent on expression of axon guidance ligands such as those of the semaphorin family, which activate their cognate receptors neuropilin (Nrp)1 and 2 on innervating nerves (33). Although much less information is available on the role of these families in controlling innervation of adult tissue, recent studies indicate that SEMA7a and SEMA3a function in reinnervation of the adult cornea. Recombinant SEMA7a was shown to promote innervation of the mouse cornea after lamellar corneal surgery (34) and inhibition of SEMA3a with a small molecule inhibitor improved nerve regeneration in a mouse model of corneal transplantation (35). We therefore determined whether these molecules were altered in the *Aire* KO cornea. As shown in **Figs. 4A-B**, expression of *Sema3a* and *7a* significantly increased in KO corneas compared to WT controls. We also found a number of other axon guidance and neurotrophic factors were altered. These included *Slit2*, a neurorepulsive factor (**Fig. 4A-B**), the neurotrophic factor neurotrophin 5 (*Ntf5*), and glial cell line-derived neurotrophic growth factor (*Gdnf*), which promotes target innervation during development (36) and has been shown to repress IL-17 induced inflammation in the cornea (37).

Similar to the cornea, loss of innervation to the lacrimal gland and increased CD4+ T cell infiltration was accompanied by upregulation of axon guidance factors (i.e., *Sema3a, Sema7a, Slit2*) and neurotrophic/guidance factors (neurturin (*Nrtn*); **Fig. 5**). Consistent with a loss in lacrimal gland function, the secretory protein amylase (*Amy1*) was downregulated in *Aire* KO mice. This alteration in guidance factors suggests that these ocular organs react in a similar fashion and, as such, may be denervated by similar mechanisms during dry eye disease.

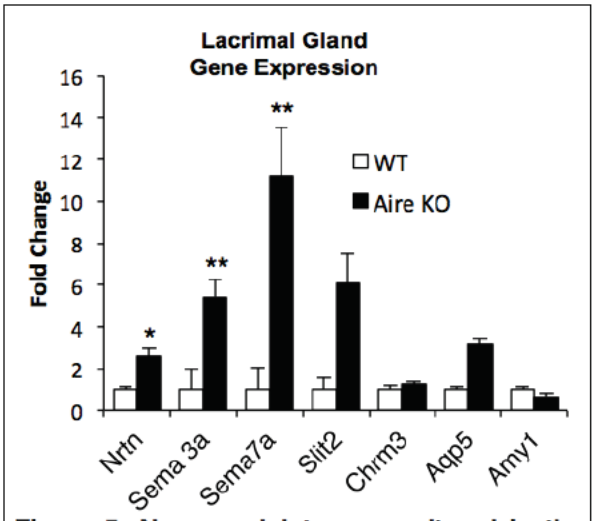
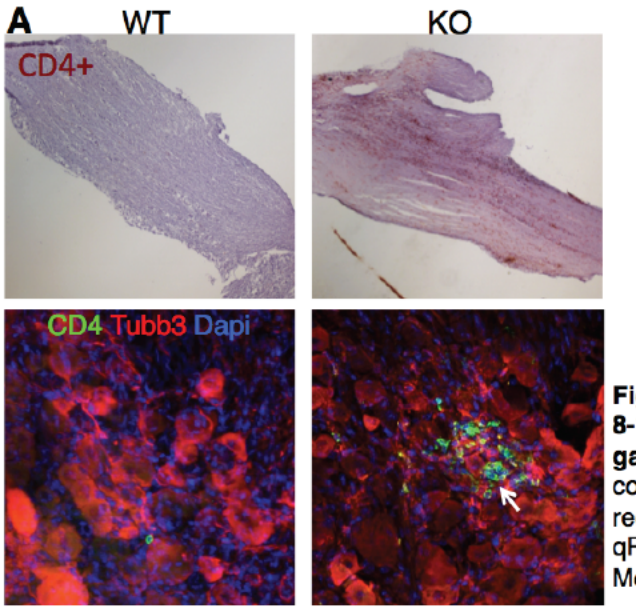


Figure 5: Neuromodulators are altered in the Aire KO lacrimal gland. Expression of axon guidance factors (i.e., Sema3a, Sema7a, Slit2,) neurotrophic factors (neurturin) and water channel protein, Aquaporin 5 (Aqp5), are upregulated whereas amylase1 is downregulated in Aire KO mice compared to WT control; mean±SEM, n= 3 WT vs. 6 KO glands per gene. *P<0.05; **P<0.01

CD4+ immune cells infiltrate the trigeminal ganglia in *Aire* KO mice

Previous studies have demonstrated sciatic neuropathy in the *Aire*-deficient mouse, with extensive lymphocytic infiltration at late stages of the disease (9). Similar to these findings, we observed abundant CD4+ T cell populations in the trigeminal ganglia of *Aire* KO mice at 8-10wks compared to control (**Fig. 6A**), indicating that during severe disease, the trigeminal ganglion is adversely affected. These results suggested that denervation may result from the inability of

sensory nerves to respond to axon guidance factors. To determine if trigeminal ganglia (TG) neurons from *Aire* KO mice were capable of responding to neurotrophic factors secreted by the cornea, we analyzed gene expression of neuromodulator receptors. The semaphorin receptors *Nrp1* and *Nrp2* and the NTF5 receptors *Ngfr* and *Ntrk2* were upregulated in the *Aire* KO TG (**Fig. 6B**) and *Robo1*, the receptor for SLIT2, was expressed at similar levels as in the WT TG. Together, these data indicated that there is a dysregulation of neuromodulators that may inhibit corneal reinnervation.



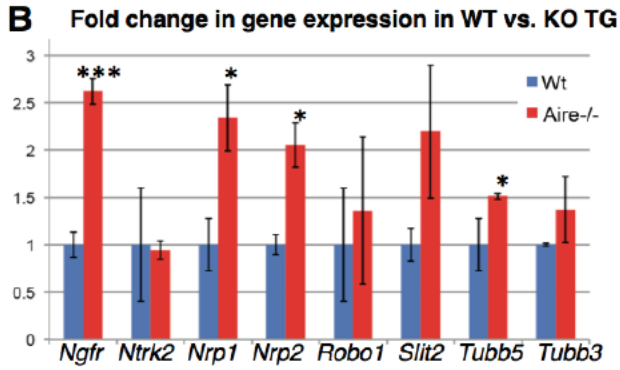


Figure 6: CD4+ cells infiltrate the Aire trigeminal ganglia (TG) at 8-10 wks and neuromodulators are altered in Aire KO trigeminal ganglia. (A) TG were fixed and immunostained for CD4+ cells and co-stained with H&E (top panel) or for nerves (lower panel, Tubb3, red). Arrow indicates T cells interacting with neuronal cell bodies. (**B**) qPCR of neuromodulators in the Aire WT vs. KO trigeminal ganglia. Mean ±SD; *P<0.05, ***P<0.0001; N=3 per genotype.

Topical application of steroids partially rescues corneal innervation.

Recently, it was shown that ablation of IFNγ signaling inhibited autoimmune peripheral neuropathy in *Aire* KO mice (9). Therefore, we tested the hypothesis that innervation would be rescued in *Aire* KO corneas when local inflammation was suppressed. As predicted, treatment of adult *Aire* KO corneas with topical, dexamethasone (Dex) 2x daily for 14 days resulted in a partial rescue of innervation in the inflamed cornea (**Fig. 7**). Of note, the lacrimal gland did not show an increase in nerve supply, as would be expected following local (topical) administration of the steroid (data not shown). Importantly, we observed the restoration of the sub-epithelial nerve plexus suggesting that the basement membrane was also rescued to some extent. In this proposal we will utilize Dex treatment at various stages of disease progression to identify molecules regulated by the immune system that promote denervation of the cornea.

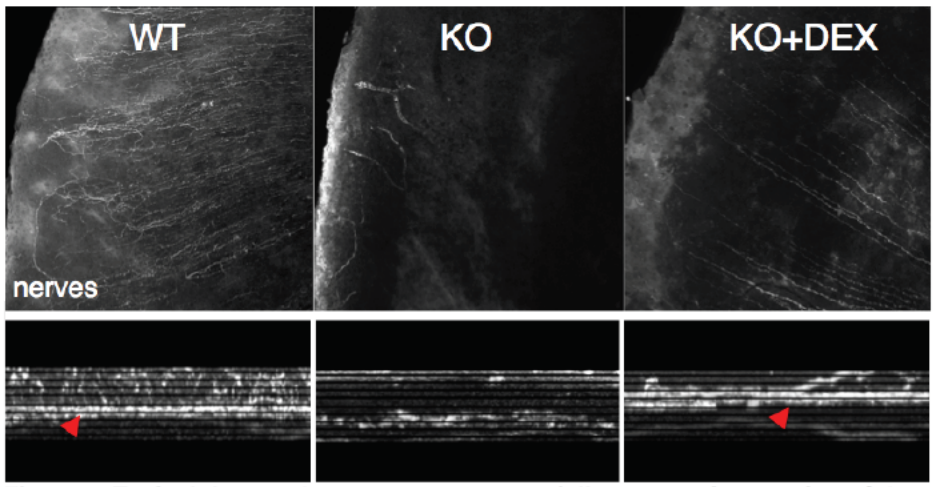


Figure 7. Topical dexamethasone treatment partially rescues innervation of the cornea. Aire WT vs KO Aire mice were treated 2x daily with topical dexamethasone (DEX) for 2 weeks and the cornea fixed and immunolabeled for nerves. Lower panel shows nerve fibers accumulating at basement membrane separating epithelium from stroma (arrow heads). Upper panels are 80μm projections of 1μm confocal sections. Lower panel is a z-projection through the y plane of upper panel.

Genetic ablation of the IL1β receptor, IL1R1, rescues cornea innervation and the basement membrane. Previously, we used impression cytology to demonstrate increased ocular surface expression of IFNγ and IL1β in SS patients (38). More recently, we have gone on to define the functional roles of these cytokines in dry eye pathogenesis. Specifically, we showed that the infiltration of autoreactive, INFγ-secreting CD4+ T cells, provoked the accumulation of IL1β in the epithelium and basement membrane of *Aire* KO corneas

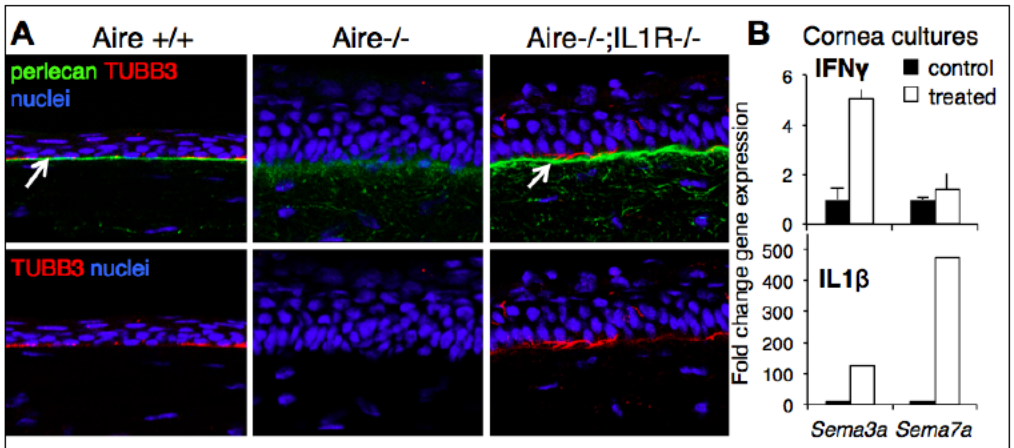


Figure 8. Genetic ablation of IL1R in the Aire deficient cornea rescues the basement membrane and innervation. (**A**) WT and Aire KO immunostained for basement membrane using antibodies to perlecan (green), nerves (TUBB3, red) and nuclei (blue). Arrow indicates basement membrane. Images are 1μm confocal sections. (**B**) IFNγ and IL1β differentially affect expression of neuromodulators *Sema7a* and *3a*. IFNg increases Sema3a but not Sema7a whereas IL1β increases both. Cornea cultured with or without IFNγ (0.5ng/mL) or IL1β (10ng/mL) for 24h. 3 cornea per treatment.

where it disrupted epithelial integrity, induced pathological keratinization and altered the glycosylation of goblet cell mucins (23). In support of this, we showed that both ablation of the IL1β receptor, IL1R1, in *Aire*-deficient mice (23) and topical treatment with the FDA-approved IL1R1 antagonist, Anakinra, (39) resulted in reduced corneal damage, even though the accumulation of CD4+ T cells was similar to that of untreated *Aire* KOs with intact IL1R1. Thus, local activation of the IL1/IL1R1 signaling pathway appears to mediate the interplay between IFNγ-secreting, effector T cells and ocular resident cells to promote ocular surface disease in SS (16). To determine if ablation of the IL1R1 in the *Aire*-deficient mouse reduced denervation and disruption of the basement membrane in the cornea, we immunostained for nerves (TUBB3) and perlecan, respectively, and found that both were substantially less affected compared to the *Aire* KO cornea (**Fig. 8**). These results suggested that IL1β produced by the epithelial cells rather than IFNγ produced by the CD4+ T cells was the major factor altering neuromodulators in the *Aire* KO cornea. To test this notion, we performed *ex vivo* cornea cultures in the presence of IFNγ or IL1β and found IL1β to dramatically increase both *Sema3a* and *7a* whereas IFNγ increased *Sema3a* only. *In this proposal, we seek to identify the effects of IFNγ and IL1β on epithelial cells in the cornea and lacrimal gland, as well as their innervating ganglia.*

RESEARCH STRATEGY

Rationale: Patients with immune-mediated aqueous-deficient dry eye as occurs in patients with SS often develop corneal neuropathies (5), where loss of neuronal inputs upsets the complex reflex network connecting the ocular mucosal tissues (e.g. cornea, limbus, conjunctiva) and the tear secreting machinery (e.g. lacrimal and meibomian glands) that maintain ocular surface homeostasis, known as the Lacrimal Functional Unit. In addition to its role in corneal sensation, this neuronal-epithelial communication network is critical for maintenance of the limbal stem cell niche (40), as well as epithelial migration and proliferation (41, 42). Consistent with this, our preliminary studies in human patients with SS as well as in the *Aire* knockout (KO) mouse model of SS demonstrate that the cornea (mouse and human) and lacrimal glands (mouse) have reduced innervation (**Figs. 1&2**). However, the timing and the mechanisms by which denervation is achieved are not known. Based on our preliminary data that IFNγ-secreting T cells damage the cornea and lacrimal glands in the *Aire* KO mouse (**Fig. 1B**), and that structural (basement membrane) and molecular cues (neuromodulators) thought to be required for innervation are altered in these organs, we will now test the hypothesis that IL1β and IFNγ promote denervation during disease progression from the early (mild) to late (severe) stages by altering neuronal-epithelial communication. Thus, in **Aim 1** *we will first define the time course of dry eye development by quantifying changes in innervation, tear secretion, epithelial/basement membrane integrity, neuromodulator expression, cytokine production (i.e., IL1β and IFNγ) and immune cell infiltration of the epithelia (i.e., cornea and lacrimal gland) and ganglia (i.e. trigeminal/pterygopalatine) of Aire KO mice at multiple time points* (**Aim 1A**). In addition, we will identify molecules that have a role in innervation of the adult cornea through transcriptional profiling during disease progression (**Aim 1A**) and by using an in vivo rescue assay (**Aim 1B**). **In Aim 2**, we will determine the impact of IFNγ and IL1β on neuromodulators and basement membrane components produced by epithelial cells of the cornea and lacrimal gland, as well as their effects on neuronal cell outgrowth and function using *ex vivo* and *in vivo* assays (**Aim 2A**). We will then determine the downstream signaling pathways activated by IFNγ and IL1β that lead to altered expression of these molecules. In **Aim 2B,** we will *determine the extent to which manipulating neuromodulators and the*

downstream signaling molecules identified above can maintain or restore innervation of the inflamed cornea using ex vivo co-cultures and the Aire KO mouse. Results will determine the time course of denervation, the mechanisms by which this is achieved and will also identify potential biomarkers associated with corneal/lacrimal neuropathy in autoimmune-mediated dry eye.

Specific Aim 1: Define the timing of denervation and pathological changes in the cornea, lacrimal gland and associated nerves during dry eye development.

***Aim 1A*:** In order to define the effects of CD4+ T cells on ocular health and innervation during disease progression, we must first characterize the timing of pathological changes associated with dry eye development in *Aire* KO mice. Alterations in innervation, neuromodulators, inflammation, tear secretion, cytokine production (i.e., IL1β and IFNγ) and epithelial and basement membrane integrity will be quantified in the corneas and lacrimal glands of female *Aire* KO and WT mice at 4, 6, 8, and 10 wks of age. Time points are selected based on historical data indicating ≥ 25% of *Aire* KO Balb/c mice beyond the age of 9 weeks experience a reduction in body weight >15% and, therefore, must be euthanized. Innervation and basement membrane integrity will be quantitatively measured in the cornea and lacrimal gland at each time point by confocal microscopy using ImageJ/Fiji (as in **Fig. 1**). Neuronal alterations and the expression of neuromodulators and cytokines will also be measured via qPCR using neuronal markers *Tubb3* and the synaptic marker Synapsin2 (*Syn2*) along with our candidate neuromodulator genes (identified in **Figs. 4&5**). Stimulated tear secretion and epithelial integrity will be measured by phenol red tear threads and lissamine green staining, respectively, as previously described (39, 43). CD4+ T cell infiltration of the cornea, lacrimal gland, trigeminal ganglia and pterygopalatine ganglia will be assessed by immunohistochemistry. Positively stained cells will be quantified using NIS Elements BR2.30 image-analysis software as previously described (20). Focal CD4+ T cell infiltration of the lacrimal gland will be manually counted as the number and size of foci per millimeter square within the tissue area (150μmx150μm). Similar to studies of the sciatic nerve in *Aire* KO mice (9) we will also determine changes in the myelination of nerve fibers innervating the lacrimal glands of *Aire* KO mice via immunofluorescent analysis of protein zero (P0) typical of myelinated nerves (corneal nerves are unmyelinated to preserve transparency and, thus, will not be assessed with P0). Assessment of changes in each of these features will be carried out by masked, well-trained individuals.

Results from these studies will inform us of when denervation takes place and will highlight molecules that correlate with this denervation. Given this is a biased approach, we will supplement this with an unbiased screen based on RNAseq. Due to cost and RNA quantity requirements of RNAseq, initially this will be performed on epithelial cells (isolated via FACS) derived from lacrimal gland of mice aged 4, 6 and 8 wks (we will collect an n=5). Differential expression analysis on the 3 time points will be performed using the *limma* package (44) followed by Ingenuity Pathway Analysis to reveal neuromodulators/neuronal pathways potentially involved in promoting axon guidance and outgrowth. The unbiased approach will also facilitate the exploration of alternative pathways that could link inflammation to loss of neuronal function and reduced lacrimal gland secretion in SS, (as reviewed in (45)). For example, In the MRL/Mpj-Fas^{lpr} mouse model of SS, the proinflammatory cytokine IL1β prevented the release of neurotransmitters from the nerve terminals thereby impairing neural stimulation of lacrimal gland secretion (46). Moreover, the chronic interaction of autoantibodies directed against the lacrimal gland muscarinic acetylcholine receptor M3 (M_3AchR), that is present in the sera of SS patients, has been postulated to inhibit secretory function by provoking an IL1β-mediated release of cellular nitric oxide that damages the acinar tissues (47, 48). Thus, cytokine-mediated inhibition of neurotransmitter release from efferent nerves and/or the presence of function-blocking antibodies to M_3AchR could account for decreased efferent nerve stimulation in SS. Pathways/factors of interest from both biased and unbiased approaches will be tested in future *in vivo* and *ex vivo* experiments of *Aire* KO mice to confirm their functional significance and to define the signaling pathways that link them to T cell-mediated inflammation (as in **Aim 2).**

Aim 1B: Here, we will determine if the molecules identified in **Aim 1A** have a role in innervating the adult cornea by testing whether they are rescued/diminished upon inhibition of chronic inflammation in the *Aire* KO cornea. WT and *Aire* KO mice will be treated before the onset of ocular surface disease at 4 wks and during disease progression at 6 and 8 wks with topical Dex (as in **Fig. 7**), Anakinra (IL1R1 antagonist (39)) or IFNγ function blocking antibody (this antibody is commercially available (clones R4-6A2 and XMG1.2 (49)) and has been used to ameliorate disease in similar models (50)) or vehicle control (both preservative free from Leiter's compounding pharmacy, San Jose CA). Mice will be treated twice daily for 2 weeks. Changes in

neuromodulator expression, basement membrane, epithelial barrier (lissamine green), innervation and inflammation will be quantified by qPCR and immunofluorescence (IF)/confocal microscopy (as in **Figs.1, 3-7**). If expression of the candidate molecules return to near basal levels, we will conclude that they have a role in immune-induced denervation of the cornea and these will be further tested in **Aim 2**.

***Expected Outcomes and Alternative Approaches*:**

In **Aim 1** we will identify basement membrane proteins and neuromodulators that maintain innervation during homeostasis and characterize how they are modulated during inflammation. In preparation for these studies, we have identified candidate molecules that are consistently altered in *Aire* KO mice and will be our primary focus. For example, expression of axon guidance ligands SEMA7a and SEMA3a, which activate their cognate receptors neuropilin (NRP)1 and 2 on innervating nerves (33), are differentially expressed in *Aire* KO mice. Both are known to function in reinnervation of the adult cornea (33), with recombinant SEMA7a promoting innervation of the cornea after lamellar corneal surgery (34) and SEMA3A inhibiting nerve regeneration in a mouse model of corneal transplantation (35). Similarly, SLIT2 (which is upregulated in *Aire* KO mice) has been shown to inhibit axon growth (51). We have also identified candidate basement membrane components including perlecan, and collagen IV (which are disrupted in *Aire* KO mice) that have been shown to promote axon targeting (52-54), suggesting their altered expression may contribute to the loss of innervation in dry eye. We have also included in our analysis non-biased transcriptome profiling (RNAseq/ Ingenuity Pathway Analysis) from *Aire* KO and WT controls to identify other factors that may also contribute to innervation of the adult eye. This is particularly necessary for the lacrimal gland as little is currently known regarding the effects of chronic inflammation on innervation of this organ in SS-associated dry eye, (rev in (45)), and even less about the identities of factors that control reinnervation of the adult lacrimal gland. In all cases, pathways/factors of interest will be tested in future *in vivo* and *ex vivo* experiments of *Aire* KO mice to confirm their functional significance and downstream signaling pathways in **Aim 2**. A caveat for **Aim 1B** is that topical application of Dex only partially reinnervates the cornea and does not reinnervate the lacrimal gland. As an alternative, we will treat mice systemically with Dex using the same regimen for topical application. Since it is also possible that Dex treatment causes off target effects resulting in false positives or negatives, we will compare Dex with the more targeted approaches i.e., treating mice with function blocking IFNγ antibody or IL1R1 antagonist. In future studies we will also characterize the impact of the loss of neuromodulators on the speed of denervation in mice where neuromodulators have been genetically ablated in the cornea or lacrimal gland (e.g. *Sema3a* floxed or Sema7a-/- mice). Conveniently, *Sema7a* null mice are commercially available on the Balb/c background and, thus, can be directly crossed with *Aire* KO mice. We can also use adoptive transfer to assess the extent to with autoreactive T cells from *Aire* KO mice alter innervation of *Sema7a-/-, Sema3-/-* and/or *Slit2-/-* mice. We have several years experience using this technique to study the immunopathogenesis of dry eye disease in *Aire* KO mice and, thus, do not anticipate technical difficulties.

To complement studies proposed **Aim 1** we will also explore an alternative approach to quantify innervation. While manual methods of nerve quantification, such as Image/NeuronJ, provide a powerful tool to quantify innervation, they are time intensive and subject to inter-grader variability. Recently, an automated nerve quantification software, ACCMetrics, has become available from Professor Rayaz Malik at the University of Manchester. ACCMetrics automatically captures corneal c-fibers from confocal images, measuring nerve fiber length and density as well as nerve branch density. Although the software was developed for the Heidelburg *in vivo* confocal microscope, to capture images of the human cornea, we are working to utilize this software for the purposes of our studies. To date, we have successfully used ACCMetrics to capture corneal nerve fibers in a projection image of mouse cornea, with the processing of each image taking less than a minute. We are currently working to optimize the software and staining technique to enhance the detection of nerves in the sub-basal region of the mouse cornea, and are planning to continue this optimization so that automated analysis using ACCMetrics can be used in conjunction and directly compared with established quantification protocols using Image/NeuronJ.

Specific Aim 2: Determine the mechanisms by which T cell-mediated inflammation alters innervation of the cornea and lacrimal gland.

***Aim 2A**:* Recently, it was shown that inhibition of IFNγ signaling prevented autoimmune peripheral neuropathy in *Aire* KO mice (9), suggesting IFNγ has a direct effect on nerves. In our preliminary data we show that the cornea and lacrimal glands of *Aire* KO mice have reduced innervation, abundant IFNγ-secreting CD4+ T cells, altered expression of neuromodulators and basement membrane proteins, and that genetic ablation of IL1R1 improves innervation and restores the basement membrane. This suggests that IFNγ and IL1β cooperate to

control sensory and autonomic denervation by either acting directly on the nerves or adversely affecting the target organ synthesis of molecules that maintain innervation. In support of this, using *ex vivo* cornea cultures we found IFNγ and IL1β increased expression of axon guidance factors that positively and negatively regulate innervation, although they did so to differing degrees with IL1β having the greatest impact (**Fig. 8**). Now we will use *ex vivo* epithelial and neuronal cultures to determine the effects of IFNγ and IL1β on neuromodulators and basement membrane proteins in the cornea, lacrimal gland, and their innervating ganglia (trigeminal/pterygopalatine) that were identified in the preliminary data and those further discovered in **Aims 1A**&**B**. We will further determine the signaling systems downstream of IFNγ and IL1β that regulate their expression using similar assays.

Ex vivo epithelial cultures: Here, *we will determine whether IFNγ or IL1β has a direct effect on corneal and lacrimal epithelial cells.* Changes in neuromodulators and basement membrane components will be assessed in *ex vivo* corneal and lacrimal cultures treated for 4 and 24 hr with PBS or IFNγ or IL1β (5ng/mL) and compared to corneas isolated from *Aire* KO mice. Analysis will focus on components known to be required for basement membrane assembly in the cornea or other epithelial tissues (e.g. fibronectin, perlecan, nidogen1/2, laminin-1/5, collagen IV, VI, XVIII). In preparation for these studies, we have already established corneal and lacrimal explant cultures (**Fig. 8**, data not shown) in which cornea/lacrimal glands are freshly isolated from wild type mice and placed on a filter above serum free media (cornea) or are dissected into 1mm pieces and placed in media (lacrimal gland). We have also generated organotypic cultures of human, primary corneal epithelial cells grown at an air-liquid interface to determine the impact of IFNγ on epithelial cell synthesis of neuromodulators and basement membrane in the absence of stroma and nerves (**Fig. 9C**). Changes in these components will be quantified by qPCR, western blot and/or immunofluroescence, as above. If IFNγ or IL1β has a direct effect on the epithelial cells, gene/protein expression will be altered.

Ex vivo nerve cultures: Similar to the epithelial study, here *we will determine if IFNγ or IL1β cause denervation by directly affecting the growth and function of nerves that innervate the cornea (trigeminal ganglia, TG) and lacrimal gland (pterygopalatine, PG)*. TG and PG from adult mice will be extracted and compartmental cultures performed as previously described (34). In this system, cell bodies are located in the central compartment and growing axons in the side compartments along tracks etched into collagen coated culture dishes; a configuration that allows us to independently analyze the effects of IFNγ or IL1β on the somas and axons. To determine the effects of these cytokines on axon outgrowth, axons will be treated with recombinant SEMA7a-Fc or neurturin (NRTN) to promote outgrowth of sensory nerves i.e. TG (34) and parasympathetic nerves (Knox et al.,(15)), respectively, in the presence or absence of cytokine. Axon outgrowth along tracks will be imaged by confocal microscopy and nerve fiber length measured in each track using NeuronJ. To determine if IFNγ alters neuronal function, cell bodies or axons with be treated with SEMA7a-Fc/NRTN, with or without IFNγ or IL1β for 1-5 days and expression of genes involved in neuronal function (e.g. neuropeptides *Cgrp* and *Npy*, the GDNF co-receptor *Ret*, and the synaptic marker synapsin 2 (*Syn2*)) will be measured by qPCR.

Next, we will define signaling pathways activated by IFNγ or IL1β in epithelia and nerves that alter the production of neuromodulators and basement membrane components identified in ***Aim 1****, as well as axon growth/function*. We will use the *ex vivo* cultures stated above (epithelial and neuronal) incubated with and without inhibitors of pathways known to be downstream of IL1β or IFNγ. Cytokine-activated signaling pathways included in our analysis will include p38/MAPK, JAK/STAT and PI3K/AKT. Involvement of these pathways will initially be assessed with chemical inhibitors, such as PD98059 (MEK), U0126 (Erk), Wortmannin (PI3K), and a JAK/STAT inhibitor panel (all available through EMB Millipore). Changes in basement membrane, neuromodulators and genes involved in axon outgrowth and function will be quantified by qPCR, immunofluroescence or western blotting. If these pathways act downstream of IFNγ or IL1β, then inhibition will rescue expression. Confirmation studies will be performed using siRNAs or blocking antibodies directed against relevant pathway components, most of which are commercially available. If none of these signaling pathways are involved, we will identify new pathways using an intracellular phosphoprotein array (Pathscan antibody array, Cell Signaling) and test whether these are required for IFNγ and/or IL1β's activity in nerves and epithelial cells.

Aim 2B: *Here we will determine the extent to which manipulating neuromodulators and downstream signaling molecules identified above can maintain or restore innervation of the inflamed cornea using ex vivo co-cultures and the Aire KO mouse.*

Ex vivo nerve-cornea co-cultures: To confirm that these components are required for reinnervation of the inflamed cornea we will overexpress or inhibit neuromodulators/signaling pathways in our cornea/nerve co-cultures in the presence or absence of IL-1β/IFNγ. For example, we can use siRNA to reduce *Sema7a* expression. Functional readouts to assess innervation will include the quantification of neurite outgrowth as demonstrated in **Fig. 9**. In these assays, cornea and TG are placed in 3D laminin matrix floating on a filter over serum free media and cultured for 3-5 days. Axon outgrowth towards the cornea is measured by immunostaining for the neuronal marker, TUBB3 and quantifying the distance travelled compared to TG in the absence of cornea. Neurite projections of the explants (~25–30 projections for each treatment group) will be manually counted and axonal spread i.e. the area covered by the longest neurites will be quantified using Image J, as well as changes in nerve function by measuring gene/protein expression of sensory neuropeptide CGRP and the synaptic marker, Syn2 via semi-quantitative PCR and western blot.

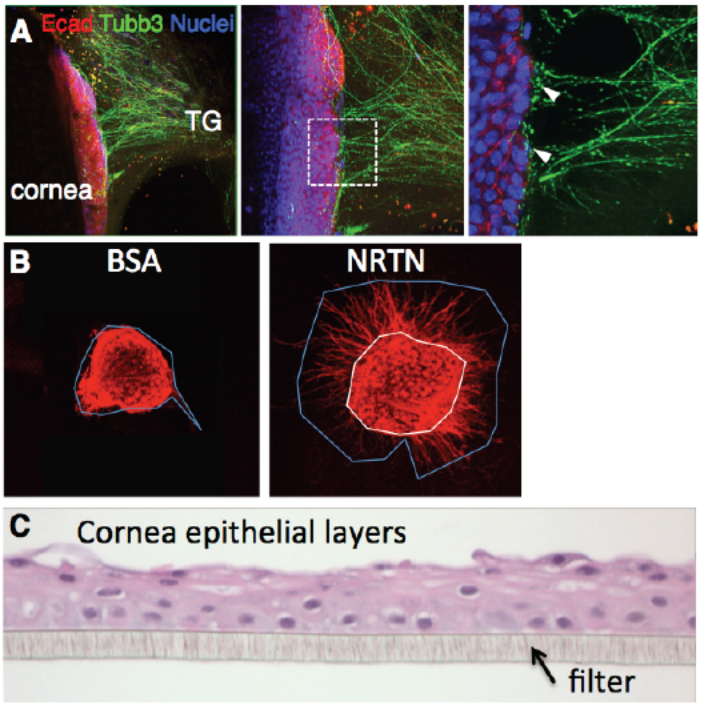


Figure 9: Ex vivo culture assays. (A) ex vivo co-culture of sensory nerve/cornea B) sensory ganglia culture, and (C) human, primary corneal epithelial cells grown at an air-liquid interface. Cultures in A and C were immunostained for nerves (Tubb3, green) and epithelial cells (Ecadherin, red). Arrowheads indicate synapses. Blue and white lines indicate neurite spread. NRTN = neurturin

In vivo studies in the Aire KO: To confirm that neuromodulators and/or signaling pathways can reinnervate the inflamed cornea, we *will test whether over-expression or inhibition of neuromodulators and/or signaling pathways that we have identified to maintain innervation during homeostasis and are altered in response to inflammation can rescue innervation.* For example, overexpression of axon guidance molecules in the cornea has been accomplished through stromal injection of a commercially available pCMV expression vector containing mouse Semaphorin cDNA (55), as well as through the topical application of recombinant proteins such as SEMA7a-Fc (34). Inhibition will be accomplished using chemical antagonist and/or siRNA as previously described (56-58). *We anticipate that overexpression of neuroattractive factors, inhibition of axon repellent factors and/or restoration of the basement membrane will rescue corneal innervation despite the presence of persistent inflammation*.

***Expected Outcomes and Alternative Approaches*:** Outcomes here will identify factors that regulate innervation of the adult cornea and lacrimal gland during homeostasis and after injury in response to IFNγ. Given the significant work involved in **Aim 2B** we have restricted our analysis to the cornea, however, in the future similar experiments will also be applied to the lacrimal gland to identify the signaling pathways of lacrimal acinar cells that are altered in SS-associated dry eye. A potential caveat for *in vivo* experiments in **Aim 2B** is that overexpression or knockdown of neuromodulators, and/or signaling molecules identified using the *ex vivo/in vivo* model may not be sufficient to inhibit innervation during homeostasis or rescue innervation during inflammation. For example, inflammation may be too severe for overexpression to be effective. To circumvent this, we can treat animals with neuromodulators before the onset of disease or test them as adjuncts to anti-inflammatory therapies by using them in conjunction with Dex treatment (systemic or topical), IL1R1 antagonist or IFNγ function blocking antibodies. We also do not anticipate difficulties with overexpression studies since we have identified several approaches, many of which have already been demonstrated in the cornea, including the injection of cDNA-containing expression vectors, or the topical application of exogenous factors (e.g. recombinant Sema3a or Sema7a; R&D systems). In the future, we will characterize the effects of inflammation on axon guidance/neurotrophic factors using a human *ex vivo* culture

system as a follow up to our *ex vivo* mouse cornea assays. This approach is supported by preliminary data that suggests pathological changes observed in the *Aire* KO cornea are similar to those induced in human corneal epithelial cells exposed to IL1β and IFNγ (38). We are currently working in collaboration with [redacted] to develop and characterize stratified cultures of human embryonic corneal epithelial cells for this purpose.

Finally, we appreciate that some of these neuromodulators have other functions not related to innervation including angiogenesis (e.g., neuropilin1 and 2 are increased under pathological conditions to induce angiogenesis (59)), and emerging evidence suggests that some members of the semaphorin family are involved not only in innervation but also in immune responses. For example, SEMA7a is a potent immunomodulator that is expressed on activated T cells where it stimulates cytokine production including interferon and IL-17, through an interaction with α1β1 integrin. As such, in the cornea, SEMA7a can stimulate both nerve regeneration and inflammatory cell influx suggesting this immune semaphorin may link neuronal and inflammatory processes. Thus, to isolate the neuronal function of semaphorins (or other candidates) from their potential immune function, we will use gain of function assays in *ex vivo* culture (as in **Aim 2B**) where proteins are overexpressed in epithelia in the absence of T cells as well as in mice deficient in Semaphorins (e.g., *Sema7a*-/-) where endogenous T cells are devoid of SEMA7a. Of course similar studies are required to explore SEMA7a's role as an immunomodulator in dry eye disease to determine whether exploitation of molecular regulators that straddle the immune and nervous system can be used to obtain an optimal level of inflammation that promotes nerve regeneration in the diseased cornea.

Timeline:

SPECIFIC AIM (YEAR)	1	2	3	4	5
Aim 1A. Determine when denervation appears in relation to other pathological events during dry eye development.	X	X			
Aim 1B. Confirm that immune-mediated changes in corneal innervation correspond to altered expression of neuromodulators using topical steroids.		X			
Aim 2A. Co-culture studies to determine the direct effects of IFNγ/IL1β on corneal epithelia cells and innervating nerves. Define cytokine-activated signaling pathways that alter neuromodulators and axon growth/function.			X	X	
Aim 2B. Manipulate neuromodulators and signaling molecules identified in Aim 2A to maintain or restore innervation in ex vivo co-cultures and *Aire* KO mice.			X	X	X

Vertebrate animals

TOTAL number of animals used in proposal ~ 900

1. Species, strains, ages, sex and numbers:

Genetic background for the Aire mouse is Balb/c. Age of animals will be 4-10 weeks. These inbred strains decrease biological variation between animals, thus increasing statistical power of the results. The number of animals reflects the need to maximize this statistical power. In all studies only females will be used. Heterozygous animals from Aire mating will not be needed and will be used for breeding, other purposes or humanly euthanized.

Breeding pairs: We will utilize the *Aire*-deficient mouse model. To generate mice for each of the studies described below we will need 10 breeding trios to generate female Aire KO mice. Breeders will be replaced every 6 months = 30 mice x 2 x 5 years = **300 mice total for breeding**.

For Aim1A-B, total number of mice to be used = 250

Aim1A: *Timing of pathological changes*: Number of mice needed: 4 time points x 2 groups x 5 mice per group = **40 mice**
FACS derived cells from lacrimal gland: Number of mice needed: 3 time points x 2 groups x 5 mice per group **= 30 mice**

Aim1B*: Dex treatment*: Number of mice needed: 3 time points x 2 groups x 10 mice per group = **60 mice**
*IL1R1-antagonist treatment***:** Number of mice needed: 3 time points x 2 groups x 10 mice per group = **60 mice**
IFNγ *blocking antibody***:** Number of mice needed: 3 time points x 2 groups x 10 mice per group = **60 mice**

For Aim2A-B, the total number of mice to be used = 340

Aim 2A: *Ex vivo epithelial cultures:* We will confirm that IFNγ and IL1β have a direct effect on corneal and lacrimal epithelial cells treated for 4 and 24 hr. Number of mice needed: 3 conditions x 5 mice per condition x 2 time points = **30 mice.**
Ex vivo nerve cultures: We will determine if IFNγ or IL1β causes denervation. Number of mice needed: 9 conditions x 5 mice per condition x 2 time points = **90 mice**
Aim 2B: *Ex vivo epithelial/ nerve co-cultures: We* will define signaling pathways activated by IFNγ and IL1β in epithelia and nerves. Number of mice needed: 9 conditions x 5 mice per condition x 2 time points x 2 tissues = **180 mice**
In vivo studies in the Aire KO: For confirmation that over-expression or inhibition of neuromodulators and/or signaling pathways re-innervates the inflamed cornea we will test 4 conditions x 10 KO mice = **40 mice**

Description of animal use:
Mice will be anesthetized with isoflurane during any examinations of the ocular surface (Lissamine green staining, tear measurement, subconjunctival injection or topical treatment with antagonists or antibodies). All mice will be observed daily for any changes in their health and then sacrificed at the proposed time point for tissue collection. Mice will be euthanized by inhalation of CO2 from a pressurized tank in an uncrowded chamber, followed by cervical dislocation and bilateral excision of the eyes, lacrimal gland, spleen and cervical lymph nodes as needed for the individual Aims.

2. Justification for the use of animals: We will study the functional relationships between immune cells, nerves and corneal epithelial/progenitor cells during homeostasis and repair in response to chronic inflammation in DED. Cross verification between *in vivo* and *in vitro* models is essential to accurately define the disease mechanisms and identify new therapeutic targets. Confirmation of these drug targets in animals is necessary prior to consideration as possible therapeutics for human disease. **The number of animals we intend to use is the minimum necessary to achieve adequate sample size for the various experimental manipulations and for the variety of sample preparations required**. Multiple mice in each experimental

group (generally six to ten) are needed to ensure the accuracy of results in topical ocular therapy or transgenic animal experiments.

3. Veterinary care: All mice will be housed in the UCSF Animal Care Facility in barrier housing under temperature and humidity control. There will be no food or water restrictions and bedding is changed daily. The facility provides veterinary care and consultation including surgical, analgesic and anesthetic support, advice to researchers on species needs, necropsy and pathology assistance and staff training. There are 6 veterinarians on staff and 7 veterinary nurses. A Heath Surveillance Report (HSR) system exists to monitor the health of research animals. Through this system investigators are contacted regarding any health conditions that are inconsistent with those listed as part of the approved protocol. They also provide 24hr emergency veterinary care. If needed, animals deemed at risk will be treated according to the recommendations of the veterinary staff.

4. Limited discomfort: Mice will be anesthetized with isoflurane during examinations of the ocular surface or prior to any topical treatments. The veterinarians at the UCSF Animal Care Facility, as well as the UCSF Animal Care and Use Committee (IACUC) have approved our anesthesia protocols, experimental protocols and procedures for monitoring and managing any possible side effects. Animals will be monitored daily and if any side effects appear, the animals will be anesthetized and will undergo euthanasia. There will be no post-surgical survival animals.

5. Euthanasia: Our euthanasia protocols have been approved by the UCSF Animal Care and Use Committee and are in accordance with the AVMA Guidelines for the Euthanasia of Animals: 2013 Edition. Mice will be euthanized by inhalation of CO_2 from a pressurized tank in an uncrowded chamber, followed by cervical dislocation. The flow rate must be set to displace 10-30% of the chamber or cage volume/minute, allowing CO_2 to enter the chamber slowly so that unconsciousness and complete narcotization occur prior to death. CO_2 flow is maintained for at least one minute after respiratory arrest; animals are left in the chamber for a sufficient time so that death has occurred prior to performing a physical method.

SUMMARY: In this application we have taken a series of steps to ensure the success of the *in vivo* experiments as well as the humane care of the animals involved in these experiments. All protocols required for these experiments have been approved by the IACUC at UCSF (#AN089075-03C (NM), AN087515-02 and AN088943-02).

MULTIPLE PD/PI LEADERSHIP PLAN:
The goal of this proposal is to determine the cellular processes that lead to denervation of target organs in Sjögren's syndrome-associated dry eye with a specific focus on epithelial-neuronal cell interactions in the cornea and lacrimal gland. The PIs at UCSF bring complementary skills to this proposal.

Nancy McNamara, OD, PhD, Associate Professor of Ophthalmology and Anatomy, is a clinician scientist with expertise in both the clinical management and immunopathological study of dry eye disease. Her lab has been exploring the interplay of epithelial cells with the mucosal and adaptive immune systems for over a decade, including the characterization of aqueous-deficient dry eye development in the autoimmune regulator (Aire)-deficient mouse model of Sjögren's syndrome and the identification of novel clinical biomarkers in human patients with Sjögren's syndrome. She maintains an active clinical practice at the Proctor Medical Group (UCSF) and is Chief of the Dry Eye Clinic at UC Berkeley, where she routinely works with patients who suffer from Sjogren's syndrome, as well as other autoimmune diseases affecting the eyes. She will be responsible for oversight, coordination, and project management for studies of corneal innervation in Aims 1 and 2. She will oversee the generation and treatment of Aire KO mice to define the timing of denervation and corresponding pathological changes in the cornea, lacrimal gland and associated nerves during dry eye development in Aim 1 and the extent to which innervation can be rescued in Aim 2. In this regard, she will direct and supervise [redacted] (Postdoctoral Scholar) and [redacted] (Staff Research Associate), who will carry out in vivo dexamethasone treatment and rescue studies, including in vivo measurements of corneal staining and tear secretion, tissue harvesting, sectioning, immunofluorescence image analysis, qPCR and western blots. Dr. Stephens will also assist with transcriptome profiling of lacrimal glands from Aire KO mice using RNAseq and Ingenuity Pathway analysis, as well as manuscript preparation. Dr. McNamara will further coordinate and assist with the analysis and interpretation of data during manuscript preparation in collaboration with Dr. Knox and biostatisticians in the UC Clinical Research Center core facility.

Sarah Knox, PhD, Assistant Professor of Cell and Tissue Biology, is an expert in epithelial-neuronal interactions during organ development and regeneration, with exceptional expertise in the study of exocrine gland development. Using novel ex vivo culture systems, Dr. Knox discovered a role for peripheral nerves in regulating stem cell proliferation, thereby establishing a new paradigm in stem cell biology and regenerative medicine (Knox et al., 2010 Science). Dr. Knox will be responsible for oversight and coordination of project management for studies of lacrimal gland innervation in Aims 1 and 2, as well as leading the ex-vivo co-culture studies in Aim 2. She will provide her expertize in the generation and analysis of innervation in explant cultures and will conduct all the confocal imaging and analysis of corneas and lacrimal glands. To accomplish these studies she will direct and supervise [redacted] (Postdoctoral Scholar), who will carry out experimentation, data analysis and manuscript preparation relevant to the imaging and analysis of in vivo innervation and ex vivo cultures.

Study goals	Task	(McNamara-PI)	(Knox-PI)
Aim 1A	Determine when denervation of the cornea and lacrimal glands appears in relation to other pathological events during dry eye development in Aire-deficient mice.	✓	(✓)
Aim 1B	Confirm that immune-mediated changes in neuromodulators correspond to altered innervation by rescuing disease with steroid treatment.	✓	(✓)
Aim 2A	Co-culture studies to confirm the direct effects of IFNγ on epithelial cells and innervating nerves. Define signaling pathways activated by IFNγ that alter epithelial production of neuromodulators and axon growth/function.	(✓)	✓
Aim 2B	Determine the extent to which manipulating neuromodulators and the downstream signaling molecules identified in Aim 2A can maintain or restore innervation of the inflamed cornea using ex vivo co-cultures and the Aire KO mouse.	✓	✓

Drs. McNamara and Knox have worked effectively together for the past year and a half conducting the

preliminary studies reported in this application. With both labs located on the UCSF Parnassus campus, they meet regularly and communicate several times weekly via email. Their collaboration is enriched through significant interaction between the members of their laboratories, including the sharing of scientific expertise and reagents. It is anticipated that while each PI will be primarily responsible for those aims that take best advantage of their expertise and their institutional strengths, they will nevertheless be fully engaged with all aspects of the project as an iterative process where experimental results at each stage feed back to improve the ultimate goal of identifying a novel approach to treat dry eye disease through the restoration of corneal innervation.

For the odd years of the grant award, Dr. McNamara will serve as the contact PI and will assume fiscal and administrative responsibilities, including maintaining communication between the two PIs and amongst key personnel. She will be responsible for communication with the NIH and submission of annual reports. The responsibilities of the contact PI will be rotated to Dr. Knox in even years of the grant award.

The PIs and laboratory personnel will attend weekly joint-lab meetings to discuss experimental design, data analysis, and to plan research activities. The PIs will discuss all administrative responsibilities using the mechanisms described above and supplemented as needed by one-on-one meetings and frequent email. Both PIs will share their respective research results with each other and key personnel. They will work together to discuss any changes in the direction of the research projects and the reallocation of funds, if necessary. Publication authorship will be based on the relative scientific contributions of the PIs and key personnel.

INTELLECTUAL PROPERTY

The Technology Transfer Offices at UCSF will be responsible for preparing and negotiating an agreement for the conduct of the research, including any intellectual property. They will work together with the PIs to ensure the intellectual property developed is protected according to the policies established in the agreement.

CONFLICT RESOLUTION

If a potential conflict develops, the PIs shall meet and attempt to resolve the dispute. If they fail to resolve the dispute, the disagreement shall be referred to an arbitration committee consisting of one impartial senior executive from each PI’s department and a third impartial senior executive mutually agreed upon by both PIs. No members of the arbitration committee will be directly involved in the research grant or disagreement.

CHANGE IN PI LOCATION

No changes in PI location are anticipated for the duration of the proposed studies. If a PI moves to a new institution, attempts will be made to transfer the relevant portion of the grant to the new institution. In the event that a PI cannot carry out his/her duties, a new PI will be recruited as a replacement, subject to the approval of the institution.

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MODEL ORGANISMS 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://ott.od.nih.gov/NewPages/Rtguide_final.html. Specifically, 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. 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.

There will be no transgenic mice generated in this proposal.

Requests for Aire-deficient mice will be handled by our institution's technology transfer office. All licensing shall be subject to distribution pursuant to our institution's policies and procedures on royalty income. The technology transfer office will report any invention disclosure submitted to them to the appropriate Federal Agency.

"Other Research Resources" generated with funds from this grant, such as transcriptome profiling data generated by RNAseq, will be made available to qualified investigators following peer-reviewed publication.

UCSF
University of California
San Francisco

Sarah M. Knox, Ph.D
Assistant Professor
Cell and Tissue Biology
UCSF School of Dentistry
513 Parnassus, HSE1510
San Francisco, CA 94143-0440
tel: 415/502-0811
e-mail: sarah.knox@ucsf.edu

Tuesday, June 04, 2013

Center for Scientific Review
National Institutes of Health
Bethesda, MD 20892-4878

Funding Opportunity Announcement (FOA) Number: PAR-11-260

FOA Title: Research Project Grant (Parent R01)

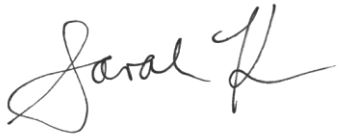
Grant title: "Neuronal regulation of salivary stem cells"

Dear NIH officer,

Please find enclosed my grant application entitled "**Neuronal regulation of salivary stem cells**". In this application, we seek to **identify human salivary gland stem/progenitor cells and neuronal signaling mechanisms that regulate progenitor cell fate** in order to develop new therapeutic strategies to reverse salivary dysfunction.

I believe the proposal's content would be of significant interest to the **National Institute of Dental and Craniofacial Research** and ask that it be assigned to the **Oral, Dental and Craniofacial Sciences (ODCS)** Scientific Review Group. The proposal includes collaborations with stem cell biologists and head and neck cancer clinicians at UCSF, as well as imaging specialists at NIDCR, with the aim of promoting the translation of discoveries.

Sincerely,



Sarah M. Knox, Ph.D.

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

2. DATE SUBMITTED	Applicant Identifier
3. DATE RECEIVED BY STATE	State Application Identifier

1. * TYPE OF SUBMISSION

○ Pre-application ● Application ○ Changed/Corrected Application

4. a. Federal Identifier

b. Agency Routing Number

5. APPLICANT INFORMATION *** Organizational DUNS** [redacted]

* Legal Name: The Regents of the University of California, San Francisco

Department: Research Services Coordinator | Division: Office of Research

* Street1: 3333 California Street, Suite 315 | Street2: Box 0962

* City: San Francisco | County/Parish: San Francisco | * State: CA: California

Province: | * Country: USA: UNITED STATES | * ZIP / Postal Code: 94143-0962

Person to be contacted on matters involving this application

Prefix: | * First Name: [redacted] | Middle Name: | * Last Name: [redacted] | Suffix:

* Phone Number: [redacted] | Fax Number: [redacted] | Email: [redacted]

6. * EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)*:
1-946036493-A6

7. * TYPE OF APPLICANT
H: Public/State Controlled Institution of Higher Education

8. * TYPE OF APPLICATION: ● New

○ Resubmission ○ Renewal ○ Continuation ○ Revision

Other (Specify):

Small Business Organization Type

○ Women Owned ○ Socially and Economically Disadvantaged

If Revision, mark appropriate box(es).

○ A. Increase Award ○ B. Decrease Award ○ C. Increase Duration

○ D. Decrease Duration ○ E. Other *(specify)*:

9. * NAME OF FEDERAL AGENCY:
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:
TITLE:

* Is this application being submitted to other agencies? ○ Yes ● No

What other Agencies?

11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:
Neuronal regulation of salivary stem cells

12. PROPOSED PROJECT:

* Start Date	* Ending Date
04/01/2014	03/31/2019

13. CONGRESSIONAL DISTRICT OF THE APPLICANT:
CA-012

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix:	* First Name:	Middle Name:	* Last Name:	Suffix:
	Sarah	Monica	Knox	PhD

Position/Title: Assistant Professor | * Organization Name: The Regents of the University of California, San Francisco

Department: Cell and Tissue Biology | Division: School of Dentistry

* Street1: [redacted] | Street2: Box 0442

* City: San Francisco | County/Parish: San Francisco | * State: CA: California

Province: | * Country: USA: UNITED STATES | * ZIP / Postal Code: 94143-0442

* Phone Number: 415-502-0811 | Fax Number: 415-476-1499 | * Email: Sarah.Knox@ucsf.edu

15. ESTIMATED PROJECT FUNDING	
a. * Total Federal Funds Requested	$1,993,399.00
b. Total Non-Federal Funds	$0.00
c. * Total Federal & Non-Federal Funds	$1,993,399.00
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: Mime Type:

19. Authorized Representative

Prefix: * First Name: [redacted] Middle Name: * Last Name: [redacted] Suffix:

* Position/Title: Contracts and Grants Officer
* Organization Name: The Regents of the University of California, San Francisco
Department: Research Management Services
Division: Office of Research
* Street1: [redacted]
Street2: Box 0634
* City: San Francisco
County/Parish: San Francisco
* State: CA: California
Province:
* Country: USA: UNITED STATES
* ZIP / Postal Code: 94143-0634
* Phone Number: [redacted]
Fax Number [redacted]
* Email: [redacted]

*** Signature of Authorized Representative** *** Date Signed**

20. Pre-application File Name: Mime Type:

Project/Performance Site Location(s)

Project/Performance Site Primary Location

Organization Name: The Regents of the University of California, San Francisco

* Street1: [redacted]		Street2: Box 0442
* City: San Francisco	County: San Francisco	* State: CA: California
Province:	* Country: USA: UNITED STATES	* Zip / Postal Code: 94143-0442
DUNS Number: [redacted]	* Project/Performance Site Congressional District: CA-012	

Project/Performance Site Location 1

Organization Name: Blood Systems, Inc. dba Blood Systems Research Institute

* Street1: [redacted]		Street2:
* City: [redacted]	County:	* State: [redacted]
Province:	* Country: USA: UNITED STATES	* Zip / Postal Code: [redacted]
DUNS Number: [redacted]	* Project/Performance Site Congressional District: CA-012	

File Name	Mime Type

Additional Location(s)

RESEARCH & RELATED Other Project Information

1. * Are Human Subjects Involved? ● Yes ❍ No

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ● Yes ❍ No

If yes, check appropriate exemption number

Exemption Number: _ 1 _ 2 _ 3 ✔ 4 _ 5 _ 6

If no, is the IRB review Pending? ❍ Yes ❍ No

IRB Approval Date:

Human Subject Assurance Number 00000068

2. * Are Vertebrate Animals Used? ● Yes ❍ No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ● No

IACUC Approval Date: 02-27-2013

Animal Welfare Assurance Number A3400-01

3. * Is proprietary/privileged information included in the application? ❍ Yes ● No

4.a. * Does this project have an actual or potential impact on the environment? ❍ Yes ● 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? ❍ Yes ❍ No

4.d. If yes, please explain:

5.a. * Is the research performance site designated, or eligible to be designated, as a historic place? ❍ Yes ● No

5.b. If yes, please explain:

6.a. * Does this project involve activities outside the U.S. or partnership with International Collaborators? ❍ Yes ● No

6.b. If yes, identify countries:

6.c. Optional Explanation:

7. * Project Summary/Abstract	Project_Summary1026915007.pdf	Mime Type: application/pdf
8. * Project Narrative	Project_Narrative1026864068.pdf	Mime Type: application/pdf
9. Bibliography & References Cited	Bibliography_and_References_Cited1026914988.pdf	Mime Type: application/pdf
10. Facilities & Other Resources	Facilities_and_Other_Resources1026915147.pdf	Mime Type: application/pdf
11. Equipment	Equipment1026864326.pdf	Mime Type: application/pdf

Project Summary/Abstract

Salivary gland dysfunction occurs as a result of pathological injury after radiotherapy for head and neck cancer and significantly compromises the oral health and quality of life of patients. A potential regenerative approach for restoring salivary function is stem cell therapy, where autologous stem/progenitor cells are transplanted into the injured organ or stem cells within the tissue are reactivated. However, the identity of human stem/progenitors and how they are regulated are unknown. In this application we propose to determine the contributions of putative progenitors in the mouse and human salivary gland to tissue repair and regeneration and to determine the influence of neuronal signals on their behavior. Parasympathetic nerves are essential to salivary function and regeneration, as well as the maintenance of epithelial progenitor cells, and are severely reduced in human salivary glands after radiotherapy. We hypothesize that discrete epithelial progenitor cells in the adult mouse and human salivary gland regenerate acini during homeostasis and injury in response to neuronal signals. Thus, the Specific Aims of this project are to: 1) Define the mechanisms by which parasympathetic nerves regulate progenitor cell fate, 2) Determine the contribution of salivary progenitor cells and neuronal signaling to adult homeostasis and repair and 3) Identify epithelial progenitor cells in the human salivary gland. These aims will be achieved using a combination of human salivary glands and mouse genetics in conjunction with genetic, biochemical, immunochemical, and fluorescence imaging techniques. Our rationale for investigating this hypothesis is that understanding progenitor cell identity in the salivary gland and the mechanisms that regulate salivary tissue regeneration will enable the design of targeted regenerative approaches to reverse salivary dysfunction.

Project Narrative

Salivary glands are irreversibly damaged after treatment with radiotherapy for head and neck cancer, and this substantially reduces oral health and quality of life. Stem cell therapy is a promising strategy to regenerate damaged tissue but the identity of stem cells and the mechanisms that regulate their behavior are not known. This project aims to identify salivary gland stem/progenitor cells that contribute to repair and regeneration after damage as well as the signals that control these events in order to develop targeted regenerative approaches to reverse salivary dysfunction.

Facilities and Other Resources

University of California, San Francisco

Laboratory: The main laboratory is in [redacted]. The P.I. has dedicated laboratory space (1500 ft^2) consisting of 4 benches and associated laboratory and office support space assigned to her. There is [redacted]. This space is in [redacted].

Animal: The animal facility at the UCSF is [redacted] and is fully accredited with the American Association for Accreditation of Laboratory Animal Care (AAALAC). All experiments involving mice are reviewed by the UCSF Institutional Animal Care and Use Committee, and are subject to annual review. Within the barrier facility, one large room shared by multiple investigators will house 280 cages in ventilated racks assigned for use by the Knox lab. In the animal facility, there are rooms designated for procedures including the non-invasive procedures outlined in this application.

Computer: Our lab possesses two shared Dell laptop computers for general usage and has access to 2 Dell desktop computers designated for imaging purposes located in a computer room adjacent to the lab. A tablet PC is used for mouse colony maintenance, and the PI has an Apple desktop computer for her own use.

Office: Postdoctoral fellows, graduate students, and technicians have use of desk space in the main lab. An office of 150 ft^2 has been set aside for the use of the PI.

Clinical: The UCSF Head and Neck Cancer Clinic is situated at the Mt. Zion campus. The campus is accessible by UCSF shuttle running every 15 min, allowing for fast retrieval of human samples.

Administrative: The department of Cell and Tissue Biology has five full-time administrators shared between eleven faculty members. These provide post-award support in purchasing, accounting, as well as 15% of a personal administrative assistant. In addition to this department-based support, UCSF provides centralized pre-award administration for the preparation of grants, and centralized human resources assistance.

Other: Additional facilities include a library, oligonucleotide and peptide sequencing facilities, and many core laboratories including sequencing, microarrays, proteomics and mouse transgenic and gene targeting cores. Microarray hybridization and imaging instruments are available for use at the UCSF Sandler Center Functional Genomics Core Facility at Mission Bay campus. Two gamma-producing irradiators are on site at Parnassus and will be used with the support of the Radiation Office.

Scientific Environment: The laboratory is part of the Department of Cell and Tissue Biology http://ctb.ucsf.edu/ at University of California San Francisco School of Dentistry. The department brings together 15 cell, developmental and molecular biologists interested in cancer biology, mesenchymal tissues, stem cells, craniofacial development and mechanisms of pathogenesis. The department ([redacted]) provides an outstanding and collegial environment to perform the proposed work. Several colleagues in the department work on topics directly relevant to our labs interests, and will provide excellent resources in the work funded by this proposal. The laboratory of [redacted] is adjacent to the Knox Lab and post-docs within both labs are highly interactive. [redacted] is a mouse geneticist who works on the role of Ephrin signaling in craniofacial development and disease. The P.I. and [redacted] have established a collaboration exploring Ephrin signaling in the salivary gland. [redacted] works on mechanisms of signaling as they regulate cell movement and proliferation. The laboratory of [redacted] uses high-resolution confocal fluorescence microscopy combined with biochemistry and molecular biology to elucidate molecular mechanisms by which

signaling controls cytoskeleton dynamics and regulates cell behavior. The laboratory of [redacted] focuses on TGF-β signaling in epithelial and mesenchymal differentiation, and in epithelial-mesenchymal transition.

In addition, our lab is a part of the Program in Craniofacial and Mesenchymal Biology http://cmb.ucsf.edu/, a program which brings together basic, translational and clinical researchers, who are interested in the development and function of the craniofacial complex and how deregulation of craniofacial development gives rise to craniofacial anomalies. The program brings together 33 researchers from different departments and graduate programs and provides a unique opportunity for interaction. Of these researchers, the laboratories of [redacted] and [redacted] with whom we share open lab space (see above) have research interests particularly similar to those of our lab, and as such are an outstanding resource. The [redacted] works on questions related to stem cell biology, with a particular focus on the morphogenesis and regeneration of the mammalian tooth. In addition, [redacted] is a specialist in pediatric medical genetics that treats children with craniofacial and dental anomalies. [redacted] is a plastic surgeon that specializes in pediatric plastic and reconstructive surgery including the treatment of craniofacial anomalies such as craniosynostosis, whose laboratory focuses on skeletal muscle regeneration through the use of stem cells. We have significant interaction with these labs including a monthly seminar series and a work in progress seminar that meets every other week. This atmosphere is therefore ideal for the work we propose.

The Knox Lab is also affiliated with the Eli and Edyth Broad Center for Regeneration Medicine and Stem Cell Research (http://stemcell.ucsf.edu/). This program consists of 125 labs focused on understanding and developing treatment strategies for such conditions as heart disease, multiple sclerosis, Parkinson's disease, Lou Gehrig's disease, spinal cord injury and cancer. The Center encourages cross-pollination of ideas among scientists of different disciplines to promote discovery through collaboration. UCSF has a Clinical and Translational Science Institute (http://ctsi.ucsf.edu/) to support the translation of basic research into human therapies. The institute provides infrastructure, services, and training programs including clinical and translational science and early translational research.

Institutional Investment in the Success of the Investigator: The P.I. on this grant is an early stage investigator and received a tenure-track appointment as assistant professor, step III. This position includes partial salary support in the form of a state-of California FTE as well as a start-up package for expenses related to her research program. Teaching and administrative requirements are minimal with at least 80% protected research time.

UCSF has an extremely active faculty mentoring system in which faculty are paired with senior faculty career mentors to provide career guidance and support. Meetings take place frequently, with a minimum of twice yearly, with the goals of assisting faculty with career advancement, increasing productivity, and career satisfaction. Through this program, the P.I. is formally mentored by [redacted], a highly successful scientist in the fields of placenta and salivary gland biology and by [redacted], a top craniofacial physician/scientist in the field of tooth development. In addition, the P.I. receives constant mentorship from the chair of the CTB department, [redacted], as well as guidance in grant writing and manuscript preparation by [redacted], an eminent physician/scientist in breast cancer research and a senior member of the CTB.

UCSF also offers a number of career enrichment programs such as faculty development day from the Chancellor's council on faculty life. This program covers a variety of topics including advancement and promotion, grant writing, and management workshops. Other seminars related to these topics are offered throughout the year by the UCSF Academic Affairs office.

Biohazards: This work involves handling of human tissue that has not been screened for human pathogens. These are fresh tissue biopsies taken from adult and fetal humans and are essential for the identification of human stem/progenitor cells in this study. Specimens are manipulated under BSL2 conditions with the appropriate approvals from the UCSF IBC.

Blood Systems Inc. dba Blood Systems Research Institute

Dr. Muench's main laboratory [redacted]

[redacted]

[redacted]

Equipment

Located at University of California, San Francisco

Microscopy and FACS: The lab is equipped with four small dissection scopes, an inverted Axiovert M200 with digital camera for imaging tissue as well as one Zeiss Discovery V12 automated stereo microscope for fluorescence, bright field and dark field microscopy. This scope is equipped with an MrC5 camera for whole mount color imaging, and an MrM camera for whole mount fluorescence imaging. The lab also is equipped with a Zeiss Imager Z2 automated upright microscope for viewing slides. This microscope is also equipped with an MrC5 camera for color imaging and an MrM camera for fluorescence. These are all located within a designated imaging room that adjoins our main lab. For confocal and FACS use, we have access to the UCSF Stem Cell imaging core equipment http://stemcell.ucsf.edu/research/imaging-core, which is located in an adjacent building which is a five minute walk from our lab. This core provides access to two Leica SP5 upright confocal microscopes: one Leica SP5 inverted confocal microscope, and one Leica SP5 multiphoton microscope, as well as Velocity and Imaris image rendering and analysis software. These are all available to members of the institute for regenerative medicine for a subsidized hourly recharge fee (around $30/hour for most equipment). This facility also contains two FACS Aria II high-speed digital sorters and one FACS LSRII cell analyzer from Becton Dickinson (~$45/hour). We also have access to a FACS Aria II high-speed digital sorter located at BSRI (15 min drive from UCSF Parnassus) for no cost. In addition, our department has a shared Nikon Ti with Yokogawa CSU-X1 Borealis spinning disk confocal that is available for our use at no charge.

Molecular Biology and Biochemistry: The lab is equipped with one CFX96 Touch™ Real-Time PCR Detection System (BioRad) and two 96 well Bio-Rad thermocyclers for PCR and other molecular biology applications. Our lab has a full complement of equipment for molecular cloning and western blotting as well as a GE imager for western blot development and quantitation. We have access to two shared Nano-drops for quantification of nucleic acids.
Irradiators: The lab has access to two gamma irradiators: a cell irradiator is located within the same building as the Knox lab and a whole animal irradiator within the animal facility. The PI and trained personnel have secured access to both instruments at no charge.
Histology: The lab is equipped with one Leica RM2235 microtome for cutting paraffin sections along with associated water bath and slide warmers, and a Microm HM550 cryostat for cutting cryosections. These are both located in a shared histology space adjacent to our main laboratory. We have access to a shared paraffin embedding machine a short walk from our lab.

Computers: Our lab possesses two shared Dell laptop computers for general usage and has access to 2 Dell desktop computers designated for imaging purposes located in a computer room adjacent to the lab. A tablet PC is used for mouse colony maintenance, and the PI has an iMac desktop computer for her own use.

Located at Blood Systems Inc. dba Blood Systems Research Institute

[redacted]

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix	* First Name	Middle Name	* Last Name	Suffix
	Sarah	Monica	Knox	PhD

Position/Title: Assistant Professor

Department: Cell and Tissue Biology

Organization Name: The Regents of the University of California, San Francisco

Division: School of Dentistry

* Street1: [redacted]

Street2: Box 0442

* City: San Francisco

County: San Francisco

* State: CA: California

Province:

* Country: USA: UNITED STATES

* Zip / Postal Code: 94143-0442

*Phone Number	Fax Number	* E-Mail
415-502-0811	415-476-1499	Sarah.Knox@ucsf.edu

Credential, e.g., agency login [redacted]

*** Project Role:** PD/PI

Other Project Role Category:

Degree Type: PhD

Degree Year: 2002

	File Name	Mime Type
***Attach Biographical Sketch**	Biosketch_KnoxS1026915010.pdf	application/pdf
Attach Current & Pending Support		

PROFILE - Senior/Key Person

Prefix	* First Name	Middle Name	* Last Name	Suffix
	Marcus	O.	Muench	

Position/Title: Senior Scientist

Department: Cell Therapy Core

Organization Name: Blood Systems, Inc. dba Blood Systems Research Institute

Division:

* Street1: [redacted]

Street2:

* City: [redacted]

County: [redacted]

* State: [redacted]

Province:

* Country: USA: UNITED STATES

* Zip / Postal Code: [redacted]

*Phone Number	Fax Number	* E-Mail
[redacted]	[redacted]	[redacted]

Credential, e.g., agency login: [redacted]

*** Project Role:** Co-Investigator

Other Project Role Category:

Degree Type: PhD

Degree Year: 1992

	File Name	Mime Type
***Attach Biographical Sketch**	Biosketch_Muench1026864788.pdf	application/pdf
Attach Current & Pending Support		

PROFILE - Senior/Key Person

Prefix	* First Name	Middle Name	* Last Name	Suffix
Dr.	William	R	Ryan	M.D.

Position/Title: Assistant Professor | Department: Otolaryngology

Organization Name: The Regents of the University of California, San Francisco | Division: School of Medicine

* Street1 [redacted] | Street2: Box 1703

* City: San Francisco | County: San Francisco | * State: CA: California | Province:

* Country: USA: UNITED STATES | * Zip / Postal Code: 94143-1703

*Phone Number	Fax Number	* E-Mail
415-885-7491	415-885-7711	wryan@ohns.ucsf.edu

Credential, e.g., agency login:

*** Project Role:** Co-Investigator | **Other Project Role Category:**

Degree Type: MD
Degree Year: 2005

	File Name	Mime Type
***Attach Biographical Sketch**	Biosketch_RyanW1026864789.pdf	application/pdf
Attach Current & Pending Support		

PROFILE - Senior/Key Person

Prefix	* First Name	Middle Name	* Last Name	Suffix
	Roberto		Weigert	

Position/Title: Chief | Department:

Organization Name: National Institute of Dental and Craniofacial Research | Division: Oral and Pharyngeal Cancer

* Street1: 30 Convent Drive | Street2:

* City: Bethesda | County: | * State: MD: Maryland | Province:

* Country: USA: UNITED STATES | * Zip / Postal Code: 20814-4340

*Phone Number	Fax Number	* E-Mail
301-496-9969	301-496-1966	roberto.weigert@nih.gov

Credential, e.g., agency login:

*** Project Role:** Other (Specify) | **Other Project Role Category:** Other Significant Contributor

Degree Type: PhD
Degree Year: 2000

	File Name	Mime Type
***Attach Biographical Sketch**	Biosketch_WeigertR1026864790.pdf	application/pdf
Attach Current & Pending Support		

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

Additional Senior/Key Person Form Attachments

When submitting senior/key persons in excess of 8 individuals, please attach additional senior/key person forms here. Each additional form attached here, will provide you with the ability to identify another 8 individuals, up to a maximum of 4 attachments (32 people).

The means to obtain a supplementary form is provided here on this form, by the button below. In order to extract, fill, and attach each additional form, simply follow these steps:

- Select the "Select to Extract the R&R Additional Senior/Key Person Form" button, which appears below.
- Save the file using a descriptive name, that will help you remember the content of the supplemental form that you are creating. When assigning a name to the file, please remember to give it the extension ".xfd" (for example, "My_Senior_Key.xfd"). If you do not name your file with the ".xfd" extension you will be unable to open it later, using your PureEdge viewer software.
- Using the "Open Form" tool on your PureEdge viewer, open the new form that you have just saved.
- Enter your additional Senior/Key Person information in this supplemental form. It is essentially the same as the Senior/Key person form that you see in the main body of your application.
- When you have completed entering information in the supplemental form, save it and close it.
- Return to this "Additional Senior/Key Person Form Attachments" page.
- Attach the saved supplemental form, that you just filled in, to one of the blocks provided on this "attachments" form.

Important: Please attach additional Senior/Key Person forms, using the blocks below. Please remember that the files you attach must be Senior/Key Person Pure Edge forms, which were previously extracted using the process outlined above. Attaching any other type of file may result in the inability to submit your application to Grants.gov.

1) Please attach Attachment 1
2) Please attach Attachment 2
3) Please attach Attachment 3
4) Please attach Attachment 4

ADDITIONAL SENIOR/KEY PERSON PROFILE(S)

Filename

MimeType

Additional Biographical Sketch(es) (Senior/Key Person)

Filename

MimeType

Additional Current and Pending Support(s)

Filename

MimeType

BIOGRAPHICAL SKETCH

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

NAME	POSITION TITLE
Knox, Sarah, Monica	Assistant Professor
eRA COMMONS USER NAME (credential, e.g., agency login) [redacted]	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of New South Wales, Sydney, Australia	Ph.D.	April 2002	Biomedical Engineering
University of New South Wales, Sydney, Australia	B.S (Hon)	April 1998	Biochemistry
Post-Doctoral Fellow, Department of Molecular, Cell and Developmental Biology, Mount Sinai School of Medicine, New York, NY, USA		2002-2003	Cell Biology
Post-Doctoral Fellow, Center of Genetics, Cell Biology and Development, University of Minnesota, MN, USA		2003-2005	Developmental Biology
Post-Doctoral Fellow, Graduate School of Biomedical Engineering, University of NSW, Sydney, Australia		2005-2006	Biochemistry
Postdoctoral Visiting Fellow, Laboratory of Cell and Developmental Biology, NIDCR, NIH, Bethesda, MD, USA		2006-2011	Developmental Biology

A. Personal Statement

My labs research program is to investigate how neuronal-epithelial interactions control glandular development and regeneration, and how radiation damage influences these processes.

Our work primarily utilizes the mouse embryonic submandibular gland ex vivo model system to examine the role of the parasympathetic ganglion in epithelial branching morphogenesis and regeneration. The submandibular gland has an autonomic ganglion located in and around the glandular epithelium from the earliest stages of development, and remains with the gland after dissection for ex vivo organ culture. During my postdoctoral training I discovered that the parasympathetic ganglion plays a critical role in submandibular gland morphogenesis by maintaining keratin 5+ epithelial stem/progenitor cells through an acetylcholine /muscarinic/epidermal growth factor receptor pathway. In addition, I found this pathway maintains progenitor cells in the developing prostate and the adult submandibular gland. I have also established that the neurotrophic factor neurturin secreted by the submandibular gland epithelium is required for parasympathetic ganglion function and survival, and increases organ regeneration after injury induced by therapeutic radiation.
Our current projects continue to elucidate the mechanisms by which nerves control development and regeneration of salivary glands. We use mouse genetics to explore the relationship between nerves and progenitors during homeostasis and injury in the fetal and adult system, and recently we have established a novel approach based on confocal microscopy, flow cytometry and gene expression analysis to identify progenitor cells in human fetal tissue. By combining studies in human salivary glands with mouse genetics to investigate progenitor cell identity and behavior we aim to identify new therapeutic applications for reversing salivary dysfunction.

B. Positions and Honors

Positions and Employment

2002-2003 Post-Doctoral Associate, Molecular, Cell and Developmental Biology, Feb 2002-May 2003, Mount Sinai School of Medicine, New York, NY, USA. Supervisor: Dr. Robert Krauss

2003-2005 Post-Doctoral Associate, Genetics, Cell Biology and Development, July 2003-July 2005, University of Minnesota, MN, USA. Supervisor: Dr. Scott Selleck

2005-2006 Post-Doctoral Fellow, Graduate School of Biomedical Engineering, August 2005-May 2006, University of NSW, Sydney, Australia. Supervisor: Dr. John Whitelock

2006-2011 Senior Postdoctoral Visiting Fellow, Matrix and Morphogenesis Unit, Laboratory of Cell and Developmental Biology, November 2006-present. NIDCR, NIH, USA. Supervisor: Dr. Matthew Hoffman

2012-present Assistant Professor, Department of Cell and Tissue Biology, School of Dentistry, University of California, San Francisco, CA, USA.

Honors

1995 CRC Cardiac Technology Summer Scholarship (UNSW)
1996 School of Pharmacology and Physiology Summer Scholarship (UNSW)
1998 Pacific Dunlop Postgraduate Scholarship ($30000)
1998 Australian Postgraduate Award (APA)
2005 Australian Post-Doctoral Industrial Fellowship (APDI)
2005 UNSW Faculty of Engineering Research Grant ($20000)
2008 NIH Pathway to Independence Award (K99/R00)
2008 Poster Award at Oral Biology Conference, Rochester, NY
2008 Pathway to Independence Award (K99/R00)

Other Experience and Professional Memberships

Memberships

1999-05 Matrix Biology Society of Australia and New Zealand
2005 Society for Neuroscience
2011 Society for Developmental Biology
2011-12 American Association for Dental Research, member

Professional Activities

2004-08 Proteoglycans Gordon Research Conference, Post-doctoral coordinator
2009 Referee for Journal of Chemical Technology and Biotechnology
2011 Referee for American Journal of Physiology - Cell Physiology
2011 7th International Conference on Proteoglycans, Discussion Leader
2012 Proteoglycans Gordon Research Seminar, Chair
2012 Society for Craniofacial Genetics, Organizing committee member
2012-13 Salivary Gland Group, Junior Director

Activities within the NIDCR DIR:

2007 Co-organizer of NIDCR Post-doctoral Retreat

C. Selected Peer-reviewed Publications

Original Research Papers

2001 **Knox, S. M**, Melrose, J., and Whitelock, J. Electrophoretic, Biosensor and bioactivity analysis of perlecans of different cellular origins. *Proteomics* 1 (12): 1534-41. PMID: 11747213

2002 **Knox, S. M.**, Merry, C., Stringer, S., Melrose, J., and Whitelock, J.M. Not all perlecans are created equal: interactions with fibroblast growth factor (FGF) 2 and FGF receptors. *J. Biol. Chem.* 277 (17): 14657-65. PMID: 11847221

2002 Melrose, J., Smith, S., **Knox, S. M.**, and Whitelock, J. Perlecan, the multidomain HS-proteoglycan of basement membranes is a prominent pericellular component of ovine hypertrophic vertebral growth plate and cartilaginous endplate chondrocytes. *Histochem. Cell Biol.* 118 (4): 269-80. PMID: 12376823

2003 Kang, S., Feinlab, J., **Knox, S. M.**, Ketteringham, M., and Krauss, R. Promyogenic members of the Ig and cadherin families associate to positively regulate differentiation. *Proc. Natl. Acad. Sci. USA* 100(7): 3989-94. PMCID: PMC153035

2005 **Knox, S. M.**, Fosang, A., Last, K., Melrose, J., and Whitelock, J.M. Perlecan from epithelial cells is a hybrid heparin/chondroitin/keratin sulfate proteoglycan. *FEBS Lett* 579 (22): 5019-23. PMID:16129435

2006 Melrose, J., Roughley, P., **Knox, S. M.**, Smith, S., Lord, M. and Whitelock J. The structure, location, and function of perlecan, a prominent pericellular proteoglycan of fetal, postnatal, and mature hyaline cartilages*. J. Biol. Chem*. 281(48): 36905-14. PMID: 16984910

2006 **Knox, S. M.**, Kirkpatrick, C., Staaz, W., Fox, B., Lercher, D., and Selleck, S. The function of a Drosophila glypican does not depend entirely on heparan sulfate modification. *Dev. Biol.* 300(2): 570-82. PMID: 17055473

2007 **Knox, S.M**, Ge, H., Ren, Y., Dimitroff, B., Howe, K., Arsham, A., Easterday, M., Neufeld, T., O'Connor, M., and Scott B. Selleck. Mechanisms of TSC-mediated Control of Synapse Assembly and Axon Guidance. *PLoS ONE*. 2:e375. PMCID:PMC1847706

2007 Patel, V.N., **Knox, S.M.**, Likar, K. M., Lathrop, C.A., Hossain, R., Eftekhari, S., Elkins, M., Vlodasky, I., Whitelock, J. M., and Hoffman, M. P. Heparanase cleavage of heparan sulfate regulates FGF10 function during submandibular gland branching morphogenesis, *Development* 134:4177-86. PMID: 17959718

2008 Whitelock, J., Ma, JL., Davies, N., Nielsen, N., Chuang, C., Rees, M., Iozzo, RV., **Knox, S**., Lord, M. *J* Recombinant heparan sulfate for use in tissue engineering applications. *Chem Tech Biotech.* (83(4):496-504.

2009 Chuang, C. Melrose, J., **Knox, S**., Iozzo, R., and Whitelock. J. The role of heparan sulfate on chondrocyte perlecan - to proliferate or not? *Int J Clin Exp Pathol.* 90(2):A102-103

2010 **Knox, S.M.**, Lombaert, I., Reed, X., Vitale-Cross, L., Gutkind, J.S., and Hoffman, MP. Parasympathetic innervation maintains epithelial progenitor cells during salivary organogenesis, *Science* 329 (5999): 1645 – 1647. PMCID: PMC3376907

2013 **Knox, S.M.**, Lombaert, I.M., Haddox, C.L., Abrams, S.R., Cotrim, A., Wilson, A.J., and Hoffman, M.P. Parasympathetic stimulation improves epithelial organ regeneration. *Nature Comm.* 4:1494. DOI: 10.1038. PMCID: PMC3582394

Non-experimental papers

2006 Di Giusto, D.,**Knox, S. M**., Lai, Y., Tyrelle, G., Aung, M., and King, G. Multitasking by Multivalents Circular Aptamers. *Chembiochem*. 7(3):535-44

2006 **Knox, S. M.** and Whitelock, JM. Perlecan: how does one molecule do so many things? *CMLS* 63(21): 2435-45

2008 **Knox, SM**., and Hoffman, MP. *Saliva Diagnostics.* Ed. Wong, D. Book Chapter: Salivary Gland Development and Regeneration.

2010 Lombaert, I., **Knox, S.M**., and Hoffman, MP. Salivary gland progenitor cell biology provides a rationale for therapeutic salivary gland regeneration, *Oral Diseases* 17(5):445-9

2011 Knosp, WM., **Knox, S.M**., and Hoffman, MP. Salivary Gland Organogenesis. *WIRES Developmental Biology*. DOI: 10.1002/wdev.4.

2013 Klein, O., Bush, J., **Knox, S.M**., Jheon, A., Cordero, D.R, and Richtsmeier, J.T. The Society of Craniofacial Genetics and Developmental Biology 35th Annual Meeting. *Am J Med Genet Part A* 9999:1–15

D. Research Support

Ongoing Research Support

R00 DE018969 (Knox) 01/24/2011 – 12/31/2014
NIH
Neuronal-epithelial interactions that regulate mouse salivary gland development
The major goal of this project is to investigate how the parasympathetic nervous system regulates submandibular gland development.

R21 DE022951 (Knox) 07/01/2012 – 06/30/2014
NIH
Salivary gland repair and regeneration via Schwann cell-nerve interactions
The major goals of this project are to 1) determine the impact of gamma radiation on Schwann cell-neuronal cell interactions, 2) to identify the effects of Neuregulin-1 on Schwann cell and neuronal survival, nerve function, and epithelial regeneration after irradiation, and 3) to determine the ability of Schwann cells to restore salivary function after irradiation.

Resource Allocation Program (Knox) 02/01/2013 – 01/31/2014
UCSF
Parasympathetic innervation and the role of neuropeptide-Y in lacrimal gland development.
The major goals of this project are to: 1) Define the timing of innervation of the LG and the expression profile of NPY and its receptors (Npyr1,2,3,4 and 5) during embryonic and post-natal development; and 2) Assess NPY-mediated epithelial and neuronal morphogenesis using an ex vivo LG culture system

Completed Research Support

5K99DE018969 Knox (PI) 12/01/2008-11/04/2011
Neuronal-epithelial interactions that regulate mouse salivary gland development
Pathway to Independence Award: to investigate how the parasympathetic nervous system regulates submandibular gland development
Role: PI

Early Career Research Award Knox (PI) 07/01/2005-07/01/2006
Proteoglycan Proteomics
UNSW Faculty of Engineering Research Award: to develop platform methodology to identify proteoglycan biomarkers for the early detection of osteoarthritis
Role: PI

BIOGRAPHICAL SKETCH

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

NAME	POSITION TITLE
Marcus O. Muench, Ph.D.	Senior Scientist (BSRI) & Associate Professor (UCSF)
eRA COMMONS USER NAME [redacted]	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
College of Marin, Kentfield, CA	A.S.	1981-1983	Physical Sciences
University of California, Davis, CA	B.S.	1983-1986	Genetics
Cornell University Medical College, NYC, NY	Ph.D.	1986-1992	Cell Biol. & Genetics
DNAX Research Institute, Palo Alto, CA	Post-Doc.	1992-1995	Human Immunology

A. Personal Statement.

I have been engaged in the study of hematopoiesis since the beginning of my graduate training in 1986. My graduate work focused on the cytokine regulation of hematopoietic stem cells and ex vivo expansion of hematopoietic precursors for transplantation. My postdoctoral training focused on identification of human fetal hematopoietic stem cells and their growth regulation by cytokines. I have been a faculty member of UCSF since 1995 with a primary focus on the early ontogeny of the human hematopoietic system and the development of novel stem cell transplantation strategies to treat birth defects. I have extensive experience in the isolation and culture of human stem cells of various origins and recent research projects have included the study of gene expression during erythroid development with a comparison of genes expressed during erythropoiesis among fetal, adult and embryonic-stem cell derived cells. My extensive experience studying hematopoiesis, stem cell development and 18-years experience with human into animal xenogeneic transplant models qualifies me to conduct the proposed research.

B. Positions and Honors.

Positions and Employment

1986-1992	Graduate Student. Laboratory of Developmental Hematopoiesis; Sloan-Kettering Institute, New York, NY.
1992-1995	Post-Doctoral Research Fellow. Department of Human Immunology; DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA.
1995-2000	Assistant Research Cellular Biologist. The Fetal Treatment Center Research Laboratory. Department of Surgery, University of California at San Francisco (UCSF).
2000-2001	Assistant Research Biologist. Department of Laboratory Medicine, UCSF.
2001-2006	Assistant Professor. Department of Laboratory Medicine, UCSF.
2006-Present	Associate Professor. Department of Laboratory Medicine, UCSF.
2006-2008	Associate Investigator, Blood Systems Research Institute (BSRI), San Francisco, CA.
2009-Present	Senior Scientist, BSRI

Other Experience and Professional Memberships (selected)

1995-1999	Research Committee, Department of Surgery, UCSF
1999	NIH-NHLBI, Ad Hoc Reviewer
2001	Swiss National Science Foundation, Ad Hoc Reviewer
2005-present	Editorial Board Member for *Current Stem Cell Research & Therapy*
2005-present	Member UCSF Liver Center
2006, ‘08, ‘11, ‘13	Singapore National Medical Research Council, Ad Hoc Reviewer
2006-07, 2011-12	NIH-NIAID, Ad Hoc Reviewer
2008-2013	Editorial Board Member for *The Open Bone Journal* and *The Open Stem Cell Journal*
2010-2011	Reviewer Scientist-Solutions Indian Graduate-Students Project Grants

2010-Present	Editorial Board Member for *ISRN Hematology, Stem Cell Studies*
2011-Present	Editorial Board Member for *Current Tissue Engineering*
2011-Present	City College San Francisco, Advisory Committee for the NSF's Stem Cell Pipeline and California Institute of Regenerative Medicine's Bridges to Stem Cell grants.
2012-Present	Editorial Board Member for *Dataset Papers in Medicine*.
2012-Present	Editorial Advisory Board Member for the journal *Niche.*
2013-Present	Editorial Board Member for *World Journal of Hepatology.*
Present	Current Member: American Association of Blood Banks, International Society for Experimental Hematology-Society for Hematology and Stem Cells, American Association for Laboratory Animal Science

Honors (selected)

2001	NIH Research Career Award from NIDDK

C. Selected Peer-reviewed Publications (from 67 total).

1. **Muench MO**, Cupp J, Polakoff J, Roncarolo MG. Expression of CD33, CD38, and HLA-DR on CD34+ human fetal liver progenitors with a high proliferative potential. Blood. 1994; 83:3170-81.
2. **Muench MO**, Roncarolo MG, Namikawa R. Phenotypic and functional evidence for the expression of CD4 by hematopoietic stem cells isolated from human fetal liver. Blood. 1997; 89:1364-75.
3. **Muench MO**, Roncarolo MG, Rosnet O, Birnbaum D, Namikawa R. Colony-forming cells expressing high levels of CD34 are the main targets for granulocyte colony-stimulating factor and macrophage colony-stimulating factor in the human fetal liver. Exp Hematol. 1997; 25:277-87.
4. Golfier F, Bárcena A, Harrison MR, **Muench MO**. Fetal bone marrow as a source of stem cells for in utero or postnatal transplantation. Br. J. Haematol. 2000; 109:173-181.
5. **Muench MO**, Bárcena A. Broad distribution of colony-forming cells with erythroid, myeloid, dendritic cell, and NK cell potential among CD34(++) fetal liver cells. J Immunol. 2001; 167:4902-9.
6. **Muench MO**, Ratcliffe JV, Nakanishi M, Ishimoto H, Jaffe RB. Isolation of definitive zone and chromaffin cells based upon expression of CD56 (neural cell adhesion molecule) in the human fetal adrenal gland. J. Clin. Endocrinol. Metab. 2003; 88:3921-3930.
7. Suskind DL, **Muench MO**. Searching for common stem cells of the hepatic and hematopoietic systems in the human fetal liver: CD34+ cytokeratin 7/8+ cells express markers for stellate cells. J. Hepatol. 2004; 40:261-268.
8. **Muench MO**, Ohkubo T, Smith CA, Suskind DL, Bárcena A. Maintenance of proliferative capacity and retroviral transduction efficiency of human fetal $CD38^{-}/CD34^{++}$ stem cells. Stem Cells Dev. 2006; 15:97-108.
9. Ishimoto H, **Muench MO**, Higuchi T, Minegishi K, Tanaka M, Yoshimura Y, Jaffe RB. Midkine, a heparin-binding growth factor, selectively stimulates proliferation of definitive zone cells of the human fetal adrenal gland. J. Clin. Endocrinol. Metab. 2006; 91:4050-4056.
10. Mold JE, Michaëlsson J, Burt TD, **Muench MO**, Beckerman KP, Busch MP, Lee TH, Nixon DF, McCune JM. Maternal alloantigens promote the development of tolerogenic fetal regulatory T cells in utero. Science 2008; 322:1562-5. PMCID:PMC2648820
11. Bárcena A, Kapidzic M, **Muench MO**, Gormley M, Scott MA, Weier JF, Ferlatte C, Fisher SJ. The human placenta is a hematopoietic organ during the embryonic and fetal periods of development. Dev. Biol. 2009; 327:24-33. PMCID:PMC2668662
12. Varga NL, Bárcena A., Fomin ME, **Muench MO.** Detection of human hematopoietic stem cell engraftment in the livers of adult immunodeficient mice by an optimized flow cytometric method. Stem Cell Studies 2010; 1:e1. PMCID: PMC3098741
13. Fomin ME, Tai L, Bárcena A, **Muench MO**. Coexpression of CD14 and CD326 discriminate hepatic precursors in the human fetal liver. Stem Cells Dev. 2011; 20:1247-57. PMCID: PMC3121933 [Available on 2012/7/1]
14. Schonemann MD, **Muench MO**, Tee MK, Miller WL, Mellon SH. Expression of P450c17 in the human fetal nervous system. Endocrinology 2012;153:2494-2505. PMCID: PMC3339640

15. Ye L, **Muench MO**, Fusaki N, Beyer AI, Wang J., Qi Z, Yu J, Kan YW. Blood cell derived induced pluripotent stem cells free of reprogramming factors generated by Sendai viral vectors. Stem Cells Transl. Med. 2013. (In Press). (PMCID pending)

D. Research Support.

Ongoing Research Support

[redacted]

Past Research Support

R01 HL083388 Busch (PI) 03/01/09–07/31/12
NIH / NHLBI
Mechanisms and Clinical Effects of Microchimerism in Transfused Trauma Patients.
Retrospective and prospective studies investigating the persistence of donor leukocytes in patients transfused following severe injury.
Role: Investigator, responsible for determining if chimerism extends to the hematopoietic stem cell compartment.

R21 HD055328 Bárcena (PI) 02/01/08-03/31/11
NIH / NICHHD
The human placenta as a novel source of hematopoietic stem cells.
The major goal of this project is to determine if the human placenta is a hematopoietic organ.
Role: Investigator, primarily responsible for performing hematopoietic stem cell assays in immunodeficient mice.

R43 NS062494 Punnonen (PI) 01/01/09-06/30/09
NIH / NINDS
Low Molecular Weight Erythropoietin Mimetics For Improved CNS Penetrance And Treatment of Ischemic Stroke.
The major goal of this project is to develop small molecule erythropoietin mimetics.
Role: Investigator, responsible for testing small molecule erythropoietin mimetics on hematopoietic cells.

Muench (PI) 07/01/06-06/30/08
National Blood Foundation
In Utero Transplantation of the Hematopoietic Microenvironment.
The major goals of this project are to determine the survival and fate of hematopoietic stem cells, progenitors and hematopoietic organoids transplanted into the fetal peritoneal cavity.
Role: Principal Investigator

R21 DK068441 Muench (PI) 09/30/04-05/31/08
NIH / NIDDK
Ontogenic changes in erythroid gene expression.
The major goals of this project are to determine the expression patterns of genes in embryonic, fetal and adult erythropoiesis.
Role: Principal Investigator

BIOGRAPHICAL SKETCH

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

NAME	POSITION TITLE
Ryan, William, Russell	Assistant Professor of Clinical Otolaryngology - Head and Neck Surgery
eRA COMMONS USER NAME (credential, e.g., agency login)	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Washington, Seattle, WA		06/94	Freshman/Honors Program
Wesleyan University, Middletown, CT	B.A.	06/97	Phi Beta Kappa, American Studies
University of Pennsylvania, Philadelphia, PA		06/98	Post-baccalaureate Program
Stanford University, Stanford, CA	M.D.	06/05	
Stanford University, Stanford, CA	Intern	06/06	Otolaryngology-Head and Neck Surgery
Stanford University, Stanford, CA	Resident	06/10	Otolaryngology-Head and Neck Surgery
University of California-San Francisco, CA	Fellow	06/11	Head and Neck Oncologic Surgery

A. Personal Statement

I have specialized in Head and Neck Oncologic Surgery since 2006, initially at Stanford and currently at the University of California, San Francisco. Two primary focuses of my current research interests are surgical methods to preserve salivary function and radiation-induced xerostomia. As a clinician I regularly perform neck dissections for the removal of cancerous lymph nodes. In doing so, I often remove the submandibular salivary glands, as this is part of an established procedure. I also perform salivary gland excisions for inflammatory and neoplastic diseases. My expertise in head and neck cancer, xerostomia and salivary gland pathology will be highly beneficial to this proposed research.

B. Positions and Honors

Principals Positions Held

2010	2011	University of California-San Francisco	Clinical Instructor	Otolaryngology-Head and Neck Surgery
2011		University of California-San Francisco	Assistant Professor	Otolaryngology-Head and Neck Surgery

Honors Awards

1994	Honors Program	University of Washington
1994	Phi Eta Sigma	University of Washington
1997	Phi Beta Kappa	Wesleyan University
2000	Travel Scholars Research Grant, $12,500	Stanford
2001	Medical Scholars Research Grant, $10,000	Stanford
2002	Medical Scholars Research Grant, $12,000	Stanford
2003	Medical Scholars Research Grant, $48,000	Stanford
2004	Best Paper by a Medical Student Award	6th International Conference on Head and Neck Cancer, Washington, DC

2007	Research Grant, $15,000	Xoran Technologies
2009	Resident Travel Grant	Western Section Triological Society
2009	Honorable Mention-Scientific Content Award	Stanford Otolaryngology Resident Research Symposium
2011	Pinnacle Award, 2011, Highest Patient Satisfaction Award For A Clinical Division at UCSF (Granted to the Division of Head and Neck Oncologic/Endocrine Surgery, faculty members: Drs. Orloff, Eisele, Knott, Wang, and Ryan)	University of California-San Francisco Medical Center
2012	Pinnacle Award, 2012, Highest Patient Satisfaction Award For A Clinical Division at UCSF (Granted to the Division of Head and Neck Oncologic/Endocrine Surgery, faculty members: Drs. Orloff, Knott, Wang, and Ryan)	University of California-San Francisco Medical Center
2013	Mount Zion Health Fund Travel Grant, $1,500	University of California-San Francisco Medical Center at Mount Zion
2013	Planning Grant for a Model Integrative Medicine Program For Head and Neck Cancer. Co-Investigator with Principal Investigator: Margaret Chesney, PhD (Director of Osher Center), $30,000	The George Family Foundation

Memberships

2006	American Academy of Otolaryngology, Head and Neck Surgery
2012	American Head and Neck Society
2012	Associate Fellow of the American College of Surgeons, Applicant For Fellow of the American College of Surgeons

Service to Professional Organizations

2012	2013	Co-director, UCSF/Tripler Pacific Rim Otolaryngology-Head and Neck Update Course, Honolulu, HI, February 2013	Co-director, Lecturer (2 Lectures)
2013	2014	Co-director, UCSF/Tripler Pacific Rim Otolaryngology-Head and Neck Update Course, Honolulu, HI, planned February 2014	Co-director, Lecturer (2 Lectures)

C. Selected Peer-Reviewed Publications

1. Fisher RL, Ryan WR, Dugdale TW, Zimmerman GA. Arthroscopic ankle fusion. Connecticut Medicine. 1997 Oct; 61 (10): 643-6
2. Quigley TM, Ryan WR, Morgan S. Patient satisfaction after carotid endarterectomy using a selective policy of local anesthesia. American Journal of Surgery. 2000 May; 179 (5): 382-5.
3. Ryan WR, Ley C, Allan RN, Keighley MRB. Patients with Crohn's disease are unaware of the risks that smoking has on their disease. Journal of Gastrointestinal Surgery. 2003; 7: 1-6.
4. Ryan WR, Allan RN, Yamamoto T, Keighley MRB. Crohn's disease patients who quit smoking have a reduced risk of reoperation for recurrence. American Journal of Surgery. 2004. February; 187 (2): 219-225.
5. Ryan WR, Fee WE, Le QL, Pinto HA. Positron-emission tomography for surveillance of head and neck cancer. Laryngoscope. 2005. Apr; 115(4):645-50.
6. Ryan WR, Fee WE. Great auricular nerve morbidity after nerve sacrifice during parotidectomy. Archives of Otolaryngology-Head Neck Surgery. 2006 Jun; 132 (6): 642-9.
7. Ryan WR, Most SP. A prospective evaluation of the efficacy of topical adhesive pads for the reduction of facial rhytids. Archives of Facial Plastics Surgery. 2009. July-August; 11(4): 252-256.
8. Ryan WR, Fee WE. Long term great auricular nerve morbidity after sacrifice during parotidectomy. Laryngoscope. 2009. June, 119:1140-1146. **Cover article.**

9. Ryan WR, Hwang PH. Safety of a preservative-free acidified saline nasal spray: a randomized, double-blinded, placebo-controlled trial. Archives of Otolaryngology-Head and Neck Surgery. 2010; 136(11): 1099-1103. **Featured in Inside Stanford Medicine Online Magazine, Winter 2011.**
10. Ryan WR, Ramachandra T, Hwang PH. Correlations between symptoms, nasal endoscopy, and in-office computed tomography for post-surgical chronic rhinosinusitis patients. Laryngoscope. 2011. March; 121(3): 674-8. **($15,000 Xoran Technologies Research Grant Recipient)**
11. Ryan WR, Orloff LA. Intraoperative tumor localization with surgeon-performed ultrasound-guided needle dye injection. Laryngoscope. 2011 Aug;121(8):1651-5. doi: 10.1002/lary.21774. Epub 2011 Jun 9. **Featured in ENT Today (August 2011 Issue) in the Literature Review: a roundup of important recent studies.**
12. Ryan WR, Heaton CM, Wang SJ. Elective Regional Lymphadenectomy for Advanced Auricular Squamous Cell Carcinoma. World J Otorhinolaryngol 2013 February 28; 3(1): 16-21
13. Ryan WR, Chang J, Eisele DW. Transfacial surgeon-performed ultrasound-guided needle-catheterization and stent placement of obstructed parotid duct with sialoendoscopic visualization. Accepted for publication by the Laryngoscope.

D. Research Support: None.

BIOGRAPHICAL SKETCH

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

NAME	POSITION TITLE
Roberto Weigert	Chief, Intracellular Membrane Trafficking Unit, Oral and Pharyngeal Cancer Branch, NIDCR, National Institutes of Health
eRA COMMONS USER NAME (credential, e.g., agency login)	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Catania, Catania, Italy	M.Sc.	1992	Chemistry
Open University of London, UK	Ph.D.	2000	Life Sciences

A. Personal Statement

Intracellular membrane trafficking plays a key role in many basic cellular processes and unraveling its complex regulation at a molecular level is a fundamental step in understanding physiological and pathological events in different organs. However most of the studies in this field have been carried out in in vitro model systems, such as cultured cells. Although these model systems that can be easily imaged and manipulated have provided invaluable information on the molecular machineries regulating several subcellular events, they have major limitations since they do not always recapitulate the complex characteristics of the living tissues in their native environment. To overcome this issue, we have combined the use of live animals with light microscopy techniques, such as intravital two-photon (TPM) and confocal microscopy, which enable the direct observation of the process of interest at a subcellular resolution. This has been complemented with the development of a series of pharmacological, molecular and genetic tools that have enabled us to investigate the process of interest at the molecular level.

In the last years our effort has been directed towards the development of two experimental systems in live animals to investigate the role of membrane trafficking in the physio-pathology of the oral cavity: the first, aimed at studying membrane trafficking under physiological conditions and based on the use of the rodent salivary glands (SGs) as a model organ; the second, aimed at studying membrane trafficking under pathological conditions and based on the use of an orthotopic implant of squamous cell carcinoma in the tongue of immunocompromised mice, a well-established model for head and neck cancer. Moreover, since intravital two-photon microscopy provides the opportunity to image biological processes at high resolution without the need for exogenous labeling we are in the process of exploiting this technique as a non-invasive diagnostic tool for various diseases of the oral cavity, such as Sjögren's syndrome, radiotherapy-induced damage and detection of pre-cancerous lesions.

B. Positions and Honors.

Positions and Employment

1993-1994	Technical Brand Manager, Department of Research and Development, Procter & Gamble, Italy
1994-1999	Pre-Doctoral Fellow, Laboratory of Molecular Neurobiology, Dept. of Cell Biololgy and Oncology, Consorzio Mario Negri Sud, Italy
1995	Special Volunteer, Cell Biology and Metabolism Branch, National Institute of Child Health Development, NIH, Bethesda USA
1999-2001	Post-Doctoral Fellow, Laboratory of Molecular Neurobiology, Dept. of Cell Biololgy and Oncology, Consorzio Mario Negri Sud, Italy
2001-2006	Research Fellow, Laboratory of Cell Biology, National Institute Heart, Lung and Blood, NIH, Bethesda, USA.
2006-present	Chief, Intracellular Membrane Trafficking Unit, Oral and Pharyngeal Cancer Branch, National Institute of Dental and Craniofacial Research, NIH, Bethesda, USA

Honors

1993 Accademia Gioena Award, for the for the best thesis work in Microelectronics
1993 Consorzio Catania Ricerche Award, for the most original thesis work
1996 FORMEZ fellowship sponsored by the "Agenzia per il mezzogiorno" for the training of young scientist coming from south Italy
2007 Chesapeake Society for Microscopy, Award
2010 ASCB Press Book
2011 Distinguished Research Award - University of South Australia
2011 Elected co-chair of the Salivary Glands and Exocrine Biology Gordon Research Conference
2012 Elected co-chair of the Lysosomes and endocytosis Gordon Research Conference
2012 Olympus Bioscape award
2013 NHLBI Orloff Science Award

C. Selected peer-reviewed publications (in chronological order).

1. Masedunskas, A. and **Weigert, R**. Intravital two-photon microscopy for studying the uptake and trafficking of fluorescently-conjugated molecules in live rodents (2008) Traffic 9(10): 1801-10. PMC2711521
2. Bhirde A.A., Patel V., Gavard, J., Zhang G., , Sousa A.A., Masedunskas A., Leapman R.D., **Weigert R**., , Gutkind J.S., and Rusling, J.F. Targeted Killing of Cancer Cells *in vivo* and *in vitro* with EGF-directed Carbon Nanotube-based Drug Delivery (2009) ACS Nano 24; 3(2):307-16. PMC2665730
3. Gavard J, Hou X, Qu Y., Masedunskas A., Martin D., **Weigert R**., Li X, and Gutkind J.S., A Role for a CXCR2/PI3K{gamma} Signaling Axis in Acute and Chronic Vascular Permeability (2009) Mol Cell Biol. 29(9): 2468-80. PMC2668372
4. Sramkova M., Masedunskas A., Parente L., Molinolo A., and **Weigert R**. Expression of plasmid DNA in the salivary gland epithelium: novel approaches to study dynamic cellular processes in live animals (2009) Am. J Physiol Cell Physiol Dec 297(6):C1347-57. PMC2793061
5. **Weigert R.,** Sramkova M., Amornphimoltham P., Parente, L. and and Masedunskas A. Intravital microscopy as a novel tool to study cell biology in live animals (2010) Histochem and Cell Biol May; 133(5):481-491. PMC3027219
6. Sakurai A., Gavard J., Annas-Linhares Y., Basile J.R., Amornphimoltham P., Palmby T., Yagi Y., Zhang F., Randazzo P., Li X., **Weigert R.,** and Gutkind, J.S. Semaphorin 3E initiates antiangiogenic signaling signaling through plexin D1 by regulating Arf6 and R-Ras (2010) Mol Cell Biol 30(12):3086-98. PMC2876686
7. Sales KU, Masedunskas A., Bey A.L., Rasmussen A.L, **Weigert R.,** List K., Szabo R., Overbeek P., and Bugge T.H. Matriptase initiates activation of epidermal pro-kallikrein and disease onset in a mouse model of Netherton syndrome. (2010) Nat. Genet. 42(8):676-83. PMC3081165
8. Amornphimoltham P, Masedunskas A, and **Weigert R.,** Intravital microscopy as a tool to study drug delivery in preclinical studies (2011) Adv. Drug. Del. Rev. 63(1-2):119-28. PMC3024442
9. Masedunskas A, Sramkova M, Parente L, Sales KU, P, Bugge TH, and **Weigert R**, Role for the acto-myosin complex in regulated exocytosis revealed by intravital microscopy (2011) Proc Natl Acad Sci U S A. 108(33):13552-7. PMC3158220
10. Yagi H, Tan W, Dillenburg-Pilla P, Armando S, Amornphimoltham P, Simaan, M, **Weigert R**, Molinolo AA, Bouvier M, Gutkind JS. A Synthetic Biology Approach Reveals a CXCR4-G_{13}-Rho Signaling Axis Driving Transendothelial Migration of Metastatic Breast Cancer Cells. (2011) Science Signaling 4(191):ra60. PMC3429372
11. Patel V, Marsh CA, Dorsam RT, Masedunskas A, Amornphimoltham P, Nathan CO, Singh B, **Weigert R**, Molinolo AA, and Gutkind JS. Decreased Lymphangiogenesis and Lymph Node Metastasis by mTOR Inhibition in Head and Neck Cancer (2011) Cancer Research 71(22):7103-12. PMC3443559
12. Sramkova M, Masedunskas A, and **Weigert R.** Plasmid DNA is internalized from the apical plasma membrane of the salivary gland epithelium in live animals. (2012) Histochem and Cell Biol 138(2): 201-213. PMC3428210
13. Wu XS, Masedunskas A, **Weigert R**, Copeland NG, Jenkins NA, and Hammer JA. Melanoregulin regulates a shedding mechanism that drives melanosome transfer from melanocytes to keratinocytes (2012) Proc Natl Acad Sci U S A 109(31):E2101-9. PMC3412008

14. Porat-Shliom N, Milberg O, Masedunskas A, **Weigert R**. Multiple roles for the actin cytoskeleton during regulated exocytosis (2012) Cell Mol Life Sci. 70(12):2099-121. PMC Journal – In Process

15. Amornphimoltham P, Rechache K, Thompson J, Masedunskas A, Leelahavanichkul K, Patel V, Molinolo A, Gutkind JS, and **Weigert R**. Rab25 regulates invasion and metastasis in head and neck cancer (2013) Clin. Can. Res. 19(6):1375-88. PMC3602237

16. Weigert R, Porat-Shliom N, and Amorpnphimoltham, P. Imaging Cell Biology in Live Animals: Ready for Primetime (2013) J. Cell Biol (in press)

D. Research Support.

[redacted] Ongoing

PHS 398 Cover Page Supplement

1. Project Director / Principal Investigator (PD/PI)

Prefix:
* First Name: Sarah
Middle Name: Monica
* Last Name: Knox
Suffix: PhD

2. Human Subjects

Clinical Trial? ●No ○Yes

* Agency-Defined Phase III Clinical Trial? ○No ○Yes

3. Applicant Organization Contact

Person to be contacted on matters involving this application

Prefix:
* First Name: [redacted]
Middle Name:
* Last Name: [redacted]
Suffix:

* Phone Number: [redacted]
Fax Number: [redacted]
Email: [redacted]

* Title: Contracts and Grants Officer

* Street1: 3333 California Street, Suite 315
Street2: Box 0962
* City: San Francisco
County: San Francisco
* State: CA: California
Province:

* Country: USA:
* Zip / Postal Code: 94143-0962

PHS 398 Cover Page Supplement

4. Human Embryonic Stem Cells

* Does the proposed project involve human embryonic stem cells? ●No ○Yes

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://stemcells.nih.gov/registry/index.asp . Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): ❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

PHS 398 Modular Budget, Periods 1 and 2

OMB Number: 0925-0001

Budget Period: 1

Start Date: 04/01/2014 End Date: 03/31/2015

A. Direct Costs

	Funds Requested ($)
* Direct Cost less Consortium F&A	250,000.00
Consortium F&A	5,682.00
* Total Direct Costs	255,682.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	Sponsored Research _ On Campus	57.00	63,921.00	36,435.00
2.	Sponsored Research On Campus	58.00	191,761.00	111,221.00
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number) DHHS, (415) 437-7820

Indirect Cost Rate Agreement Date 05/23/2012

Total Indirect Costs 147,656.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($) 403,338.00

Budget Period: 2

Start Date: 04/01/2015 End Date: 03/31/2016

A. Direct Costs

	Funds Requested ($)
* Direct Cost less Consortium F&A	250,000.00
Consortium F&A	5,682.00
* Total Direct Costs	255,682.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	Sponsored Research On Campus	58.00	62,431.00	36,210.00
2.	Sponsored Research _ On Campus	58.50	187,293.00	109,566.00
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number) DHHS, (415) 437-7820

Indirect Cost Rate Agreement Date 05/23/2012

Total Indirect Costs 145,776.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($) 401,458.00

PHS 398 Modular Budget, Periods 3 and 4

OMB Number: 0925-0001

Budget Period: 3

Start Date: 04/01/2016 End Date: 03/31/2017

A. Direct Costs

	Funds Requested ($)
* Direct Cost less Consortium F&A	250,000.00
Consortium F&A	5,682.00
* Total Direct Costs	255,682.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	Sponsored Research _ On Campus	58.50	240,203.00	140,519.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number) DHHS, (415) 437-7820

Indirect Cost Rate Agreement Date 05/23/2012

Total Indirect Costs 140,519.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($) 396,201.00

Budget Period: 4

Start Date: 04/01/2017 End Date: 03/31/2018

A. Direct Costs

	Funds Requested ($)
* Direct Cost less Consortium F&A	250,000.00
Consortium F&A	5,682.00
* Total Direct Costs	255,682.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	Sponsored Research _ On Campus	58.50	240,203.00	140,519.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number) DHHS, (415) 437-7820

Indirect Cost Rate Agreement Date 05/23/2012

Total Indirect Costs 140,519.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($) 396,201.00

Tracking Number

PHS 398 Modular Budget, Period 5 and Cumulative

OMB Number: 0925-0001

Budget Period: 5

Start Date: 04/01/2018 End Date: 03/31/2019

A. Direct Costs

	Funds Requested ($)
* Direct Cost less Consortium F&A	250,000.00
Consortium F&A	5,682.00
* Total Direct Costs	255,682.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	Sponsored Research _ On Campus	58.50	240,203.00	140,519.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number) DHHS, (415) 437-7820

Indirect Cost Rate Agreement Date 05/23/2012

Total Indirect Costs 140,519.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($) 396,201.00

Cumulative Budget Information

1. Total Costs, Entire Project Period

* Section A, Total Direct Cost less Consortium F&A for Entire Project Period	$	1,250,000.00
Section A, Total Consortium F&A for Entire Project Period	$	28,410.00
* Section A, Total Direct Costs for Entire Project Period	$	1,278,410.00
* Section B, Total Indirect Costs for Entire Project Period	$	714,989.00
* Section C, Total Direct and Indirect Costs (A+B) for Entire Project Period	$	1,993,399.00

2. Budget Justifications

Personnel Justification: Personnel_Justification1026864061.pdf

Consortium Justification: Consortium_Justification1026864328.pdf

Additional Narrative Justification:

Tracking Number

Attachments

PersonnelJustification_attDataGroup0

File Name	Mime Type
Personnel_Justification1026864061.pdf	application/pdf

ConsortiumJustification_attDataGroup0

File Name	Mime Type
Consortium_Justification1026864328.pdf	application/pdf

AdditionalNarrativeJustification_attDataGroup0

File Name	Mime Type

Personnel Justification

A. Key Personnel

Sarah Knox, PhD (Principal investigator, [redacted] effort and salary support requested). Dr. Knox will have overall responsibility for the aims outlined in the project. Specifically, she will design, plan, and carry out the proposed research, analyze the results, supervise and mentor personnel, and the writing and editing of manuscripts. Dr. Knox has extensive expertise in the area of salivary gland development and regeneration using the ex vivo mouse model and human tissue, as well as high-resolution microscopy. She also has significant expertise in gene expression analysis.

William R Ryan, MD, (Co-investigator, [redacted] and salary support requested). Dr. Ryan is an Assistant Professor in the Department of Otolaryngology-Head and Neck Surgery at UCSF who specializes in head and neck oncologic, endocrine, and skull base surgery. His present focus is on surgical intervention of salivary gland tumors and on the reconstitution of salivary gland function. He will provide surgical specimens for isolating adult human salivary gland stem/progenitor cells. A letter of collaboration is included.

B. Other Personnel

[redacted] (Post-doctoral fellow, [redacted] effort and salary support requested). [redacted]

[redacted] (Post-doctoral fellow [redacted] effort and salary support requested). [redacted]

[redacted], (Staff Research Associate, [redacted] effort and salary support requested). [redacted]

C. Other Significant Contributors

Roberto Weigert, Ph.D. (NIDCR) is an expert in intra vital imaging and will provide technical support for the duct cannulation repair model as described in Aim2 of this proposal. A letter of support is attached.

Consortium Justification

Consortium with the **Blood Systems Inc. dba Blood Systems Research Institute**
{X} Domestic { } Foreign
Approximately $15,000 Total Costs for all years. [redacted])
Calculations are based on 58% F&A rate (DHHS negotiated rate agreement, date of 05/07/2013)

Marcus Muench, PhD (Co-investigator, Blood Systems Research Institute, [redacted] effort and salary support requested). [redacted]

PHS 398 Research Plan

1. Application Type:

From SF 424 (R&R) Cover Page and PHS398 Checklist. The responses provided on these pages, regarding the type of application being submitted, are repeated for your reference, as you attach the appropriate sections of the research plan.

*Type of Application:

● New ○ Resubmission ○ Renewal ○ Continuation ○ Revision

2. Research Plan Attachments:

Please attach applicable sections of the research plan, below.

1. Introduction to Application (for RESUBMISSION or REVISION only)	
2. Specific Aims	Specific_Aims1026914986.pdf
3. Research Strategy	Research_Strategy1026914984.pdf
4. Inclusion Enrollment Report	
5. Progress Report Publication List	

Human Subjects Sections

6. Protection of Human Subjects	Protection_of_Human_Subjects1026864340.pdf
7. Inclusion of Women and Minorities	
8. Targeted/Planned Enrollment Table	
9. Inclusion of Children	

Other Research Plan Sections

10. Vertebrate Animals	Vertebrate_Animals1026915184.pdf
11. Select Agent Research	
12. Multiple PD/PI Leadership Plan	
13. Consortium/Contractual Arrangements	ConsortiumContractual_Agreements1026864330.pdf
14. Letters of Support	Letters_of_Support1026864060.pdf
15. Resource Sharing Plan(s)	Resource_Sharing_Plan1026864065.pdf

16. Appendix

Attachments

IntroductionToApplication_attDataGroup0

File Name	Mime Type

SpecificAims_attDataGroup0

File Name	Mime Type
Specific_Aims1026914986.pdf	application/pdf

ResearchStrategy_attDataGroup0

File Name	Mime Type
Research_Strategy1026914984.pdf	application/pdf

InclusionEnrollmentReport_attDataGroup0

File Name	Mime Type

ProgressReportPublicationList_attDataGroup0

File Name	Mime Type

ProtectionOfHumanSubjects_attDataGroup0

File Name	Mime Type
Protection_of_Human_Subjects1026864340.pdf	application/pdf

InclusionOfWomenAndMinorities_attDataGroup0

File Name	Mime Type

TargetedPlannedEnrollmentTable_attDataGroup0

File Name	Mime Type

InclusionOfChildren_attDataGroup0

File Name	Mime Type

VertebrateAnimals_attDataGroup0

File Name	Mime Type
Vertebrate_Animals1026915184.pdf	application/pdf

SelectAgentResearch_attDataGroup0

File Name	Mime Type

MultiplePDPILeadershipPlan_attDataGroup0

File Name	Mime Type

ConsortiumContractualArrangements_attDataGroup0

File Name	Mime Type
ConsortiumContractual_Agreements1026864330.pdf	application/pdf

LettersOfSupport_attDataGroup0

File Name	Mime Type
Letters_of_Support1026864060.pdf	application/pdf

ResourceSharingPlans_attDataGroup0

File Name	Mime Type
Resource_Sharing_Plan1026864065.pdf	application/pdf

Appendix

File Name	Mime Type

SPECIFIC AIMS

There exists a significant unmet need to repair, regenerate and replace salivary glands after pathological destruction due to surgery, disease, and therapeutic radiation for cancer. Irreversible loss (partial or complete) of salivary function due to destruction of secretory acini severely depletes oral health and quality of life of patients. Transplantation of stem cells or reactivation of existing progenitor cells are promising therapeutic strategies to treat salivary dysfunction. To achieve this goal, **we must first identify adult human salivary gland progenitor cells and understand how they are regulated**.

We have previously shown that, in the developing mouse salivary gland, proliferation of progenitors marked by keratin (K)-5 is dependent on parasympathetic nerves. Furthermore, regeneration of fetal and adult salivary acini after damage requires a functional nerve supply. However, genetic characterization of putative progenitor cells in the salivary gland has been limited to embryonic development, and there is a dearth of knowledge on how these cells are regulated and what progenitor cell types are involved.

Understanding progenitor cell identity in the salivary gland and the mechanisms that regulate acinar regeneration will enable the design of targeted regenerative approaches to reverse salivary dysfunction. To this end, we wish to understand how nerves regulate salivary gland repair and regeneration by characterizing progenitor cells in the mouse and human and determining mechanisms by which parasympathetic nerves control and support their differentiation into functional glandular tissue. Thus, we will **test the hypothesis that discrete progenitor cells contribute to epithelial lineages of mouse and human salivary glands** and are regulated by neuronal signals to promote acinar regeneration after injury.

Aim 1: Define the mechanisms by which parasympathetic nerves regulate acinar cell fate. We previously reported that parasympathetic nerves promote fetal mouse acinar regeneration after irradiation. However, the mechanisms by which nerves drive acinar formation are unclear. Based on our preliminary data, we hypothesize that neuronal-derived neuregulin-1 type III (Nrg1-III) and acetylcholine (ACh) promote the regeneration of acini by directing Sox2+ and K5+ progenitor cells toward an acinar cell fate. To test this, we will define the effects of Nrg1-III and ACh on K5+ and Sox2+ cell proliferation and acinar differentiation in fetal human and mouse fetal salivary glands. We will then elucidate the signaling pathways downstream of ACh and Nrg1-III that promote acinar formation. These data will provide the first mechanistic understandings of how acini may be regenerated after damage.

Aim 2: Determine the contribution of salivary progenitor cells and neuronal signaling to adult homeostasis and repair. Our preliminary data show that Sox2 and K5 mark progenitor cell populations during mouse salivary gland development that are regulated by nerves. However, the contribution of these cells to the adult tissue during homeostasis and repair is unclear. To test this, we will perform lineage-tracing in the mouse under both homeostatic and repair conditions to determine the contribution of Sox2+ and K5+ cells to salivary gland structure. We will also utilize an ex vivo repair model to test the effect of ACh and Nrg1-III on Sox2+ and K5+ cell proliferation and differentiation. These studies will provide us with a clear picture of the contribution and requirement of Sox2+ and K5+ cells and neuronal input in the adult salivary gland.

Aim 3: Identify epithelial progenitor cells in the human salivary gland. [redacted]

RESEARCH STRATEGY

a. SIGNIFICANCE

Salivary gland (SG) dysfunction severely compromises the oral health and quality of life of patients: saliva protects the oral mucosa, fascilitates food digestion and articulation, and aids in the remineralization of dental hard tissues (1). Dry mouth or xerostomia can occur from irreversible pathological injury due to the autoimmune disease Sjogren's Syndrome (1-2 million in USA) and also from therapeutic radiation for head and neck cancer where SGs are inadvertently irradiated along with the tumor (~50,000 cases/year). Unfortunately, there are no available treatments for salivary dysfunction. A potential regenerative approach for restoring salivary function is stem cell therapy where autologous stem/progenitor cells are transplanted into the injured organ (2) or surviving stem cells within the tissue are reactivated (3). A number of putative progenitor cell populations have been identified in the mouse SG (2, 4-6) and to a much lesser extent in the human SG (7). Despite these important observations, **the identity of salivary stem/progenitor cells that contribute to tissue homeostasis, regeneration and/or repair and the regulatory mechanisms controlling their cell fate are poorly understood.**

In this proposal we seek to understand the contribution of discrete epithelial progenitor cell populations to epithelial lineages in mouse and human SGs and determine how signals from parasympathetic nerves (ACh and Nrg1-III) promote an acinar cell fate (**Fig.1**). Parasympathetic innervation is required for the maintenance of progenitor cells and the development and regeneration of acini in salivary glands (5), yet the mechanisms by which nerves achieve these outcomes and the identity of the progenitor cells involved are unclear. Our recent finding that parasympathetic innervation is severely reduced in irradiated human SGs, but that putative progenitor cells (marked by keratin (K)-5+ and Sox2+) survive (8) (**Fig.2**) highlights the critical importance of defining neuronal-progenitor cell communication for the development of regenerative stratgies to reverse salivary dysfunction. We therefore postulate that discrete epithelial progenitor cells in the adult mouse and human SG regenerate acini during homeostasis and injury in response to neuronal signals. **Testing this hypothesis will identify human salivary progenitor cells and neuronal signaling mechanisms that regulate progenitor cell fate.** Our findings will significantly impact the feasibility of targeted stem cell therapy for long-term functional restoration of SGs. In addition, the outcome will likely translate to regeneration of other organ systems innervated by the parasympathetic nervous system.

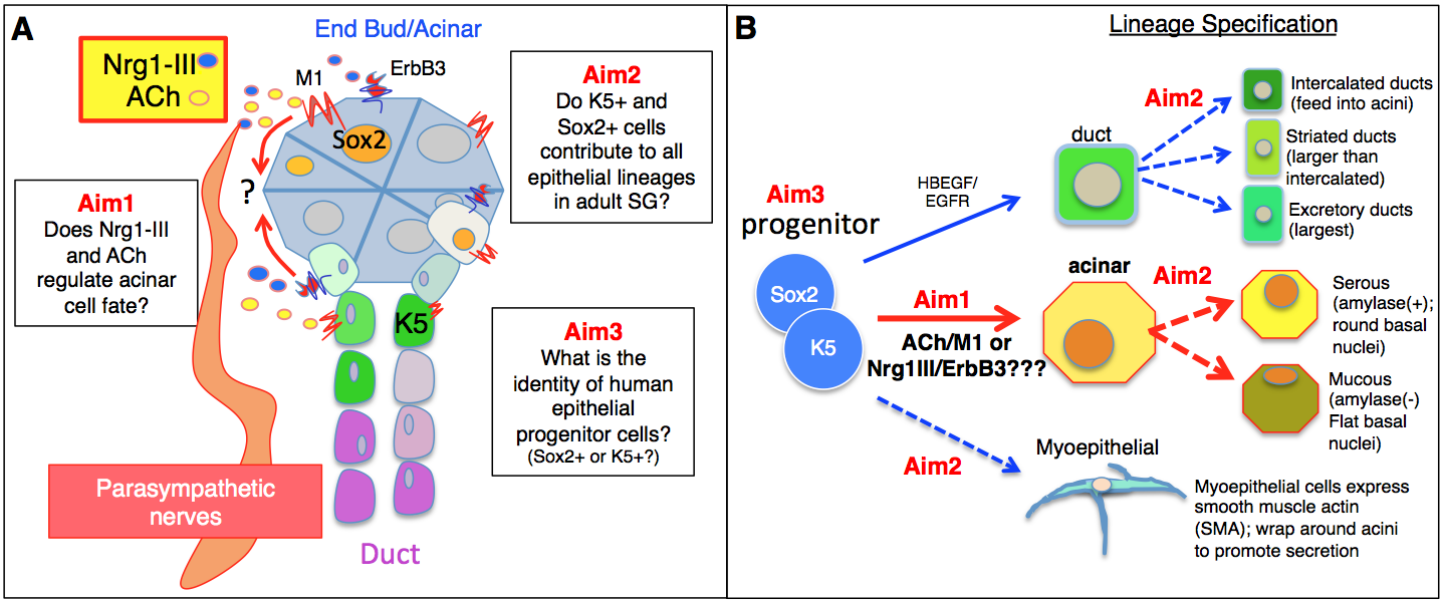


Figure 1: Aims to be tested in this proposal. A. Schematic showing acini with duct. Sox2+ cells are predominately in acini and K5+ cells in the duct. ACh and Nrg1-III are secreted by parasympathetic nerves and can interact with their receptors M1 or ErbB3, respectively, on the epithelium. **B.** Lineage specification during salivary gland development.

b. INNOVATION

First, this study is innovative in its characterization of novel progenitor cells in the **human SG**. There have been no studies of human fetal salivary progenitors, and few of human adult salivary progenitor cells. The use of fetal tissue has significant advantages in understanding human progenitor cells in that it allows for the evaluation of human developmental populations, the isolation of progenitor populations and, as fetal cells have a competitive advantage over adult cells, successful ex vivo culture and in vivo engraftment are highly feasible (9). Importantly, our strategy of isolating human progenitors is not dependent on extrapolation from mouse

models but is based on transcriptional analysis and ex vivo self-renewal/differentiation capability in human tissue. This novel and powerful approach will reveal new insights into progenitor cell identity and mechanisms regulating their behavior. **Second,** this will be the first study to characterize progenitor cells using mouse genetics in the adult system under conditions of **homeostasis and injury**. To date, only Sox2 has been genetically confirmed via lineage tracing to be a progenitor cell in the adult tissue *during homeostasis* (6), but its contribution to adult epithelial lineages has not been determined. Here we will define the epithelial lineages derived from K5+ and Sox2+ progenitor cells in the adult and determine their ability to respond and repopulate the gland after gamma radiation or mechanical-induced injury. This novel strategy will provide essential information on the ability of progenitor cell populations to populate the SG during homeostasis and repair/regeneration. **Third**, we are only beginning to appreciate parasympathetic nerve signaling as a key regulator of epithelial, and in particular, acinar regeneration after injury. This proposal is therefore innovative in its interrogation of neuronal-epithelial-progenitor signaling pathways modulating repair and regenerative processes in human and mouse SGs.

c. APPROACH

Preliminary Studies

Innervation and progenitor cells in the irradiated human SG. Patients receiving therapeutic radiation for head and neck malignancies will lose most of their salivary function through the inadvertent destruction of secretory acini (**Fig.2A**.). Unfortunately, this often results in permanent xerostomia (dry mouth) due to the tissues inability to regenerate. Why the human SG does not regenerate after IR is unclear. We recently found that parasympathetic innervation is severely reduced in human IR SGs (**Fig.2A**, middle panel), as well as the acetylcholine muscarinic receptors involved in salivary function (*CHRM1 and 3*; **Fig.2B**). These findings indicate that loss of function is due not only to acinar destruction, but also to loss of neuronal-epithelial communication. Furthermore, putative progenitor cells marked by KRT5 are abundant (**Fig.2A** and **B**), and the stem cell marker *SOX2* is maintained, albeit at reduced levels. These data are consistent with a previous study demonstrating the presence of functional progenitor cells in human IR SG biopsies (10). Thus, we postulate that regeneration of acinar cells in the human SG requires reinnervation of progenitor cells.

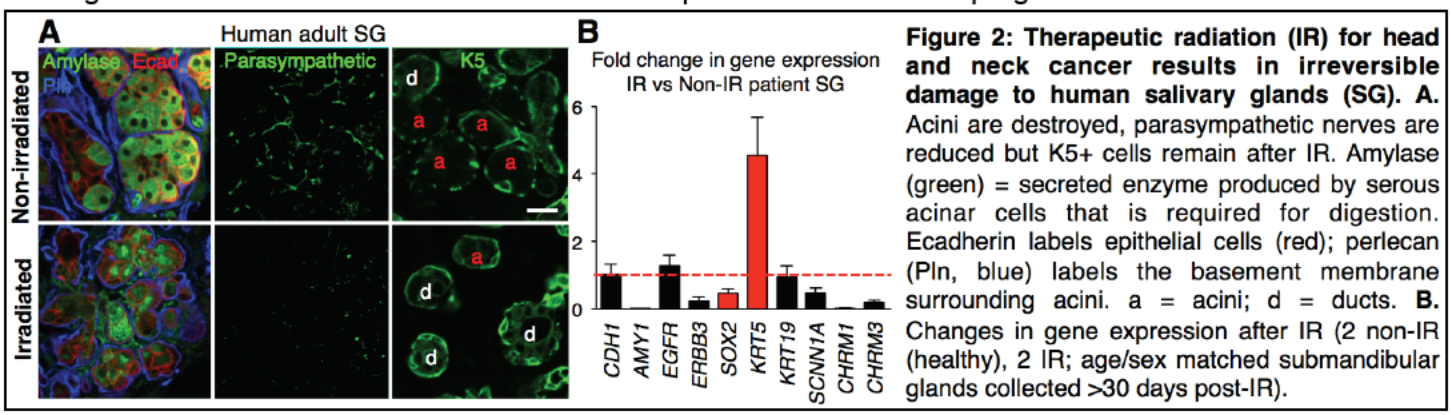


Figure 2: Therapeutic radiation (IR) for head and neck cancer results in irreversible damage to human salivary glands (SG). A. Acini are destroyed, parasympathetic nerves are reduced but K5+ cells remain after IR. Amylase (green) = secreted enzyme produced by serous acinar cells that is required for digestion. Ecadherin labels epithelial cells (red); perlecan (Pln, blue) labels the basement membrane surrounding acini. a = acini; d = ducts. **B.** Changes in gene expression after IR (2 non-IR (healthy), 2 IR; age/sex matched submandibular glands collected >30 days post-IR).

A role for parasympathetic innervation in acinar development and regeneration. Rodent SGs are capable of regenerating after injury (11-16), a process postulated to be analogous to fetal SG development (16). Prior studies in the rodent have demonstrated an essential requirement for parasympathetic innervation in adult SG regeneration: in the absence of nerves, the acini do not regenerate (rev. by (17)). Indeed, the human SG also degenerates after parasympathectomy (18). However, the mechanisms by which nerves control acini regeneration are not known. We recently reported that parasympathetic innervation is necessary for murine fetal SG development as it is required to maintain the K5+ epithelial progenitor cell population and promote acinar formation via an ACh/muscarinic receptor (M)1/EGFR pathway (5, 8) (**Fig.3A** and **D**). More recently, we demonstrated that maintaining cholinergic innervation of damaged epithelium in an irradiated fetal mouse SG model (19) or adding the acetylcholine analog carbachol (CCh) in explant assays, improves end bud (pre-acinar) regeneration and re-activates markers of acinar development (8) (*Fgf1, Fgfr1b, Etv4* and *5;* **Fig.3C**). However, it remains unknown whether acinar cell regeneration is mediated by proliferation and differentiation of K5+ progenitor cells, or whether other progenitor populations are responsible for the neuronal responsive regeneration. Furthermore, ACh/M1 signaling only partly rescues acinar regeneration after IR-induced damage, suggesting that other neuronal-derived factors are required.

Using ex vivo parasympathetic ganglia cultures, we have identified another candidate neurotrophic factor, Nrg1-III that regulates K5+ cells and acinar formation during development and after injury. Nrg1-III is an EGF

family member that binds its cognate receptor ErbB3, and is expressed predominately in epithelial end buds of the fetal mouse SG (20). Using both gain- and loss-of-function experiments, we show that Nrg1-III is required for epithelial end bud formation (**Fig.4A-C**) and regeneration (**Fig.4D**). Notably, Nrg1-III treatment also results in increased acinar gene expression (*Etv4, Etv5* and *Sox10)* while ductal gene expression is reduced (*Krt7* and *Krt19*; **Fig.4C**). Together, these data strongly suggest that acinar formation and regeneration is dependent on parasympathetic innervation via Nrg1-III/ErbB3 and ACh/M1 signaling pathways. We aim to dissect the requirement of Nrg1-III and ACh in acinar formation and regeneration, the mutual regulation of these neuronal pathways, and the downstream mediators involved.

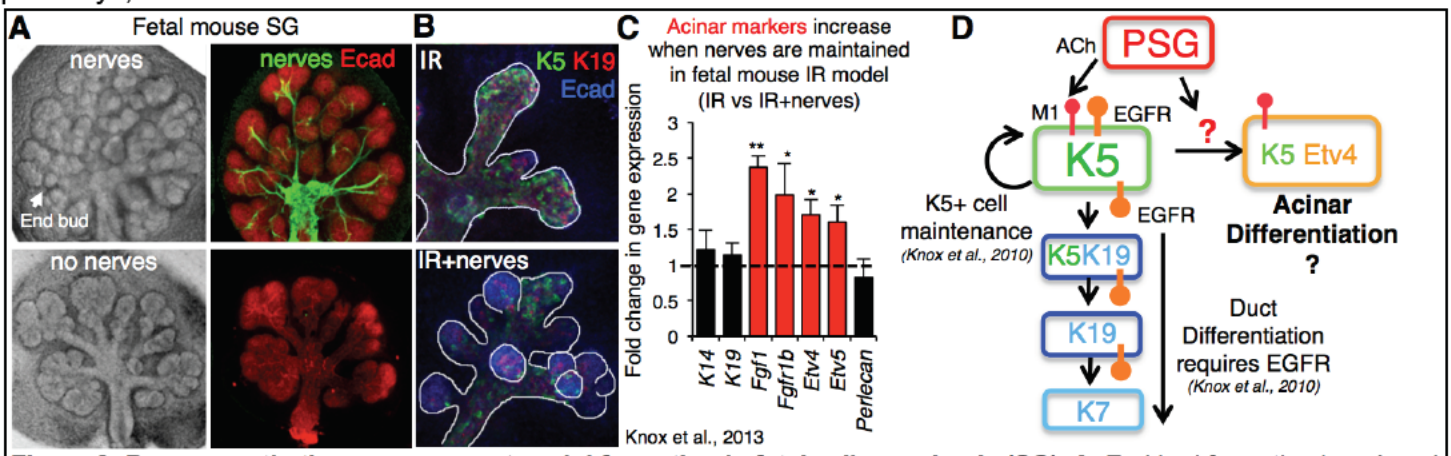


Figure 3: Parasympathetic nerves promote acini formation in fetal salivary glands (SG). A. End bud formation is reduced in fetal mouse SGs cultured without nerves for 48h. **B and C.** Maintaining innervation of the epithelium increases end bud formation (B) and expression of genes enriched in end buds (C, red bars). **D.** Diagram showing differentiation of K5+ progenitors to duct and acinar cells. PSG = parasympathetic ganglion; IR = irradiation; K = keratin; Ecad = Ecadherin

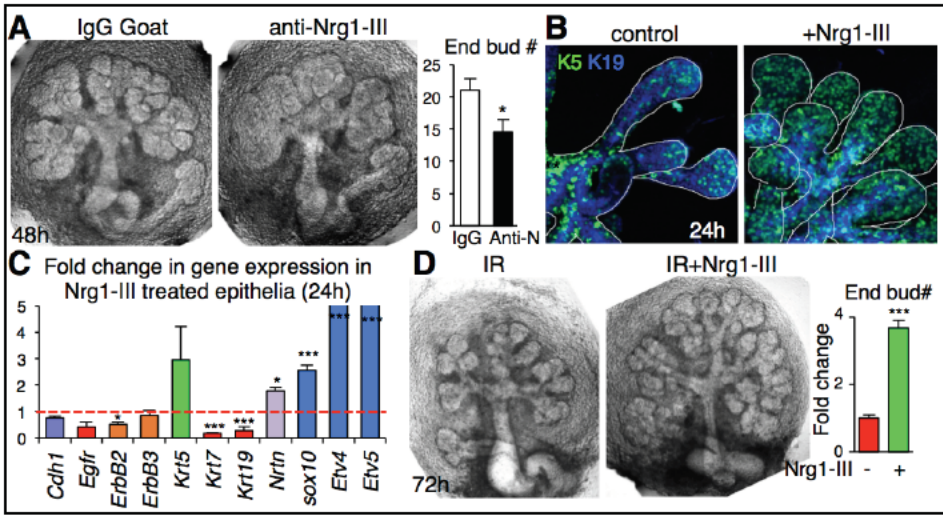


Figure 4. Nrg1-III increases epithelial end bud formation during development and after injury and regulates K5+ cells. A. Blocking Nrg1-III function reduces epithelial end bud (pre-acinar) formation. Embryonic day 12 (E12) SG were treated with Nrg1-III function blocking antibody for 48h. **B-C.** Exogenous Nrg1-III increases end buds and K5+ cells (B) as well as acinar markers (*Sox10, Etv4/5*; C) but reduces duct markers (*Krt19, Krt7*) in isolated epithelial rudiments. **D.** Nrg1-III improves end bud regeneration in IR SGs. IR E13 SG were cultured for 72h, with or without Nrg1-III. Anti-N = anti-Nrg1-III. *<0.05; **<0.01; ***<0.001

ACh positively regulates Sox2+ and K5+ cells in the fetal mouse and human SG. Studies in the SG (4), as well as other epithelial organs (21) indicate that multiple stem cell types are involved in the development, homeostasis and regeneration of a tissue. Sox2 has been identified as a progenitor cell in the fetal SG(6), but exhibits little overlap with K5 (~3% of Sox2+ cells are K5+ (22)), indicating that Sox2+ and K5+ progenitor cells are distinct populations. We therefore determined whether Sox2+ cells were also regulated by parasympathetic innervation. Data in **Figure 5** confirm that Sox2 expression requires neuronal signaling through an ACh/muscarinic receptor pathway in the developing mouse and human SG. Inhibition of ACh/M receptor signaling (4-DAMP) or removal of the nerves results in a dramatic reduction in Sox2 expression in the fetal mouse SG (**Fig.5A** and **B**). Denervation of the extensively innervated human fetal SG (**Fig.5C**) via dissection followed by ex vivo culture also resulted in reduced levels of *SOX2* that was completely rescued with CCh (**Fig. 5D**). Similar to the irradiated human SG (5) (**Fig.2**), *KRT5* expression did not change after denervation but was upregulated 2-fold with CCh (**Fig.5D**). In sum, these results suggest that Sox2+ and K5+ cells (as well as cells expressing CHRM3 and PROM1) are regulated by cholinergic innervation. In this proposal we will extend these studies to determine the influence of parasympathetic function on acinar cell fate during mouse and human SG development and regeneration.

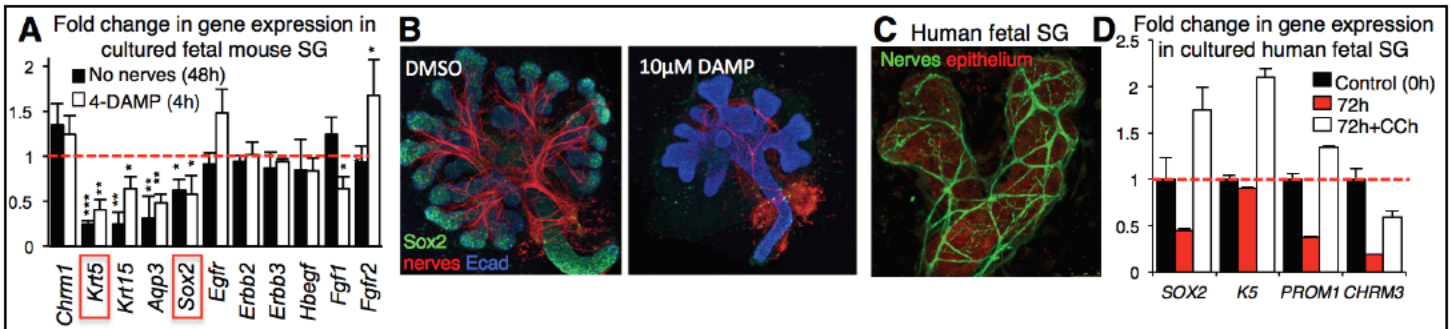


Figure 5: Sox2 expression requires parasympathetic innervation in the fetal mouse and human SG. A and B. Sox2 is reduced in SG after removal of parasympathetic nerves (A) or when cultured with the muscarinic inhibitor 4-DAMP (A and B). Mouse fetal SG was treated with or without 4-DAMP for 4h (A) or 24h (B). **C.** Human fetal SG (16w) is highly innervated. **D.** CCh rescues *SOX2, PROM1 and CHRM3* expression and increases expression of *KRT5* in human SG explant cultures.

Lineage tracing confirms Sox2+ and K5+ cells are progenitors in the mouse salivary gland.

Lineage tracing via genetic recombination is a powerful tool to identify progeny of a single cell and has been used to confirm the identity of putative progenitor cells in many adult organ systems. However, surprisingly few studies have been performed in the SG. Recent lineage-tracing studies demonstrated that Sox2+ and K5+ cells are progenitors in the fetal SG (5, 6) and that Sox2+ cells are also progenitors in the adult SG during homeostasis (6). We confirmed that Sox2 labeled progenitor cells in the fetal SG by genetically labeling Sox2-expressing cells at E9-11 with a tamoxifen-inducible *Cre-ER^{T2}/LoxP*-based tracing system (6) (**Fig.6A**). In this assay, all progeny of Sox2+ cells will be green (Sox2$^{Cre\text{-}ERT2GFP;}$ **Fig.6A**). At P1, ~50% of the tissue was GFP+ with Sox2$^{Cre\text{-}ERT2GFP}$ cells contributing to all ductal, myoepithelial and acinar cell lineages. Only rare GFP+ cells expressed K5, confirming that Sox2 and K5 mark distinct cell populations ((22) data not shown). In contrast, lineage tracing in the post-natal (differentiated) SG, revealed Sox2$^{Cre\text{-}ERT2GFP}$ cells in the acini (serous and mucous) and intercalated ducts, but not in the larger ducts or myoepithelial cells (**Fig.6B**). These results indicate that Sox2+ progenitors in the differentiated SG are lineage-restricted. Consistent with this compartmental distribution of Sox2$^{Cre\text{-}ERT2GFP}$ cells, we observed endogenous Sox2 in the adult to be expressed predominately by acinar cells and to a lesser extent by intercalated ducts. Only a subset of these Sox2+ cells was mitotically active (Ki67+; **Fig.6D**). Here we propose to determine whether the Sox2+Ki67+ cells give rise to all epithelial lineages in the adult SG.

Although K5+ cells have also been confirmed as progenitors in the fetal SG (5) and in other adult epithelial tissues during repair and homeostasis (23), they have not been confirmed as such in the adult SG. In support of Sox2 and K5 marking discrete progenitor populations, endogenous K5 expression in the adult SG is restricted to the ducts (intercalated, striated and excretory, **Fig.6C**) and

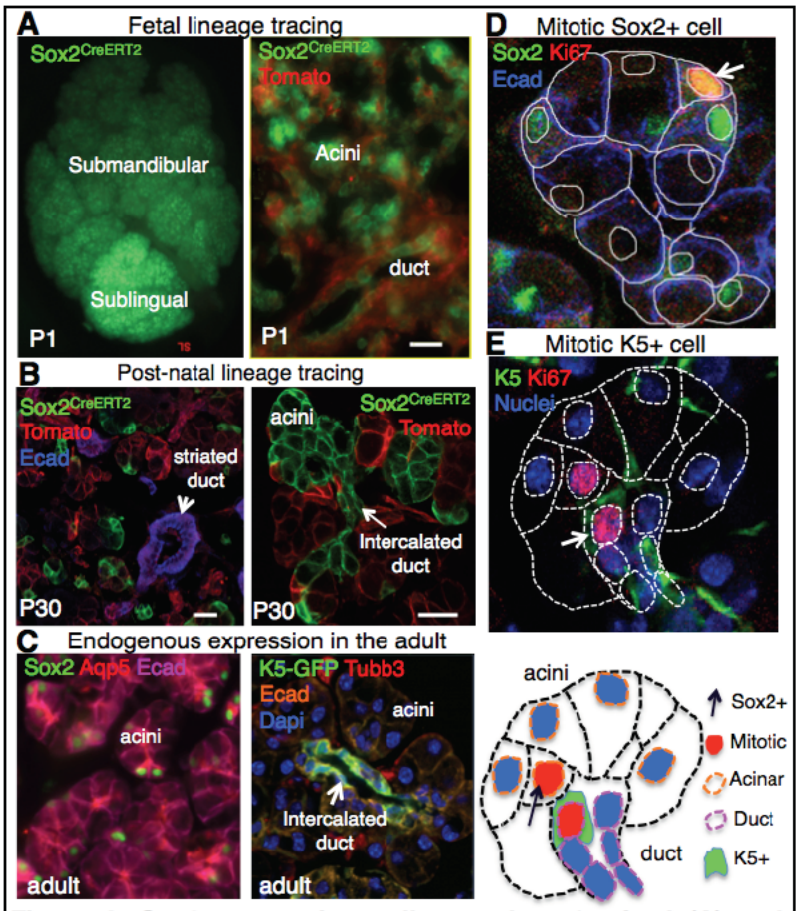


Figure 6. Sox2+ progenitor cells populate the fetal (A) and post-natal SG (B), in the adult SG Sox2 is expressed by acini and duct cells and K5 by ducts only (C) and a subset of Sox2+ (D) and K5+ (E) cells are mitotically active. A-B. Lineage tracing reveals Sox2+ cells contribute to acini and ducts during fetal (A) and post-natal (B) development. Cre-recombinase under the control of the Sox2 promoter was activated at embryonic day (E) 9-11 (A) and post-natal day 2 (P2; B). **C.** In the adult Sox2+ cells are located in the acini and ducts whereas K5+ cells are restricted to ducts. **D-E.** Sox2+ cells and K5+ duct cells are mitotically active (Ki67+, arrows). Cartoon depicts cellular compartments and mitotic cells in acini (Sox2+) and intercalated duct (K5+).

myoepithelial cells (5). We observed rare mitotic K5+ cells in the intercalated ducts (hypothesized as the primary location of salivary stem cells), suggesting that these cells also contribute to the adult SG (**Fig.6E**). In this proposal we will use genetic lineage tracing to confirm K5+ cells are progenitors in the adult and to determine the contribution of K5+ and Sox2+ cells to adult epithelial lineages during homeostasis and repair.

Sox2+ and K5+ cells respond to injury in the adult mouse SG. Rodent SGs can regenerate to some extent after mechanical or IR-induced injury (11-16) with identifiable mitotic cells in acini and intercalated ducts (13, 24). We postulated that their ability to regenerate was due to maintenance of parasympathetic innervation of progenitor cells. Thus, we established two in vivo injury models to evaluate the effects of SG damage on K5+ and Sox2+ cells and innervation. The first model is based on IR-induced damage where SGs are irradiated and acini (*but not the ducts*) undergo cell death followed by regeneration after 3 days (14). In our initial study we examined Sox2+ and K5+ cells in **wild type** C57BL/6 mice 7 days after 5 or 15 Gy (fractionated 5Gy/day/x3; the following results are representative of 15Gy but 5Gy produced similar outcomes). Although the functional markers amylase (*Amy1*) and *Smgc* were reduced at 7 days post-IR compared to the non-IR control (**Fig.7A)**, aquaporin-5 (*Aqp5),* an essential water channel located in acini, was unchanged and expression of the acini marker Sox10 (25) was substantially upregulated (2.6-fold). Furthermore, the number of acinar cells themselves (marked by Ecadherin; **Fig.7B**) appeared similar to control, indicating regeneration and/or repair had occurred. Importantly, in contrast to the IR human SG (**Fig.2**), innervation at 7 days post-IR was similar to the control, with little transcriptional change in neuronal markers *Tubb3* and *Ret* (**Fig.7B**) or GFRa2 protein (parasympathetic marker; data not shown). Lastly, there was a striking increase in Sox2+ and K5+ cells in IR SGs compared to controls (**Fig.7B**, green cells). Together, our results suggest that these putative progenitor cells could repopulate the tissue after IR-induced injury in response to signals from parasympathetic nerves.

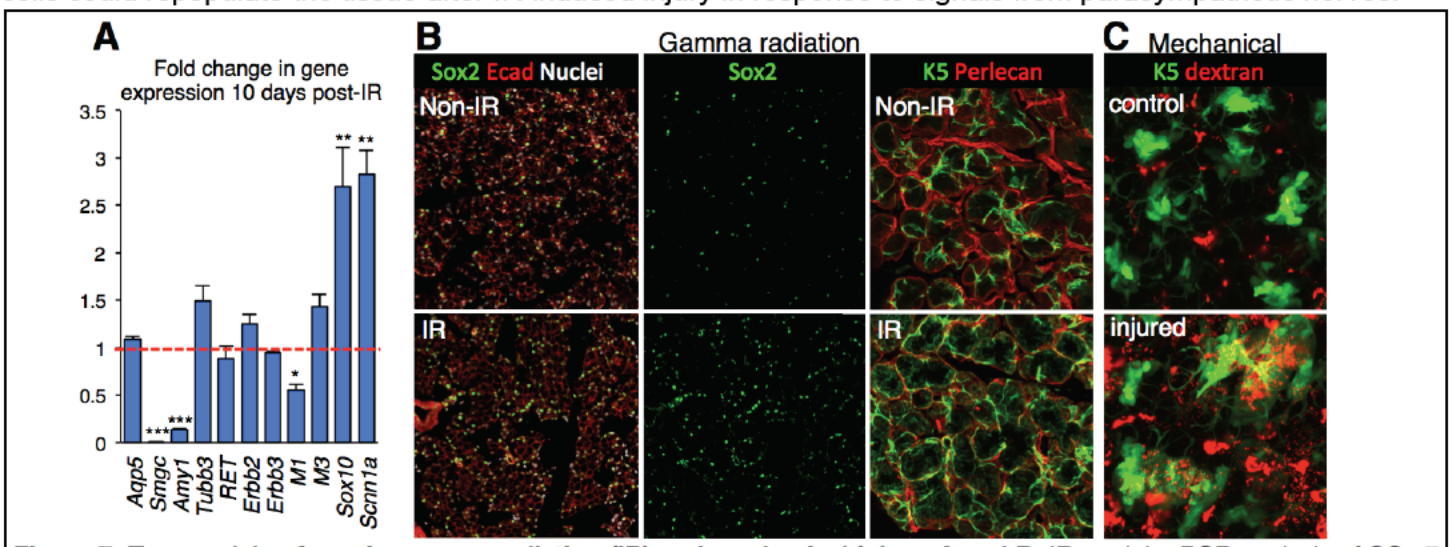


Figure 7: Two models of repair: gamma radiation (IR) and mechanical injury. A and B. IR model. qPCR analysis of SGs 7 days after 15Gy (5Gy/day) shows functional markers, amylase (Amy1) and *Smgc* were reduced (A). However, the water channel *Aqp5,* neuronal markers *Tubb3* and *Ret*, and the EGF receptors *ErbB2/ErbB3* were similar to control and the acini and duct markers, *Sox10* and *Scnn1a,* respectively, were upregulated, suggesting repair/regeneration had occurred. (B) Sox2+ and K5+ cells (green) are abundant 7 days after IR, indicating these cells respond to damage. **C**. Mechanical model. K5-GFP cells in the submandibular gland appear more numerous in the ductal regions 24h after injury. dextran = dextran-texas red (10kDa).

To analyze the effects of damage on epithelia and ductal tissue directly, we developed a second damage model system that involves mechanical injury to the ductal network (model was co-developed with Dr. Roberto Weigert, NIDCR, see letter of support). Briefly, the opening of the submandibular duct is cannulated (26) and tissue damage is induced by continuous injection of high molecular weight fluorescent dextran (>1ml/min); the contralateral submandibular gland remains intact and acts as an internal control. Importantly, innervation is not perturbed by injury (data not shown). We first examined the reorganization of K5+ cells in response to injury (10kD dextran, red) using a **K5-GFP** transgenic mouse 24h after injury. Similar to the IR model, K5-GFP cells were more numerous and accumulated within the ductal regions of the injured gland (**Fig.7C**), suggesting K5+ cells reorganized and/or proliferated in response to damage. We now seek to determine the contribution of K5+ and Sox2+ cells to the repair/regeneration of damaged salivary tissue, as well as the epithelial lineages they form, using genetic lineage tracing in these two model systems. In addition, we aim to determine how progenitor cell fate is regulated by neuronal signals in the adult SG.

Isolation and characterization of fetal human epithelial cells.

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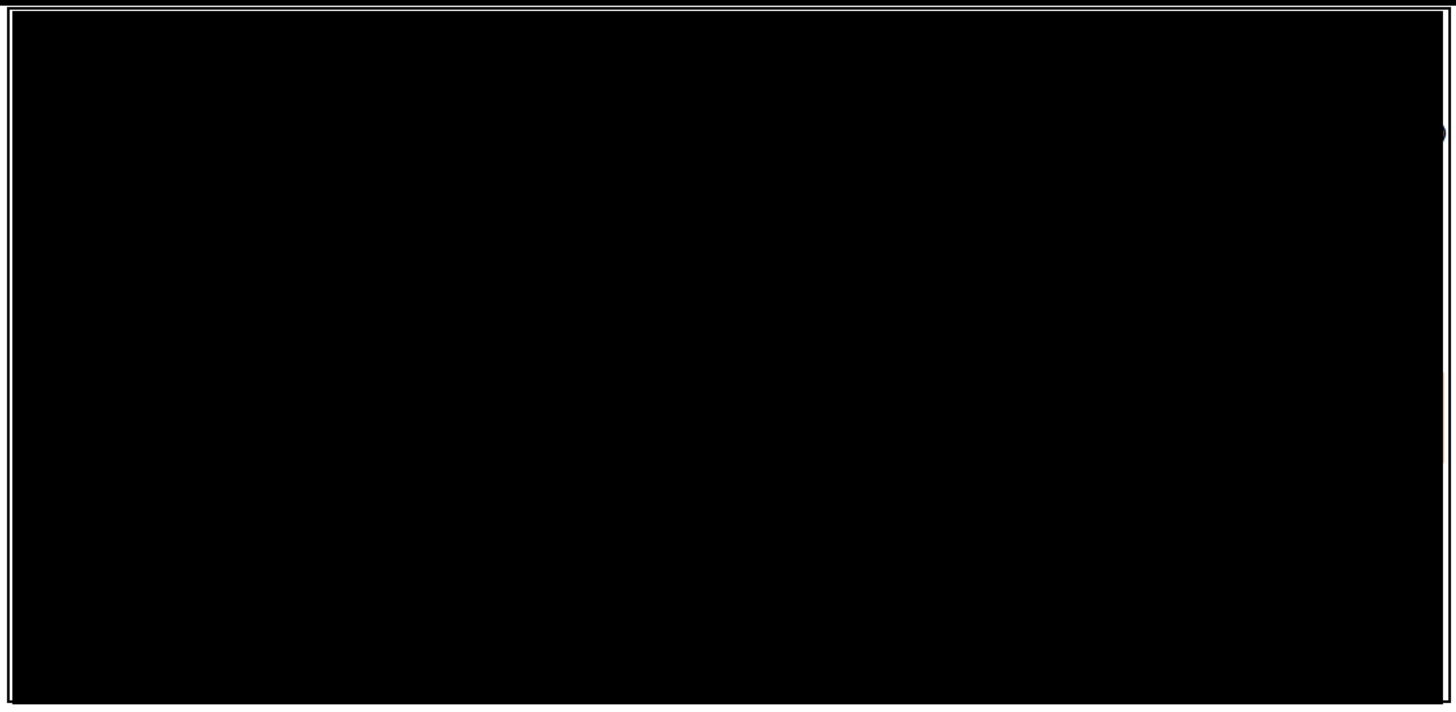

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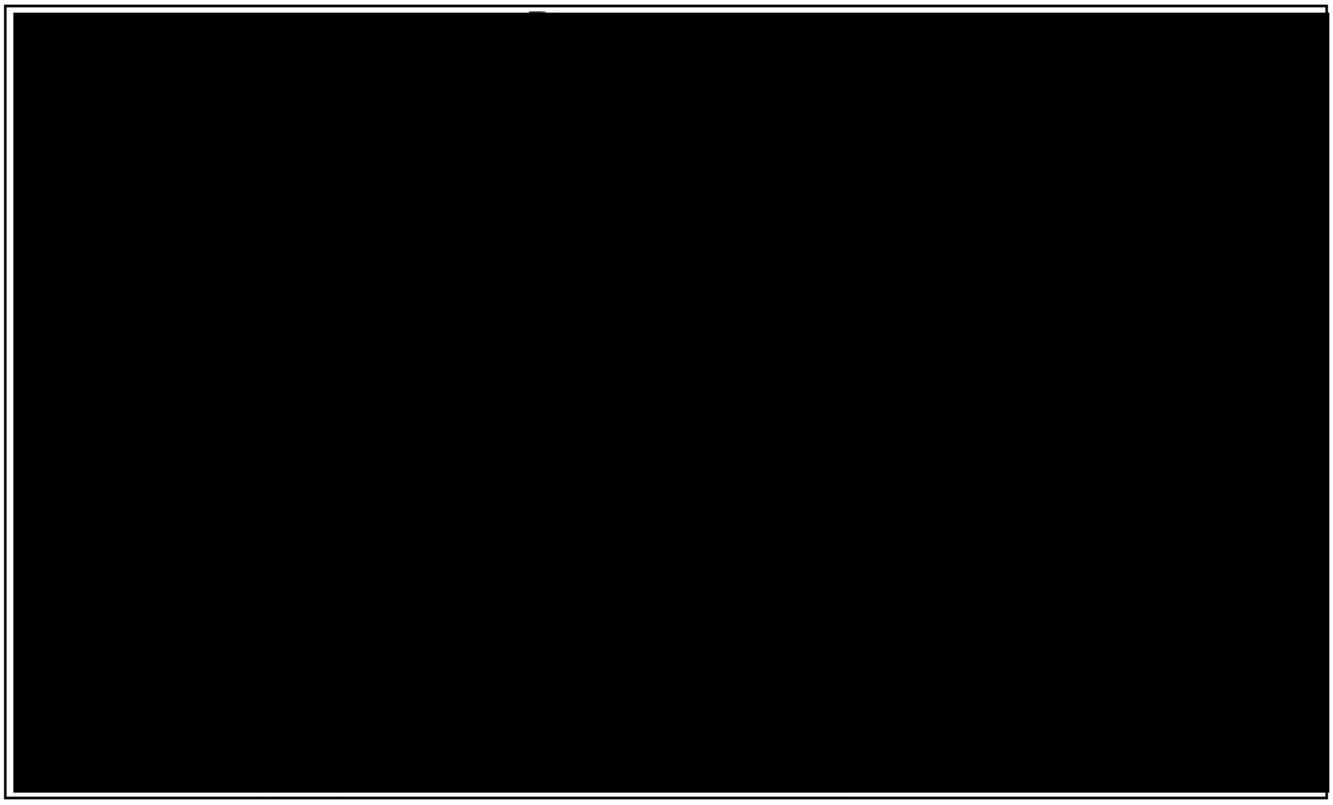
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Research Design

Specific Aim1: Define the mechanisms by which parasympathetic nerves regulate acinar cell fate

Rationale: How progenitor cells establish secretory acinar cells during development and after injury is not known. Previous reports by our group and others in the field strongly support a role for parasympathetic nerves in the generation and regeneration of acini in the fetal and adult mouse (8, 17). Our preliminary data show that ACh/M1 and Nrg1-III/ErbB3 signaling increases end bud formation during development and after injury, possibly by directly regulating Sox2+ and K5+ progenitor cell populations (**Fig.4** and **5**). Our current objective is to determine the influence of ACh and Nrg1-III on K5+ and Sox2+ progenitor cell proliferation and differentiation as well as the downstream signaling pathways promoting the acinar cell fate (**Fig.10**). Our strategy to investigate these processes using the fetal SG is based on the rationale that regenerative healing mechanisms in the SG, as well as other tissues, recapitulate those of organ development (8, 16, 28, 29).

Aim1A: Define the effects of Nrg1-III and ACh on K5+ and Sox2+ progenitor cell proliferation and differentiation in fetal salivary glands

Here *we aim to determine* whether ACh/M1 and/or Nrg1-III/ErbB3 signaling regulates K5+ and Sox2+ progenitor cell proliferation and differentiation using gain- and loss-of-function assays (**Fig.10**).

First, *we will test the prediction that ACh and Nrg1-III activate their respective receptors on Sox2+ and K5+ cells*. In our prior studies we show that K5+ cells express M1 and EGFR, suggesting ACh directly regulates these cells (5). Although M1 and ErbB3 are enriched in acini (17, 20), along with Sox2 (**Fig.6C**), it is not known if Sox2+ cells also express M1 and EGFR or if K5+ and Sox2+ cells express ErbB2/ErbB3. We will first analyze expression of these receptors on isolated Sox2-GFP and K5-GFP cells (mouse SG) using FACS,

confocal microscopy and qPCR (as in ref (5)) followed by testing receptor activation upon stimulation. If our prediction is correct, then isolated Sox2+ and K5+ cells will express the appropriate receptors and respond accordingly to CCh and Nrg1-III by upregulating Egr1 (transcription factor downstream of M1) or by increasing phosphorylation of ErbB2, as quantified by western blot or qPCR at defined time points (0-60 min).

Second, we will *determine the effects of ACh/M1 and Nrg1-III/ErbB3 signaling on Sox2+ and K5+ cell proliferation and differentiation*. We predict that in the absence of neuronal signaling, progenitor cell (Sox2+ and K5+) proliferation will be reduced, acinar differentiation will decrease and ductal differentiation will increase. In contrast, addition of CCh or Nrg1-III to epithelial rudiments will increase progenitor cell proliferation and acinar differentiation but decrease ductal differentiation. In these experiments fetal mouse SGs/epithelial rudiments (**Fig.4, 5**) or human SGs (**Fig.5**) will be treated with either ACh/M1 or Nrg1-III signaling inhibitors or with CCh or Nrg1-III for 2-48h (mouse) or 1-8 days (human). Proliferation, acinar and ductal markers will be quantified by qPCR and/or confocal microscopy (**Fig.10**)

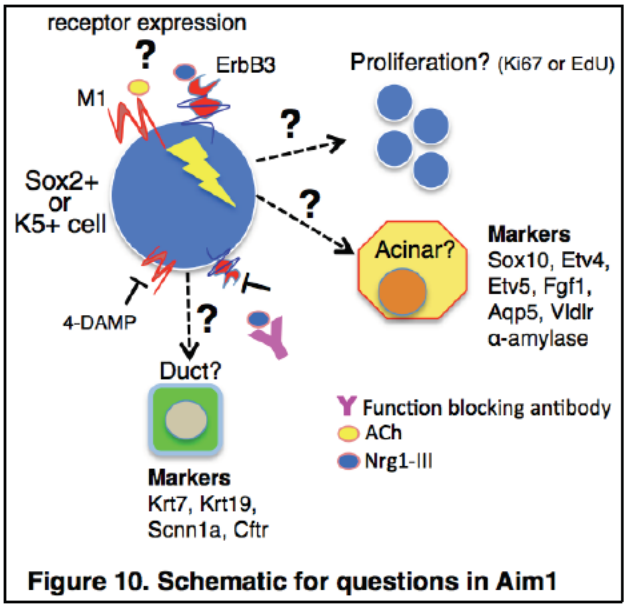

Figure 10. Schematic for questions in Aim1

Third, we *will test the prediction that ACh and Nrg1-III promote Sox2+ and K5+ cell proliferation in the injured SG*. This will inform us as to whether these cells are capable of responding to neuronal signals after damage. Fetal mouse (K5-GFP or Sox2-GFP) or human SG explants will be irradiated and cultured with neuronal factors for 24-72h(8). As a positive control, nerves (which are damaged after IR) will be maintained with the neurotrophic survival factor neurturin(8). Progenitor cell proliferation, apoptosis (activated caspase3) and epithelial differentiation (**Fig.10**) will be quantified as above. If progenitor cells respond to ACh and/or Nrg1-III after IR, then Sox2+ and K5+ cell proliferation and acini formation will increase.

Fourth, *we will determine whether ACh/M1 and Nrg1-III/ErbB3 signaling direct Sox2+ and K5+ progenitor cells towards an acinar cell fate during SG development and after injury using ex vivo genetic lineage tracing.* These experiments will also inform us of the contribution of Sox2+ and K5+ cells to fetal SG development. Genetic recombination of a subset of progenitors will be induced in vivo in Sox2CreER^{T2}- or K5CreER^{T2}; R26R-GFP after the initial bud has formed (E12.5). Lineage specification of progenitor cells (GFP+ progeny) will be traced in intact E13 SGs or IR SGs (± antagonists or CCh/Nrg1-IIIm, **Fig.4** and **5**) cultured for 1-3 days. We predict that newly formed end buds (or cells repopulating damaged end buds) will be GFP+. In contrast, in the absence of Nrg1-III or ACh/M1 signaling, if new end buds form they will be GFP-negative, and therefore derived from Sox2- and K5-negative progenitor cells (to be characterized in subsequent studies).

If our hypothesis is correct, ACh/M1 and Nrg1-III/ErbB3 will directly regulate Sox2+ and K5+ cells and cells will proliferate and differentiate towards an acinar cell fate during development and after injury.

Aim1B: Elucidate the signaling mechanisms downstream of ACh and Nrg1-III that regulate K5+ and Sox2+ cells and promote acinar formation

Our preliminary data show that ACh/M1 and Nrg1-III/ErbB3 signaling are required for K5 and Sox2 expression and increase acinar formation during development and after IR-induced injury (**Fig.4** and **5**). Here *we aim to identify the signaling events downstream of M1/EGFR and ErbB2/3 that regulate Sox2+ and K5+ cell proliferation and acinar specification*.

First, *we will define the signaling pathways downstream of ACh/M1/EGFR and Nrg1-III/ErbB2/3 that regulate K5+ and Sox2+ proliferation and promote acinar formation*. We predict that inhibition of pathways mediating the effects of Nrg1-III and ACh will reduce Sox2 and K5 expression, decrease Sox2+ and K5+ cell proliferation and acinar formation, and increase duct differentiation. To test this, human or mouse SG epithelial rudiments will be cultured with Nrg1-III or CCh and commercially available pharmacological inhibitors of pathway components downstream of M1, EGFR and ErbB2/3 (e.g. PI3K/Akt, MAPK). Expression of Sox2 and K5 transcripts and protein will be measured via qPCR and western blot (at 0-6h) and proliferation of K5+ and Sox2+ cells via EdU uptake (4-96h). Acinar differentiation will be quantified morphometrically (e.g. end bud number) and by analysis of epithelial lineage markers (**Fig.10**).

Second, *we will confirm candidate pathways are activated in K5+ and Sox2+ cells by assessing phosphorylation of corresponding signaling molecules in response to Nrg1-III or ACh.* Sox2-GFP or K5-GFP

cells (mouse SG) will be cultured ± CCh or Nrg1-III (and/or inhibitors) for 0-120 min and cell extracts subjected to western blot or phosphoprotein immunoassay (Cell Signaling). Changes in phosphorylation will be quantified by fluorescent readout captured by a Li-Cor Biosciences Odyssey imaging system (UCSF, shared equipment). We predict one or more of the signaling molecules in the candidate pathways identified above will be phosphorylated. If none of these molecules are phosphorylated, we will identify phosphorylated signaling proteins using an intracellular phosphoprotein array (Pathscan antibody array, Cell Signaling).

Thus, Aim1B will define candidate signaling pathways/molecules downstream of M1 and ErbB3 that modulate Sox2+ and K5+ cell behavior. These will be confirmed by treating IR fetal mouse SGs with pharmacological antagonists/agonists in the presence or absence of CCh or Nrg1-III. If pharmacological reagents are not available for candidate targets, we will activate and/or inhibit other members of the signaling cascade using available reagents or knockdown expression using small interfering RNA (siRNA, (5)).

Expected Results, Potential Problems and Alternative Approaches: Based on our preliminary studies, we anticipate that Sox2+ and K5+ cells are directly regulated by ACh/M1 and Nrg1-II/ErbB3 signaling resulting in proliferation and differentiation towards an acinar cell fate. We will have also identified signaling cascades controlling neuronal-mediated cell fate decisions of which the components will be analyzed further using in vivo assays. Given our published work and preliminary findings described herein, we do not anticipate any technical difficulties in performing these studies. One possible caveat in **Aim1A** is that innervation may be negatively affected by the Nrg1-III function-blocking antibody, thus reducing both Nrg1-III and ACh signaling. Therefore, we will confirm these results by knocking down ErbB3, the relevant receptor, in the epithelium using siRNA (5) (Dharmacon Inc.). The parasympathetic ganglion does not express ErbB3, such that knockdown of ErbB3 will not directly affect the nerves. Another approach would be investigating loss of Nrg1-III/ErbB3 signaling using the Nrg1-III deficient mouse (James Salzer, NYU). In **Aim1A** it is also conceivable that Sox2+ and K5+ cells do not express ErbB3 and that the effects of Nrg1-III on acinar formation are mediated indirectly via another cell type. To determine if Sox2(-) or K5(-) cells form acinar cells in response to Nrg1-III, we will isolate ErbB3(+)GFP(-) epithelial cells from Sox2-GFP and K5-GFP SGs and culture with Nrg1-III. If acinar markers are apparent, this will indicate another progenitor population (such as Ascl3 (4)) is regulated by Nrg1-III and this population will be characterized further in subsequent studies. A potential caveat for **Aim1B** is that inhibitors may affect other pathways (or cells) required for epithelial morphogenesis resulting in false positives (i.e. reduced acinar formation). One alternative approach is to culture salispheres from Sox2-GFP and K5-GFP cells in 3D matrix (differentiation assay, **Fig.9**), in the presence of CCh or Nrg1-III, and assess the effects of pharmacological inhibitors on cell proliferation and acinar differentiation.

Specific Aim 2: Determine the contribution of salivary progenitor cells and neuronal signaling to adult homeostasis and repair

Rationale: The identity of progenitor cells that contribute to epithelial lineages of the adult SG during homeostasis, repair and/or regeneration, as well as mechanisms that regulate their behavior, is not known. Based on our observations that Sox2+ and K5+ cells are mitotically active and are differentially expressed in the epithelium of the adult SG (**Fig.6**), we hypothesize that these cells are discrete populations that contribute to multiple epithelial lineages of the mouse adult SG during homeostasis and after injury. To date, only Sox2+ cells have been confirmed as progenitors in the adult tissue (6), but characterization of the cell lineages derived from these cells was not performed. Furthermore, it is not known if Sox2+ cells contribute to adult tissue after injury or if Sox2+ and K5+ cells are regulated by neuronal signals as in the fetal system (**Fig.5**). Here we will use genetic lineage tracing to confirm K5+ cells are progenitors in the adult mouse SG and to determine the contribution of Sox2+ and K5+ cells to SG epithelial lineages during conditions of homeostasis and injury. We also seek to define the effects of the neuronal signaling molecules Nrg1-III and ACh on the proliferation and differentiation of adult Sox2+ and K5+ cells, with the expectation that they will respond in a similar fashion as fetal Sox2+ and K5+ cells. These experiments will significantly enhance our understanding of how progenitor cells contribute to salivary tissue and the mechanisms that govern their behavior.

Aim2A: Determine the contribution of Sox2+ and K5+ progenitor cells to epithelial cell lineages during homeostasis and repair in the adult SG

We will *test the prediction that K5+ and Sox2+ cells contribute to multiple epithelial lineages of the adult SG during homeostasis and after injury*. To this end, we will perform lineage tracing in adult mice using Sox2CreER^{T2} and K5CreER^{T2} and the R26R-GFP reporter, as in **Figure 6**. Cre-mediated recombination will be induced at 1, 2 and 6m of age. This age range was chosen based on previous reports that younger adults have

increased mitotic activity compared to older animals (6). Mice will be sacrificed 24h after treatment ("pulse" to establish the number of single cells activated) and after 1, 2, 3, 6, 12 and 18 months post-tamoxifen injection ("chase"). For repair models, Cre-mediated recombination will be induced one day prior to damage in 2m old adults to establish GFP-labeled single cells. For the IR model (**Fig.7A**), mice will be irradiated with 5 or 15Gy (5Gy/day x3 doses) and the SGs removed for analysis at 1-90 days post-IR by confocal microscopy and FACS (as described above). For the mechanical injury model (**Fig.7B**), dextran (Texas Red or Alexa 647; 10kDa) will be injected into the cannulated duct and cell fate determined at defined time points 1-30 days post-injection. If $Sox2^{Cre\text{-}ERT2GFP}$ or $K5^{Cre\text{-}ERT2GFP}$ cells contribute to all epithelial lineages under homeostasis and repair conditions, then we will observe GFP+ acini (mucous, serous), ducts (intercalated, striated, excretory) and myoepithelial cells. All SG will be analyzed by confocal microscopy using appropriate lineage markers or morphological identifiers (e.g. nuclei shape; **Fig. 1B** and **10**). Thus, Aim2A will establish Sox2+ and K5+ cells as progenitors in the adult SG and define their contribution to epithelial lineages during homeostasis and injury.

Aim2B: Define the effects of Nrg1-III and ACh on Sox2+ and K5+ cells in the adult SG

We previously showed that ACh/M1 signaling is required for the self-renewal of K5+ cells in the fetal SG(5). We also showed that K5+ cells in adult SG increase in response to CCh (5). Now *we will determine whether ACh or Nrg1-III modulate K5+ and Sox2+ cell self-renewal in the adult SG using the salisphere culture* **(Fig.9).** In this culture system, EGF is required for progenitor cell proliferation and subsequent colony formation. Therefore, we will test whether CCh or Nrg1-III can substitute for EGF. Adult Sox2-GFP and K5-GFP cells will be isolated by FACS and cultured as salispheres ± Nrg1-III, ACh or EGF and salisphere size, proliferation and Sox2 and K5 expression quantified as in Aim1A. If ACh and Nrg1-III promote Sox2+ and K5+ cell self-renewal, GFP+ cells will proliferate to form salispheres.

Next we *will test the prediction that Nrg1-III and ACh direct adult Sox2+ and K5+ towards an acinar cell fate* using the salisphere differentiation assay (3D matrix, **Fig.9**) and ex vivo lineage tracing. K5-GFP or Sox2-GFP salispheres will be treated ± Nrg1-III or CCh and changes in acini, duct and myoepithelial markers quantified (as in Aim2A). If ACh and/or Nrg1-III promote the acinar cell fate, acini markers will increase and ductal and myoepithelial markers will decrease. In the adult ex vivo lineage tracing assay, SGs are dissected (1mm pieces) from $Sox2CreER^{T2}$- or $K5CreER^{T2}$; R26R-GFP one day after induction (to establish labeled single cells) and cultured ± CCh or Nrg1-III for 1-4 days(5). Lineages derived from $Sox2CreER^{T2}$ and $K5CreER^{T2}$ cells will be analyzed by morphological appearance as well as by immunofluorescent detection of epithelial lineage markers (as in Aim2A, **Fig.10**). If our prediction is correct then Nrg1-III and ACh signaling will increase the number of GFP+ (progeny of Sox2+/K5+ cells) acinar cells.

Expected Results, Potential Problems and Alternative Approaches: Based on our studies to date, we anticipate that Sox2+ and K5+ cells in the adult SG will give rise to multiple epithelial lineages but non-overlapping populations during homeostasis and after injury. We also expect adult K5+ and Sox2+ cells will respond to neuronal signals to self-renew as well as to form acinar cells (similar to the fetal SG). Subsequent studies will elucidate the role of neuronal signals in cell fate decisions by manipulating neuronal input in vivo (e.g. denervation, anti-muscarinics). Given the robust features of these transgenic animals, and our preliminary findings, we do not anticipate any difficulties in generating data and performing the proposed analysis. A possible caveat for **Aim2A** is that tissue damage may result in Sox2+ and K5+ cells becoming inadvertently lineage restricted. For example, the environmental changes occurring due to IR-induced damage may alter cues necessary for correct cell fate specification. If all possible lineages are not forthcoming from either Sox2+ or K5+ cells, we will isolate $K5^{CreERT2\text{-}GFP}$ and $Sox2^{CreERT2\text{-}GFP}$ cells after induction via FACS and transplant into the SGs of IR and non-IR treated immune-compromised ($CB.17^{SCID/SCID}$) mice (see letter of support, Dr. Isabelle Lombaert, NIDCR (2)). A second model system we will use to counter the same issue is the duct ligation model. In this model the duct is ligated, resulting in extensive acinar atrophy, which is reversed upon de-ligation (see letter of support, Dr. Gordon Proctor, Kings College (30)). It is also conceivable that the 3D matrix environment (which contains growth factors) may inadvertently promote one lineage over another despite the presence of CCh and Nrg1-III, resulting in false positive or negatives (**Aim2B**). Such an outcome would be countered by utilizing different 3D matrices that have altered levels of endogenous growth factors (e.g. laminin, growth factor reduced matrix etc.)

Specific Aim 3: Identify human epithelial progenitor cells in fetal and adult salivary gland

Rationale: [redacted]

[redacted]

Expected Results, Potential Problems and Alternative Approaches: [redacted]

Future Studies: [redacted]

Time Table: We plan to carry out the proposed experiments following the table below.

Specific Aims		Year1	Year2	Year3	Year4	Year5
Specifc Aim1: Mechanisms regulating acinar cell fate using fetal SG	**Aim1A**					
	Aim1B					
Specific Aim2: Adult SG lineage tracing during homeostasis and repair	**Aim2A**					
	Aim2B					
Specific Aim3: Identify human progenitors	**Aim3A**					
	Aim3B					

Protection of Human Subjects

This application proposes to study cells/tissues isolated from adult human biopsies and aborted fetal tissues. **This Human Subjects Research falls under Exemption 4** as we propose to study specimens without subject identifiers. There will be no intervention or interaction with the human subjects by the PI; and no identifiable private information associated with the samples. Privacy to research participants is achieved by the tissue being provided to the PI as either anonymized, de-identified, or as a limited data set (e.g. age, diagnosis, ethinicity, prior treatment with radiotherapy/chemotherapy) with no identifiable coding. The research is also not regulated by the FDA. The samples are obtained from Dr. Marcus Muench (IRB: 10-00768) and Dr. Dieter Gruenert (IRB #: 11-07428). The P.I. is an additional investigator on both approved IRBs. These IRBs state that the P.I.s involved will never be provided with donor identities or other identifying information. This proposal therefore meets the requirements under Exemption 4 of the 45 CFR part 46 (http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.html). As such, the NIH policies for inclusion of women, minorities and children in clinical research, and targeted/planned enrollment tables, do not apply to this research project, as covered by Exemption 4.

Vertebrate Animals

ANIMAL REQUIREMENTS AND RATIONALE FOR ANIMAL USE:

1) No adequate non-animal models exist for analyzing the molecules involved in the processes of salivary gland formation, repair and regeneration. Cell lines lack most of the architectural and regulatory features of normal embryonic development, particularly of normal morphogenesis (the development of tissue form and organization). Furthermore, homeostasis (maintenance of tissue under normal conditions) and repair of structure cannot be easily analyzed. The salivary gland contains multiple cell types including epithelial, mesenchymal, neuronal and endothelial. The processes of salivary gland formation, (e.g. branching morphogenesis and differentiation to form connected ducts and acini) and regeneration/repair involves the interactions of multiple cell types and thus has no adequate cell line models. It is hoped that this work might eventually lead to enough understanding of these highly complex processes that cell culture models may become more practical in the future.
2) The mouse is the optimal species for these studies because it has developmental and regenerative processes similar to those of humans. It is the currently preferred system for studying the extremely complex processes of mammalian craniofacial development and craniofacial anomalies. The mouse is also the organism of choice because the mouse genome is well-characterized, because mouse salivary gland culture has proven to be a powerful and efficient system for analyzing mechanisms of development, and because a number of craniofacial and other developmental mutants exist in mouse so that findings can be related to a larger existing literature on this topic than for any other non-human species. It is also the only species in which transgenic and gene substitution and induction strategies could be contemplated in the near future to mimic or to test strategies to alleviate developmental defects and regulate regeneration.
3) The number of mice is the minimum number of animals possible to obtain replication of results and to adequately measure the regulation of gland development and regeneration after irradiation or mechanical damage.

TOTAL number of adult mice to be used in this proposal is 1433. This number is derived as follows:

Timed Pregnant mice: **Total number of timed pregnant female mice to be used in the embryonic studies in Aim1A and B is 743.** Mice will be bred in the animal facility at UCSF. Each experiment (requires 2-3 pregnant mice due to natural variation in the developmental stages of the embryos obtained even from a single mouse and animal-to-animal variation. Specifically, the use of 6 salivary glands at the same stage per data point in comparative studies is the minimum number necessary to achieve sufficient power to perform statistical analyses. There are two salivary glands per embryo, and 8-10 embryos per mouse, with 10% that are usually not used due to variation in stage (i.e. too young or too old).

The table below describes all the experiments in this study using timed pregnant females and justifies the number of mice utilized.

Adult mice: Aim2 and 3 use adult mice rather than embryos. **The total number of adult mice that will be utilized in Aim2 and 3 is 690**.

For Aim2A, the total number of mice to be used is 598. For the lineage tracing studies we have generated Krt5CreERT2;R26R-mTmG and Sox2CreERT2;R26R-mTmG. For analysis of progenitor populations under homeostatic conditions we will require 210 mice (5 mice x 3 ages for Cre-activation x 7 end points x 2 genotypes). This will be matched with 210 Cre-negative control animals (total = 420). The number of transgenic mice that will be irradiated in Aim2A is 64 (8 mice x 4 end points x 2 genotypes). The total number of non-irradiated control mice will be 64. The number of mice for the irradiation study was chosen to maximize tissue for immunostaining: we will embed 4 SG in OCT and 4 in paraffin and sections probed with ~25 antibodies. For the duct injury model, we will require 50 animals (5 mice x 5 end points x 2 genotypes). Since one of the salivary glands is not cannulated (internal control), no external control mice are required. For all experiments in Aim2A, the numbers of mice were chosen to also provide significant statistical power even in the event that 1-2 mice are removed from each experimental study due to poor health or death.

For Aim2B, the total number of mice to be used is 32. The number of Sox2-GFP+ and K5-GFP+ mice required is 22 (5 mice for ex vivo experiments x 2 genotypes +6 mice for salispheres x 2 genotypes). This number is necessary to provide statistical power for analysis via confocal micrscopy and FACS (FACS requires >1-5x10^6 cells per experiment). In Aim2B, lineage tracing experiments using the ex vivo model in Aim2B will require 10 mice (5 mice x 2 genotypes). This number is the minimum number of mice that will give significant statistical power for analysis by confocal microscopy.

For Aim3 (kidney capsule model), the number of CB.17$^{SCID/SCID}$ mice required is 60. We expect to analyze the differentiation capacity of ~10 human fetal epithelial populations predicted to be progenitors from the in vitro studies. This will therefore require ~10 mice x 5 repeats to reach statistical power necessary for analysis by confocal microscopy, RNAseq and histology. In addition, we will need a further 10 mice in order to provide significant statistical power in the event that 1-2 mice are removed from each study due to poor health or death.

Irradiation of Adult mice: Targeted head and neck irradiation will be performed on 8-week old C57BL/6 mice with a 7.5 Gy dose of γ-radiation using a cesium-137 source (J.L. Shepherd & Associates, San Fernando, CA). The dose regimen has been well characterized and results in salivary gland hypofunction and tissue damage but does not compromise the health and welfare of the animals {Urek, 2005}. The irradiation set up requires that each mouse be anaesthetized (intra peritoneal injection of 0.1mg / g body weight of ketamine and 0.02 mg /g xylazine) and placed onto a piece of styrofoam set on top of a laboratory jack. The mouse's head will rest on a 5 mm thick piece of plastic, and iron shielding (6mm thick) will be used to limit radiation to a narrow, approximately 1 cm vertical beam focused on the animal's neck (submandibular glands). Following irradiation of mice, the animals are placed on warmed cotton to maintain body temperature and monitored for breathing. After regaining consciousness, these animals are returned to the animal facility and at various times following irradiation treatment salivary gland tissues are collected. Mice will be followed and if signs of illness develop (weight loss, decreased activity, paresis), mice will be euthanized.

Our studies have been designed to isolate γ-radiation exposure to the head and neck γ-radiation region only and therefore avoid the severe systemic radiation-induced illness. This level of radiation results in a ~50% decrease in the production of saliva {Limesand, 2006; Kojima, 2011}. We observed general weakness was present within the first week after irradiation; animals were fed wet chow post-irradiation to ensure their wellbeing. The criteria for removing animals from the study include, but are not limited to: veterinary opinion, weight loss more than 15% of body weight, inability to eat or drink adequately and severe reductions in mobility. These parameters will be accessed by the PI at least once/day and consultations with veterinarian staff as necessary.

Ductal cannulation of adult mice: Ductal cannulation will be performed on 8-week old C57BL/6 according to published protocols {Kuriki, 2013}. This method results in the injury to one of the pair of submandibular glands, leaving all other glands intact and therefore does not compromise the health of the animal. The mouse is anaesthetized (intra peritoneal injection of 0.1mg / g body weight of ketamine and 0.02 mg /g xylazine), and laid on a custom made platform in the ventral position with the maxillary incisors hooked on a metal wire. Using a 29-guage needle a polyethylene tube (5mm) is inserted into one of the two ductal openings located beneath the tongue and the dextran-texas red is injected at >1ml/min (0.5mL). After regaining consciousness, these animals are returned to the animal facility and at various times following cannulation salivary gland tissues are collected. Mice will be followed daily and if signs of illness develop (weight loss, decreased activity, paresis), mice will be euthanized.

Kidney capsule experiments (Aim3): Implantation of human cells under the kidney capsule of immune-compromised mice according to published protocols (see ref 33 and 34). Recipient mice are anaesthetized as above, shaved and cleaned. A small incision is made on the left flank of the mouse and the kidney is exposed. The kidney, fat, and tissue are kept moist with normal saline swab. The distal end of a piece of PE50 tubing is attached to a Hamilton screw drive syringe, containing a pipette tip, using silicone adaptor tubing. A small shallow nick is made on the right flank side of the kidney. The beveled end of the PE50 tubing is then carefully placed under the capsule, and cells/salispheres in 3D matrix are slowly delivered behind a small air bubble. Once the islets have been delivered kidney homeostasis is maintained and the knick is cauterized with low

heat. The kidney is placed back into the cavity and the peritoneum and skin are sutured and stapled. Mice are immediately treated with Flunixin and Buprenorphine subcutaneously and placed in a cage on a heating pad. After regaining consciousness, these animals are returned to the animal facility and at various times following engraftment salivary gland tissues are collected. Mice will be followed daily and if signs of illness develop (weight loss, decreased activity, paresis), mice will be euthanized.

TABLE 1

Experimental Aims	Type: embryos (from timed pregnant females) and justification	Minimum # glands	# embryos per data point	#mice
Aim1A	***Confirm*** *the presence of ErbB2, ErbB3, M1 and EGFR on Sox2+ and K5+ cells* . - Experiment: examine the expression of ErbB2/ErbB3 and M1/EGFR on Sox2-GFP and K5-GFP cells using fluorescent activated cell sorting (FACS), confocal microscopy and qPCR: 2 (Sox2GFP or K5-GFP) x (50 SMG for FACS + 20 SMG for confocal+PCR+immunostaining) x 3 repeats = 420	420	210	23
	Determine *the effects of ACh/M1 and Nrg1-III/ErbB3 signaling on Sox2+ and K5+ cell proliferation and differentiation* Experiment: gain and loss-of-function experiments - K5-GFP or Sox2-GFP fetal mouse SGs will be cultured with or without inhibitors or agonists for 2-48h and examined by confocal analysis: 4 times points x 6 SG per plate x 6 treatments x 6 analytical procedures x 3 repeats = 2592	2592	1296	144
	Determine *the effects of ACh and Nrg1-III on Sox2+ and K5+ cells after irradiation* Experiment: Irradiate SG cultures from E13.5 Sox2GFP or K5GFP and treat with CCh, Nrg1-III or both : 2 (genotypes) x 6 SG per plate x 8 treatments (with or without IR) x 3 time points x 3 repeats x 2 (analytical methods) = 1728	1728	864	96
	Define *the effects of ACh and Nrg1-III signaling on K5+ and Sox2+ cell self-renewal and differentiation using salisphere cultures* . Experiment: Sox2-GFP or K5-GFP single cells will be derived from E14 salivary glands of transgenic mice via FACS and cultured in the presence or absence of CCh or Nrg1-III ± EGF: 2 genotypes x 50 SMG per sort x 4 treatments x 2 culture conditions x 3 repeats = 2400	2400	1200	133
	Determine *whether ACh/M1 and Nrg1-III/ErbB3 signaling direct Sox2+ and K5+ progenitor cells*	288	144	16

	towards an acinar cell fate during SG development and after injury - Experiment: genetic lineage tracing using ex vivo culture system, with or without CCh or Nrg1-III or both on irradiated and non-irradiated tissue: 2 genotypes x 2 (IR vs no IR) x 4 treatments x 6 SG per plate x 3 repeats = 288			
Aim1B	***Identify*** *the time points at which ACh/M1 and Nrg1-III signaling has an effect on Sox2 and K5 gene/protein expression* . Experiment: Epithelial rudiments from fetal mouse will be treated with CCh or Nrg1-III for 0, 1, 2, 4, 6 and 24 h and changes in gene and protein expression will be quantified by qPCR and western blot: 6 SG/plate x 3 treatments x 6 time points x 2 analytical methods x 3 repeats = 648	648	324	36
	Define *the signaling pathways downstream of ACh/M1/EGFR and Nrg1-III/ErbB2/3 that increase Sox2 and K5 expression and promote acinar formation* Experiment: SG epithelial rudiment cultures will be cultured with Nrg1-III or CCh in the presence or absence of commercially available pharmacological inhibitors: 6 SG per plate x 5 time points x 16 treatments x 3 methods of analysis x 3 repeats = 4320	4320	2160	240
	Define *the signaling pathways downstream of Nrg1-III/ErbB3 or ACh/M1, we will assess phosphorylation of signaling molecules using a cell-based immunoassay* *Experiment:* Epithelial rudiments from mouse SG will be cultured with or without CCh or Nrg1-III for 0, 15, 30, 60 and 120 min and cell extracts subjected to the phosphoprotein array: 4 treatments x 5 time points x 6 SG per plate x 3 repeats = 360	360	180	20
	From experience we expect a small percentage of timed pregnant mice **are not pregnant** or have too few embryos for an experiment (<5%). Therefore 35 extra timed pregnant mice will be required.			35
Total	*Total number of timed pregnant mice*			**743**
Total	*Number of adult mice*			**690**
TOTAL				**1433**

VETERINARY CARE

Animals from newly arrived from the [redacted] Laboratory will be visually inspected by the staff of the Laboratory Animal Resource Center (LARC). These animals are observed daily by qualified persons of the LARC to verify the well being of all animals. Only animals of defined health status will be used in this research, as such the veterinarian has the authority to use appropriate treatment or control measures, including euthanasia if indicated, following diagnosis of an animal with disease or injury.

ANIMAL HANDLING

The handling of mice will be minimized to avoid stress to the animals. When handling is required, mice will be moved quickly using the proper handling techniques, as described by the IACUC. No surgical techniques (or anesthesia) will be performed on ICR pregnant females.

EUTHANASIA

The mice will be killed by CO_2 narcosis, followed by cervical dislocation, as approved by the IACUC. The embryos will be euthanized by decapitation. This is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association for the euthanasia of rodents. Carcasses will be collected in plastic bags and disposed of as Medical Pathological Waste.

SUMMARY: Salivary gland hypofunction due to radiation damage significantly reduces the oral health and quality of life of human patients. There is a substantial increase in oral infection, periodontal disease, bone loss, difficulties in speaking and eating, and dry mouth. In this application we have taken a series of steps to ensure the success of the *in vivo* experiments as well as the humane care of the animals involved in these experiments. All protocols required for these experiments have been approved by the IACUC at UCSF (#AN087515-02 and AN088943-02).

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Consortium/Contractual Arrangements

The appropriate programmatic and administrative personnel of each institution involved in this grant application are aware of the National Institutes of Health's Consortium Grant Policy and are prepared to establish the necessary inter-institutional agreement consistent with the Guidelines for Establishing and Operating Consortium Grants.

The initial Consortium Agreement will be set up with initial year funding and project period as proposed in this application. All National Institutes of Health's policies will be restated to the consortium institution; periodical reports from the consortium institution will be requested. The consortium institution will certify that neither it nor its principals nor those performing services under this agreement are presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded by any Federal department or agency from participation in this transaction and have not, within the 3 year period preceding this application, been convicted of, or had a civil judgment rendered against them or had any public transaction (Federal State or local) terminated for cause or default. The agreement will be amended annually with additional funding to the consortium institution. The estimated costs are shown in the Consortium Budget Justification; the actual amount awarded to the consortium institution will be determined after an award is made.

Resource Sharing Plan

1. Data Sharing Plan:
Research findings obtained with NIH funding will be shared prior to publication with the scientific community by presentation at scientific meetings in the form of posters and oral presentations, and personal communications. In addition, upon publication, microarray or RNAseq datasets, and any novel gene expression data will be made available upon request. We will try our best to publish the research data and findings in a timely manner.

2. Sharing Model Organisms: N/A

3. Genome Wide Association Studies (GWAS): N/A

Other research resources: Any other tools or resources generated through work funded by the NIH will be made freely available upon request in a timely fashion. These are most likely to include protocols for methodology, which will be available freely upon request. We will also 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. We will therefore deposit crucial plasmids such as transgenic constructs in a repository such as Addgene (http://www.addgene.org/), so that they are broadly available for a low handling fee.

PHS 398 Checklist

1. Application Type:

From SF 424 (R&R) Cover Page. The responses provided on the R&R cover page are repeated here for your reference, as you answer the questions that are specific to the PHS398.

* Type of Application:

● New ○ Resubmission ○ Renewal ○ Continuation ○ Revision

Federal Identifier:

2. Change of Investigator / Change of Institution Questions

☐ Change of principal investigator / program director

Name of former principal investigator / program director:

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

☐ Change of Grantee Institution

* Name of former institution:

3. Inventions and Patents (For renewal applications only)

* Inventions and Patents: Yes ○ No ○

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

* Previously Reported: Yes ○ No ○

4. * Program Income

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)

5. * Disclosure Permission Statement

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., poss ble collaborations, investment)?

●Yes ○No

PHS 398 Cover Letter

* **Mandatory Cover Letter Filename:** Cover_Letter10269150 81.pdf

Attachments

CoverLetterFilename_attDataGroup0

File Name	Mime Type
Cover_Letter1026915081.pdf	application/pdf