Notice of Award

RESEARCH
Department of Health and Human Services
National Institutes of Health

NATIONAL EYE INSTITUTE

Federal Award Date: 02/23/2017

Grant Number: 1R01EY026640-01A1
FAIN: R01EY026640

Principal Investigator(s):
Joseph B. Ciolino, MD

Project Title: Antibiotic eluting contact lenses for the treatment of bacterial keratitis

Ms. Russell, Monica
Grants & Contracts Officer
20 Staniford Street
Boston, MA 021142508

Award e-mailed to: research_administration@meei.harvard.edu

Period Of Performance:
Budget Period: 03/01/2017 – 02/28/2018
Project Period: 03/01/2017 – 02/28/2021

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $502,372 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to SCHEPENS EYE RESEARCH INSTITUTE in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Eye Institute of the National Institutes of Health under Award Number R01EY026640. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Karen Robinson-Smith
Grants Management Officer
NATIONAL EYE INSTITUTE

Additional information follows

SECTION I – AWARD DATA – 1R01EY026640-01A1

Award Calculation (U.S. Dollars)

Federal Direct Costs	$255,011
Federal F&A Costs	$247,361
Approved Budget	$502,372
Total Amount of Federal Funds Obligated (Federal Share)	$502,372
TOTAL FEDERAL AWARD AMOUNT	$502,372
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$502,372

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
1	$502,372	$502,372
2	$492,054	$492,054
3	$488,660	$488,660
4	$488,559	$488,559

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name:	Vision Research
CFDA Number:	93.867
EIN:	1042129889A1
Document Number:	REY026640A
PMS Account Type:	P (Subaccount)
Fiscal Year:	2017

IC	CAN	2017	2018	2019	2020
EY	8472436	$502,372	$492,054	$488,660	$488,559

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: 2E /GAM / **OC**: 414A / **Released**: [redacted]02/17/2017
Award Processed: 02/23/2017 12:12:41 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 1R01EY026640-01A1

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 1R01EY026640-01A1

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2. As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase VI Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots.

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01EY026640. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:
Additional Costs

MODULAR GRANTS:
This is a Modular Grant Award without direct cost categorical breakdowns issued in accordance with the guidelines published in the NIH Grants Policy Statement at: http://grants.nih.gov/grants/policy/nihgps/nihgps.pdf. Recipients are required to allocate and account for costs related to this award by category within their institutional accounting system in accordance with applicable cost principles.

FUTURE BUDGET PERIOD SUPPORT
In accordance with the NIH requirement to maintain an overall average of 4.0 years, the competing segment for this grant is being reduced by one budget period.

CONSORTIUM:
This award includes funds awarded for consortium activity with University of Pittsburgh. Consortiums are to be established and administered as described in the NIH Grants Policy Statement (NIH GPS). The referenced section of the NIH Grants Policy Statement is available at http://grants.nih.gov/grants/policy/nihgps/nihgps.pdf.

SALARY CAP:
None of the funds in this award shall be used to pay the salary of an individual at a rate in excess of the applicable salary cap. Therefore this award and/or future years are adjusted accordingly, if applicable. Current salary cap levels can be found at the following URL: http://grants1.nih.gov/grants/policy/salcap_summary.htm.

REMINIDER - REQUIREMENTS FOR THE APPROPRIATE SIGNATURES ON NIH FORMS AND OFFICIAL DOCUMENTATION:
NIH no longer accepts forms or other documentation bearing generic departmental signatures or their electronic equivalent (e.g., Department of Sponsored Research). All forms and documentation submitted to the NIH must reflect the name of the individual, electronic, or otherwise, with the appropriate institutional authority to submit such information (i.e., Authorized Organizational Official (AOR), Signing Official (SO), Business Official (BO), Principal Investigator (PD/PI). See NIH Guide Notice: NOT-OD-16-071.

PRIOR APPROVAL:
Requests which require the prior approval of the NEI must be submitted in writing to the Grants Management Specialist. All requests should reference the complete grant number and must be signed by the authorized official of the business office of the grantee organization and by the principal investigator.

GM ROLES & RESPONSIBILITIES:
If you need assistance from the National Eye Institute (NEI) during the course of this grant, please contact the grants management and program staff listed on the Notice of Grant Award (NGA). The telephone numbers of these individuals, as well as other extramural staff members of the NEI, can be located on the NEI web site, http://www.nei.nih.gov. ; The grants management and program staff members work closely with one another through all phases of the project to facilitate the award and the administration of the grant. Their functions are defined as follows:

GRANTS MANAGEMENT CONTACT: The Grants Management Specialist is responsible for all business management matters associated with the review, negotiation, award, and administration of grants. Grants Management Specialists serve as the focal point for receiving and responding to all questions and correspondence related to business management and policy matters, such as correspondence giving or denying any prior approval required by Public Health Service (PHS) policy or special Terms and Conditions of Award, transfer of the grant to another institution, a change in the period of support, or any action which commits, or may result in committing the NEI to a change in the amount of funding.

PROGRAM CONTACT: The Program Director is responsible for all scientific and technical matters associated with the grant. The program official reviews and monitors scientific progress of the project and provides advice and assistance relative to all technical problems to ensure that the scientific objectives of the research program can be pursued effectively and successfully. All questions or correspondence dealing with research progress, changes in research direction, unique scientific opportunities, or any other scientific needs should be addressed to the Program Director.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Linda Dingle
Email: ltd@nei.nih.gov **Phone**: (301) 451-2020 **Fax**: (301) 496-9997

Program Official: George Ann Mckie
Email: mckiegeo@mail.nih.gov **Phone**: 301-451-2020 **Fax**: 301-402-0528

SPREADSHEET SUMMARY
GRANT NUMBER: 1R01EY026640-01A1

INSTITUTION: SCHEPENS EYE RESEARCH INSTITUTE

Budget	Year 1	Year 2	Year 3	Year 4
TOTAL FEDERAL DC	$255,011	$255,130	$255,356	$255,496
TOTAL FEDERAL F&A	$247,361	$236,924	$233,304	$233,063
TOTAL COST	$502,372	$492,054	$488,660	$488,559

Facilities and Administrative Costs	Year 1	Year 2	Year 3	Year 4
F&A Cost Rate 1	97%	97%	97%	97%
F&A Cost Base 1	$255,011	$244,252	$240,520	$240,271
F&A Costs 1	$247,361	$236,924	$233,304	$233,063

PI: **Ciolino, Joseph B.**	Title: Antibiotic eluting contact lenses for the treatment of bacterial keratitis	
Received: 03/07/2016	FOA: PA13-302	Council: 10/2016
Competition ID: FORMS-C	FOA Title: RESEARCH PROJECT GRANT (PARENT R01)	
1 R01 EY026640-01A1	Dual:	Accession Number: 3916634
IPF: 6948301	Organization: SCHEPENS EYE RESEARCH INSTITUTE	
Former Number:	Department: Ophthalmology	
IRG/SRG: ZRG1 BDCN-J (91)S	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 250,000 Year 2: 250,000 Year 3: 250,000 Year 4: 250,000 Year 5: 250,000	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: Y Early Stage Investigator: Y
Senior/Key Personnel:	*Organization:*	*Role Category:*
Joseph Ciolino M.D.	Schepens Eye Research Institute	PD/PI
[redacted]	University of Pittsburgh	Other (Specify)-Sub-award PD/PI
	University of Pittsburgh	Co-Investigator
	Massachusetts Eye and Ear	Other (Specify)-Optometrist

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
R01EY026640

b. Agency Routing Number

2. DATE SUBMITTED | **Application Identifier**

c. Previous Grants.gov Tracking Number
GRANT12113074

5. APPLICANT INFORMATION **Organizational DUNS*:** 073826000

Legal Name*: Schepens Eye Research Institute
Department: Ophthalmology
Division:
Street1*: 20 Staniford Street
Street2:
City*: Boston
County:
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 02114-2508

Person to be contacted on matters involving this application
Prefix: Ms. First Name*: Monica Middle Name: Last Name*: Russell Suffix:
Position/Title: Grants & Contracts Officer
Street1*: 20 Staniford Street
Street2:
City*: Boston
County:
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 02114-2508
Phone Number*: 617-912-2568 Fax Number: Email: Monica_Russell@meei.harvard.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 1042129889A1

7. TYPE OF APPLICANT* M: Nonprofit with 501C3 IRS Status (Other than 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*
Antibiotic eluting contact lenses for the treatment of bacterial keratitis

12. PROPOSED PROJECT
Start Date* Ending Date*
10/01/2016 09/30/2021

13. CONGRESSIONAL DISTRICTS OF APPLICANT
MA-008

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. First Name*: Joseph Middle Name: Bowers Last Name*: Ciolino Suffix: M.D.
Position/Title: Assistant Professor
Organization Name*: Schepens Eye Research Institute
Department: Ophthalmology
Division:
Street1*:
Street2:
City*: Boston
County:
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 02114-2508
Phone Number*: 617-573-5575 Fax Number: Email*: joseph_ciolino@meei.harvard.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$2,467,314.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$2,467,314.00
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: Ms. First Name*: Monica Middle Name: Last Name*: Russell Suffix:
Position/Title*: Grants & Contracts Officer
Organization Name*: Schepens Eye Research Institute
Department: Ophthalmology
Division:
Street1*: 20 Staniford Street
Street2:
City*: Boston
County:
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 02114-2508
Phone Number*: 617-912-2568 Fax Number: Email*: research_administrator@meei.harvard.edu

Signature of Authorized Representative*
Monica Russell

Date Signed*
03/07/2016

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:1247-R01 Cover Letter Ciolino_3 4 2016.pdf

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

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OMB Number: 4040-0010
Expiration Date: 06/30/2018

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: Schepens Eye Research Institute
Duns Number: 0738260000000
Street1*: [redacted]
Street2:
City*: Boston
County:
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 02114-2508
Project/Performance Site Congressional District*: MA-008

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: University of Pittsburgh
DUNS Number: 0045143600000
Street1*: [redacted]
Street2:
City*: Pittsburgh
County:
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 15213-2588
Project/Performance Site Congressional District*: PEN 14

File Name

Additional Location(s)

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: 11-26-2014

Animal Welfare Assurance Number A3177-01

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*	1238-Project Abstract_Ciolino.pdf
8. Project Narrative*	1239-Project Narrative_Ciolino.pdf
9. Bibliography & References Cited	1240-References3 4 16_final-2.pdf
10.Facilities & Other Resources	1241-Facilities_Ciolino R01.pdf
11.Equipment	1242-Equipment.pdf
12. Other Attachments	1243-Authentication_Ciolino.pdf 1244-Biohazards_SERI Safety Program.pdf 1245-Biohazard Protection_Ciolino Lab.pdf 1246-Abbreviations_Ciolino R01.pdf

Project Summary/Abstract

Bacterial keratitis is one of the leading causes of corneal blindness. The primary medical therapy involves an intensive regimen of hourly antibiotic drops to eradicate the infection. A major unmet need for the treatment of bacterial keratitis is a sustained method of topical drug delivery to improve treatment efficacy and patient adherence. This proposal seeks to develop and test an antibiotic-eluting therapeutic contact lens (TCL) for the treatment of bacterial keratitis. The proposed research will test the hypothesis that because of continuous administration at therapeutic doses, an antibiotic-eluting TCL can safely and more effectively treat bacterial keratitis than the current standard of care, topical antibiotic drops. Moreover, we also hypothesize that TCLs can locally delivery high enough antibiotic concentrations so that it can overcome resistance and be used to treat "fluoroquinolone-resistant" bacterial keratitis. Our approach uses a TCL design that incorporates a thin drug-polymer film within the periphery of a standard contact lens; this enables the release of large amounts of drug over days to weeks, while allowing unimpeded vision through the lens, and used materials that are all FDA-approved for use on the eye. Our preliminary studies that support this approach have demonstrated that a steroid-eluting contact lens can provide continuous drug levels to the cornea that exceeds that of hourly drops. We have formulated fluoroquinolone-eluting contact lenses that demonstrated controlled drug release for 2 days. The Specific Aims for this project are designed to systematically evaluate our hypotheses and evaluate the potential of this approach. In Aim 1 we confirm *in vitro* antibacterial effectiveness, perform cytotoxicity studies, and evaluate drug flux and biocompatibility in normal rabbit eyes.To ensure that the antibiotic-eluting TCL maintains the physical properties required for safe wear, we will characterize light transmittance, O_2 transmissibility, water content, hydrophobicity, and elasticity of the TCL. In Aim 2 we will compare the efficacy of TCLs with hourly commercially available fluoroquinolone eye drops for the treatment of keratitis resulting from fluoroquinolone-susceptible Gram-negative (*Pseudomonas* aeruginosa) and Gram-positive (*Staphylococcus aureus*) bacteria, which are two of the most common causes of bacterial keratitis. In Aim 3, we will test the hypothesis that TCLs can provide enough antibiotic to overcome resistance and we will compare the treatment of TCL with vancomycin drops for the treatment of fluoroquinolone-resistant *Staphylococcus aureus* keratitis and with tobramycin drops for the treatment of fluoroquinolone-resistant *Pseudomonas aeruginosa*. Public Health Relevance: If successful, a TCL could be used as a platform to expand the treatment for bacterial keratitis and other blinding ocular diseases.

Project Narrative

A major unmet need for the treatment of bacterial keratitis is a method of sustained ocular drug delivery that could improve treatment efficacy. This proposal seeks to test an antibiotic-eluting therapeutic contact lens for the treatment of bacterial keratitis.

Facilities and Other Resources – Schepens Eye Research Institute

Laboratory:
The Ciolino Laboratory is located [redacted] and consists of 650 sq. feet of space that includes four lab benches with adjacent desk space. The laboratory has a dedicated fume hood and an adjacent, separate room that houses the ultraviolet (UV) light chamber and spin-coater. The Ciolino laboratory is also equipped other tools necessary for the study of drug delivery with contact lenses and includes an oxygen permeometer, Chiltern Contact Lens unit (used to measure contact lens dimensions), an Omni Bead Ruptor Homogenizer and other equipment used to characterize contact lenses.

Computer:
The PI, the Postdoctoral Associate, and the Technologist have networked laptop computers with ample processing and hard drive capacity to analyze and store experimental data and prepare manuscripts and presentations. There is also a computer that is dedicated to the oxygen permeometer and is used to measure contact lens oxygen permeability. In addition, a computer with printer, scanner, and DVD/CD drive are available at Dr. Ciolino's office for use for this project. The network systems are supported by a central IT department at SERI, which consists of a high-speed Local Area Network- including 8 Institute-wide servers, 1.25 external IP networks and 9 internal IP networks behind 2 routers. This network is maintained by three full-time staff at the Schepens. They provide such services as maintaining email, desktop computer staging, remote access to the network, network security (including maintaining the firewall and virus subscriptions), maintaining shared network storage, and shared printer deployment. We have now merged with the MEEI network, which will give us a 10Gb/s connection to the Internet. Our network link with the Harvard Libraries is a major resource that provides access to hundreds of research literature databases, thousands of electronic journals, and other resources.

Office:
The PI has a private 120 square foot office [redacted]. He also has resources for photocopying, faxing, and stores of supplies.

Animal Facilities:
The care and maintenance of our laboratory animals is central to the success of our research and, thus, our animal facility must conform to the highest standards and provide a first-rate program for animal surgery training, maintenance of a virus-free induced mutant colony and stable animal environment as well as the provision of veterinary care. The Schepens Eye Research Institute a registered research facility under the Animal Welfare Act, has an 8,530 square feet AAALAC accredited animal facility that is located [redacted] and has a Biosafety Level 2 (BSL-2) room. The facility is fully staffed with full-time veterinarian, animal caretakers, and facility supervisors, who provide expertise and assistance in animal surgical techniques and postoperative care, anesthesia administration, and administration of drugs and medication. The facility also provides a variety of services including: consultation on acceptable protocols for the handling of experimental animals, and training and orientation for new fellows and research technicians. The core contains 16 housing rooms serviced by a central corridor and other functional support space. There is a dedicated a rabbit surgery room, a microsurgical suite with laser systems, a special eye examination and photography room, a general prep area, and a Satellite Room.

Rabbits are housed individually, with each cage placed directly across from another for visualization. Music is played at a low volume to provide background noise and minimize startling. Rabbits are on a weekly enrichment schedule, which includes a new interactive enrichment device Monday-Friday. The schedule includes the use of autoclaved cardboard towel rolls, rattles, fresh vegetables, 'bunny blocks' on a chain, jingle balls and timothy hay. They are also held and brushed by humans at this time at least once a week.

Rabbits are housed individually, with each cage placed directly across from another for visualization. Music is played at a low volume to provide background noise and minimize startling. Rabbits are on a weekly enrichment schedule, which includes a new interactive enrichment device Monday-Friday. The schedule includes the use of autoclaved cardboard towel rolls, rattles, fresh vegetables, 'bunny blocks' on a chain, jingle balls and timothy hay. They are also held and brushed by humans at this time at least once a week.

Morphology Core Facility

The SERI morphology core provides technical assistance for light, electron, and confocal microscopy. Services include: paraffin, methacrylate, epon, and frozen sectioning; complete transmission electron microscopy service including processing of tissue, sectioning of tissue, scoping of tissue and printing of micrographs and training in microscopy. The core facility staff assists with technical problems and procedures in use of a scanning electron microscope and a confocal microscope. Within the Morphology Unit, two confocal microscopes and two Nikon Eclipse E800 Epifluorescent Microscopes are accessible. A Scanning Electron Microscope are available in house for faculty and their laboratories

Laboratory Computer Applications Unit

The SERI computer core provides support for individual and collaborative efforts by assisting in the interfacing of microcomputers with laboratory equipment and by developing software for gathering, processing and analyzing experimental data by microcomputer systems. The module provides the expertise to update and purchase new additional software, interconnect microcomputer systems between laboratories and equipment to facilitate analyses within laboratories as well as within the CORE facilities such as morphology.

Machine Shop

The SERI machine shop is available on an as-needed basis to suitable qualified personnel working on the project. It consists of a 400 sq. ft. facility with machine tools capable of performing most common machining tasks. These tools include drill presses, large sheet metal shears, a sheet metal brake (bender), a micro-drilling tool, a belt and a disc sander, arbor presses, a large commercial metal-cutting brand saw, a professional Clausing-Colchester lathe, a Bridgeport vertical milling machine, and a complete set of hand tools, taps, and machinist tools. Schepens also provides carpentry and electrical services as needed and an electronics workshop (192 sq. ft.) is available, as required.

Scientific Environment at the Schepens Eye Research Institute:

Schepens Eye Research Institute's mission is to conduct research that will lead to a better understanding, treatment and prevention of diseases that cause visual dysfunction and blindness. Our institute's environment provides supportive facilities and access to world-class ophthalmologists and researchers on the eye, its diseases, and their remediation though direct formal and casual collaboration and frequent seminars given by in-house and external experts. The recent merger of Schepens (SERI) and nearby Massachusetts Eye and Ear Infirmary (MEEI) adds a world-class teaching hospital and its access to clinical patients to our resources. Our affiliation with Harvard Medical School provides supportive facilities within the wider Harvard Community, including Harvard Catalyst (The Harvard Clinical and Translational Science Center) which provides biostatistical services, a range of other resources to support clinical and translational research and to foster new collaborations. The greater Boston/Cambridge metropolitan area is a rich source of skills and learning opportunities in all of our areas of interest, as it includes Harvard, Tufts, Boston, and Northeastern Universities, the Massachusetts Institute of Technology, and the New England College of Optometry, all of which have seminar series related to our efforts.

In summary, the scientific environment and facilities at the Schepens Eye Research Institute MEEI and Harvard affiliate are superb, and will enhance my ability to perform the proposed research.

Early Stage Career Support:
The research environment at Harvard is both highly intensive and supportive. There are many other researchers in close proximity that are interested in collaborative work. The Harvard environment is very collaborative, which fostered my initial mentorship by [redacted] at Children's Hospital Boston and has now facilitated the participation of [redacted] at Mass. Eye and Ear's [redacted] Laboratory and is the founder and PI of the Harvard-wide Program on Antibiotic Resistance, and of the academic/industry consortium Boston-Area Antibiotic Resistance Network ..Additionally, the Harvard Catalyst program has set up a number of core facilities throughout the Harvard system. Moreover, the Harvard-Massachusetts Institute of Technology (MIT) program in Health Science and Technology provides Harvard investigators additional resources at the MIT campus and this includes the trip quad liquid chromatography–tandem mass spectrometry, a Cary 500i UV-Vis/NIR spectrophotometer with diffuse reflectance accessory and integrating sphere that is used for light transmittance studies, and access to the Nuclear Reactor Laboratory that is used for sterilization procedures.

SERI, MEEI and the department of Ophthalmology are extremely dedicated in helping Early Stage Investigators, with numerous resources for classes, travel, training and exceptional collegial support. There is readily available assistance, guidance and supervision by senior Mentors in addition to logistical support and administrative management. Securing funding from federal/foundation sources will permit me to expand my program. SERI will modify the allotment of space provided as my career matures, the department will monitor my level of accomplishment with the expectation of steady promotion at Harvard Medical School. The Mass Eye and Ear is poised to build a new, modern research facility. With expanded state-of-the-art research space, SERI and MEEI will be well positioned to meet my growing space needs as my research program develops.

Major Equipment:
The PI's equipment includes: a spin coater (Best Tools, LLC Model SC EZ-6) used to fabricate thin polymeric films and a Loctite ZETA® 7401 ultraviolet (UV) light chamber that has a 400 watt metal halide bulb used for hydrogel polymerization and contact lens fabrication; instruments that

are required for measuring contact lens thickness and oxygen permeability including ET-3 electronic thickness gauge, a polarographic cell, (Rehder Development Company), and a Model 201T oxygen permeometer (Createch, Inc); a Chiltern Contact Lens unit (Optimec® Limited), which is used to measure the curvature of hydrated contact lenses; and a bench top incubator shaker (Genemate) used for drug release studies. The PI also has use of all SERI and MEEI Core Facilities, which includes HPLC with ultraviolet detection (ThermoFisher Scientific) and a spectrophotometer (Tecan).

On the MIT campus (see support letter from [redacted] the PI has access to additional equipment such as a Cary 500i UV-Vis/NIR spectrophotometer with diffuse reflectance accessory and integrating sphere that is used for light transmittance studies that is located at Building 13 (Vannevar Bush building). Also available is a triple quad liquid chromatography–tandem mass spectrometry (Agilent 6410) and tissue homogenizer (Omni Bead Ruptor 24) both available in building 68. Within MIT's Koch Institute, the PI has access to a Drop Shape Analyzer (DSA100, Kruss GmbH), which is used to measure water contact angle of the contact lens surface, a centrifuge (Eppendorf 5174R), a gel permeation chromatography system (Agilent 1260) with a triple detector array (Viscotek). Finally, within MIT's Nuclear Reactor Laboratory the PI and his team have access to a Gamma Cell 220E Cobalt 60 Irradiation Unit (Atomic Energy of Canada LTD, Ottawa, Can.) that they use for terminal sterilization of the drug-eluting contact lenses.

The [redacted] Ophthalmic Microbiology Laboratory in the Department of Ophthalmology, University of Pittsburgh School of Medicine

Laboratory: The laboratory is part Ophthalmology and Visual Sciences Research Center (OVSRC), located on [redacted] at the University of Pittsburgh. The OVSRC has shared access to cold rooms, freezer storage, conference rooms and common equipment (21,000 Sq ft reserved for basic science research), including state of the art microscopy systems and real-time PCR equipment. The [redacted] Laboratory of Ophthalmic Microbiology has approximately 1600 sq ft of fully equipped laboratory space. Our laboratory is BSL-2 rated.

Clinical: Part of the [redacted] Laboratory includes the Clinical Microbiology Laboratory, complete with thousands of frozen excess sample clinical isolates. These isolates are de-identified (no patient information) ocular clinical isolates and used to evaluate antibiotic efficacy and diagnostic methods, and include approximately 2000 bacteria (*Pseudomonas, Serratia, Staphylococcus, Streptococcus*, etc.), and other microorganisms. Our group includes fellowship trained corneal specialists, clinical microbiologists, and basic scientists to help us achieve our research goals. Work will occur in BSL-2 rooms with appropriate biosafety ratings.

Computer: The [redacted] Laboratory has Macintosh and PC computers and all of the data analysis software necessary for the proposed study.

Office: The [redacted] Laboratory has 200 sq ft office immediately adjacent to the laboratory.

Others: The PI has access to the research modules of the National Eye Institute P30 CORE grant awarded to the Department of Ophthalmology at University of Pittsburgh. Investigators at the University of Pittsburgh have access to numerous core facilities including Sequencing, RNA-Sequencing, NanoString services, and electron microscopy at the Center for Biological Imaging.

Animal: The lab members have full access to the BSL2 animal suite of the main Division of Laboratory Animal Resources (DLAR) facility in [redacted] at the University of Pittsburgh. This AAALAC International accredited animal program and animals are housed are according to the USDA, PHS and NIH guidelines as monitored and rigorously enforced by the Institutional Animal Care and Use Committee of the University of Pittsburgh. The [redacted] Laboratory has digital camera equipped slit-lamps, humane animal restraint devices, and other related equipment.

Equipment: The [redacted] laboratory has a Biotek Synergy 2 plate reader with fluorescence, absorption and luminescence reading capabilities, three thermal cyclers and a 3 channel real-time thermalcycler, a nanodrop spectrophotometer, two full size incubators equipped with New Brunswick Tc-7 tissue culture rollers, pumps and flow cell apparatus for preparation and imaging of biofilms including a Bioflux 200 microfluidic device, bioreactors, water baths, pH meters balances and general equipment required by a functioning molecular biology/biochemistry laboratory. The laboratory has shared access to three BSL2 culture hoods, large rotary incubators, an autoclave, transilluminator, three CO2 incubators, spectrophotometers, centrifuges, an ultracentrifuge with several rotors, a Carestream Gel Logic 212 Pro fluorescent detection system, Li-cor IR scanner, Aligent Bioanalyzer, and other equipment relevant for molecular and biochemical techniques. The Shanks laboratory has all of the equipment necessary for the proposed studies, such as tissue homogenizers, microbiological equipment, slit lamps, and photography equipment. The OVSRC has Applied Biosystems Prism 7700 Analyzer and Step One RT-PCR machines; a Nikon Confocal Microscopy System; Fryer TE2000E Real Time Imaging System with Metamorph Software; Nikon T100 inverted and Olympus BHY2 and AH2 microscopes, all equipped with UV fluorescent imaging detection. Our imaging equipment falls under a departmental P30 grant that funds an imaging expert to maintain and assist PIs with their imaging needs.

Major Equipment:
The PI's equipment includes: a spin coater (Best Tools, LLC Model SC EZ-6) used to fabricate thin polymeric films and a Loctite ZETA® 7401 ultraviolet (UV) light chamber that has a 400 watt metal halide bulb used for hydrogel polymerization and contact lens fabrication; instruments that are required for measuring contact lens thickness and oxygen permeability including ET-3 electronic thickness gauge, a polarographic cell, (Rehder Development Company), and a Model 201T oxygen permeometer (Createch, Inc); a Chiltern Contact Lens unit (Optimec® Limited), which is used to measure the curvature of hydrated contact lenses; and a bench top incubator shaker (Genemate) used for drug release studies. The PI also has use of all SERI and MEEI Core Facilities, which includes HPLC with ultraviolet detection (ThermoFisher Scientific) and a spectrophotometer (Tecan).

On the MIT campus (see support letter from [redacted]) the PI has access to additional equipment such as a Cary 500i UV-Vis/NIR spectrophotometer with diffuse reflectance accessory and integrating sphere that is used for light transmittance studies that is located [redacted] Also available is a triple quad liquid chromatography–tandem mass spectrometry (Agilent 6410) and tissue homogenizer (Omni Bead Ruptor 24) both available in building 68. Within MIT's Koch Institute, the PI has access to a Drop Shape Analyzer (DSA100, Kruss GmbH), which is used to measure water contact angle of the contact lens surface, a centrifuge (Eppendorf 5174R), a gel permeation chromatography system (Agilent 1260) with a triple detector array (Viscotek). Finally, within MIT's Nuclear Reactor Laboratory the PI and his team have access to a Gamma Cell 220E Cobalt 60 Irradiation Unit (Atomic Energy of Canada LTD, Ottawa, Can.) that they use for terminal sterilization of the drug-eluting contact lenses.

Authentication of Key Biological and/ or Chemical Resources

Cell Lines. The proposed study employs telomerase-immortalized human corneal-limbal epithelial (HCLE) cell lines, which were originally developed in the laboratory of Dr. Ilene K. Gipson.[1] HCLE cells have been cryopreserved using freezing medium containing a cryoprotectant (DMSO), and by storage in liquid nitrogen. These cells are currently in use by several laboratories worldwide, including our collaborators at the University of Pittsburgh;[2] to date, no issues have been reported. While recovering these cells for use in routine experiments, they are checked for 1) their ability to stratify, differentiate, and express membrane-associated mucins (MAMs) at levels comparable to their native counterparts, and 2) any contamination.

New Zealand White (NZW) Rabbits. Male and female NZW rabbits will be obtained from Covance. Rabbit are given a unique identification number, which is tattooed on the inside of the one of the ears by the animal vendor prior to arrival. The veterinary staff will have already made cage cards prior to their arrival. Rabbits will have individual paper records prepared. Once in the housing area, the crates are opened one at a time. The animal's overall appearance and health is assessed. Rabbits are then weighed and sex is checked. They are than placed in a new cage. The rabbit's clinical records are then updated accordingly.

Fortified Tobramycin (14 mg/ml) and Vancomycin (25 mg/ml). Fortified compounds will be obtained from Mass. Eye and Ear's compounding pharmacy and made using commercially available sterile products so they do not require a certificate of analysis confirming identification and chemical validity. Tobramycin is compounded from Tobramycin 80 mg/2 mL injection, USP NDC 63323-306-02 (Fresenius Kabi, USA) and Vancomycin is compounded from 500 mg injection, USP NDC 67457-611-00 (Novaplus, USA). All these ingredients are produced using FDA Current Good Manufacturing Practices (CGMPs) and conform with United States Pharmacopoeia (USP) standards. The pharmacy compounds all sterile medications using the standards outlined in USP Chapter <797>.

Bacterial Isolates. The following bacterial isolates have been isolated from human eyes and will be supplied from our collaborators at the University of Pittsburgh: FQ-susceptible *S. aureus* (K1181), FQ-susceptible cytotoxic *P. aeruginosa* (PA14), FQ-susceptible invasive *P. aeruginosa* (PA01), FQ-resistant *S. aureus* (E277), and FQ-resistant *P. aeruginosa* (K1278). The bacterial isolates are de-identified (no patient identifiers) and saved as part of a clinical laboratory bank for validation of new anti-infectives. The clinical bacterial isolates were identified in a College of American Pathologists (CAP) certified laboratory. The Laboratory (The [redacted] Ophthalmic Microbiology Laboratory) is a fully certified independent clinical laboratory (CAP, CLIA, and State of Pennsylvania) that uses standard methods for the identification of bacteria.

Drug-eluting therapeutic contact lenses (TCLs). The TCLs are manufactured with standard reagents and chemicals listed above and using the method previously described.[3] Each TCL is given a unique identification number that it maintains throughout the manufacturing and study process. After the final manufacturing step and before sterilization, each TCL is examined grossly, microscopically, and by ocular coherence tomography (OCT). Under a microscope we measure the inner and outer diameters of the drug-polymer film using digital calipers and the thickness of the film is measured by OCT. The quality of the TCL is also evaluated under a microscope in an effort to detect any flaws, such as chips in the periphery of the lens.

Reagents and chemicals. All the remaining reagents and chemicals that are included in the application are standard and therefore not expected to vary. As per NIH guidelines, these standard chemicals do not need to be described in this plan. However, for the sake of completeness, we have included the vendors for the following proposed reagents and chemicals: Besifloxacin 0.6% solution (B&L), besifloxacin HCl (LKT Laboratories), PLGA (Evonik), HEMA with methacrylic acid (Mitsubishi Rayon Co., Ltd.), Irgacure® 2959 [2-Hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone] (Sigma Aldrich), 1,1,1,3,3,3-Hexa-2-fluoropropanol (Sigma Aldrich), and Phosphate Buffered Saline (Life Technologies).

1. Gipson, I. K. *et al.* Mucin gene expression in immortalized human corneal-limbal and conjunctival epithelial cell lines. *Invest Ophthalmol Vis Sci* **44,** 2496–2506 (2003).
2. Wingard, J. B. *et al.* A novel cell-associated protection assay demonstrates the ability of certain antibiotics to protect ocular surface cell lines from subsequent clinical Staphylococcus aureus challenge. *Antimicrobial Agents and Chemotherapy* **55,** 3788–3794 (2011).
3. Ciolino, J. B. *et al.* In vivo performance of a drug-eluting contact lens to treat glaucoma for a month. *Biomaterials* **35,** 432–439 (2014).

Biohazards

We will be using BL2 at Schepens Eye Research Institute (SERI), which has a comprehensive Health and Safety Program in place for all personnel. This includes having access to an occupational health physician and a full time safety officer. Much of the safety program is organized around the needs of the Institutional Animal Care and Use Committee (IACUC), which is AAALAC certified. No animal work is permitted without hazard analysis. The Institutional Biosafety Committee has direct input into the hazard analysis of the laboratory safety program. Hazard identification and oversight is accomplished primarily by the safety officer. The safety officer sits on the Institutional Biosafety Committee and reviews all protocol submissions for the IACUC with regard to hazardous agents.

Occupational hazards are minimized through risk assessment by the SERI Safety Review Committee (SRC) in conjunction with the safety officer. The SRC is responsible for the creation of Special Animal Safety Protocols (SASPs) and Laboratory Safety Protocols (LSPs). The use of engineering controls and personal protection equipment is identified in these protocols. Currently there are 25+ SASPs and 21 LSPs in place for the most commonly utilized hazards.

The use of biologic, recombinant or synthetic nucleic acid molecules are reviewed and approved by the IBC prior to submission of IACUC protocols. Schepens labs are equipped to handle RG2 agents at BSL-2 and ABSL-2. Chemical hazards are identified on the IACUC protocols by the investigator. If a new hazardous agent is identified, the investigator is directed to work with the safety officer to review, perform a risk assessment and identify appropriate controls. There is a robust hazardous waste program.

The safety officer provides a comprehensive training program which includes new employee training and annual retraining on bloodborne pathogens, the use of the biosafety cabinet, GHS HazCom, best practices in the laboratory, sharps management, disinfection and emergency procedures. Additionally there is a monthly mini training on current safety topics which are presented at the technical staff meeting and disseminated through the laboratories. Separate trainings on animal handling and safety in the animal facility, inclulding occupational health and zoonoses, is accomplished by the animal facility staff. The safety officer inspects the labs and hazardous waste areas weekly and performs a more formal laboratory inspection annually. All biosafety cabinets and fume hoods are certified annually

All personnel are required to report all injuries, work related illness, bites, needle sticks or possible allergic reactions to their supervisors within 48 hours. They must also submit a Report of Injury or Illness to Human Resources within 7 calendar days. A copy is provided to the safety office. Exposures are discussed with an Occupational Medicine Physician.

BIOHAZARD PROTECTION IN THE LABORATORY

1. A mandatory blood borne pathogen training is given to all laboratory personnel at Schepens.
2. Every laboratory has a written exposure control plan that is read by all staff.
3. All laboratory personnel are required to wear laboratory coats, gloves, and eye protection when dealing with biological material and chemicals.
4. A biosafety cabinet is used in the laboratory whenever there is potential for aerosol generation while handling samples.
5. The use of sharps (needles, blades, other sharp instruments) in the laboratory is minimized; however, when used, laboratory personnel are instructed to discard sharps in designated sharps containers.
6. Solid biological waste is always autoclaved.
7. Disinfectants such as 10% bleach are used for disinfecting liquid waste whenever necessary.
8. Universal precautions are followed at all times.
9. All accidents are reported to the PI, Biosafety Officer, and the Biosafety Institutional Committee.

Abbreviations

ANOVA	Analysis of variance
AUC	Area under the curve
C_{AVE}	Average hourly concentration
C_{MAX}	Maximum concentration
C_{SS}	Steady state concentration
CLSI	Clinical and Laboratory Standards Institute
CFU	Colony forming unit
CL	Contact lens
CMV	Cytolmegalovirus
FDA	Food and Drug Administration
FQ	Fluoroquinolone
FQ-R	Fluoroquinolone-Resistant
FQ-S	Fluoroquinolone-Susceptible
HCLE	Human corneal epithelial-limbal cells
HPLC	High Precision Liquid Chromatography
IOP	Intraocular pressure
ISO	International Organization for Standardization
Kgy	Kilogrey
LC-MS/MS	Liquid chromatography-tandem mass spectroscopy
MIC	Minimum inhibitory concentration
MPa	Megapascal
MRSA	Methicillin resistant *staphylococcus aureus*
MTT	(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NEI	National Eye Institute
O_2	Oxygen
OCT	Optical coherence tomography
P. aeruginosa	*Pseudomonas aeruginosa*
PBS	Phosphate buffered saline
pHEMA	Poly(hydroxyethylmethacrylate)
PLGA	Poly(lactic-co-glycolic acid)
S. aureus	*Staphylococcus aureus*
SD	Standard deviation
TCL	Therapeutic contact lens
UV	Ultraviolet
UV/Vis-NIR	Ultraviolet-visible spectrum-near infrared

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*: Joseph Middle Name Bowers Last Name*: Ciolino Suffix: M.D.

Position/Title*: Assistant Professor
Organization Name*: Schepens Eye Research Institute
Department: Ophthalmology
Division:
Street1*: [redacted]
Street2:
City*: Boston
County:
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 02114-2508

Phone Number*: 617-573-5575 Fax Number: E-Mail*: joseph_ciolino@meei.harvard.edu

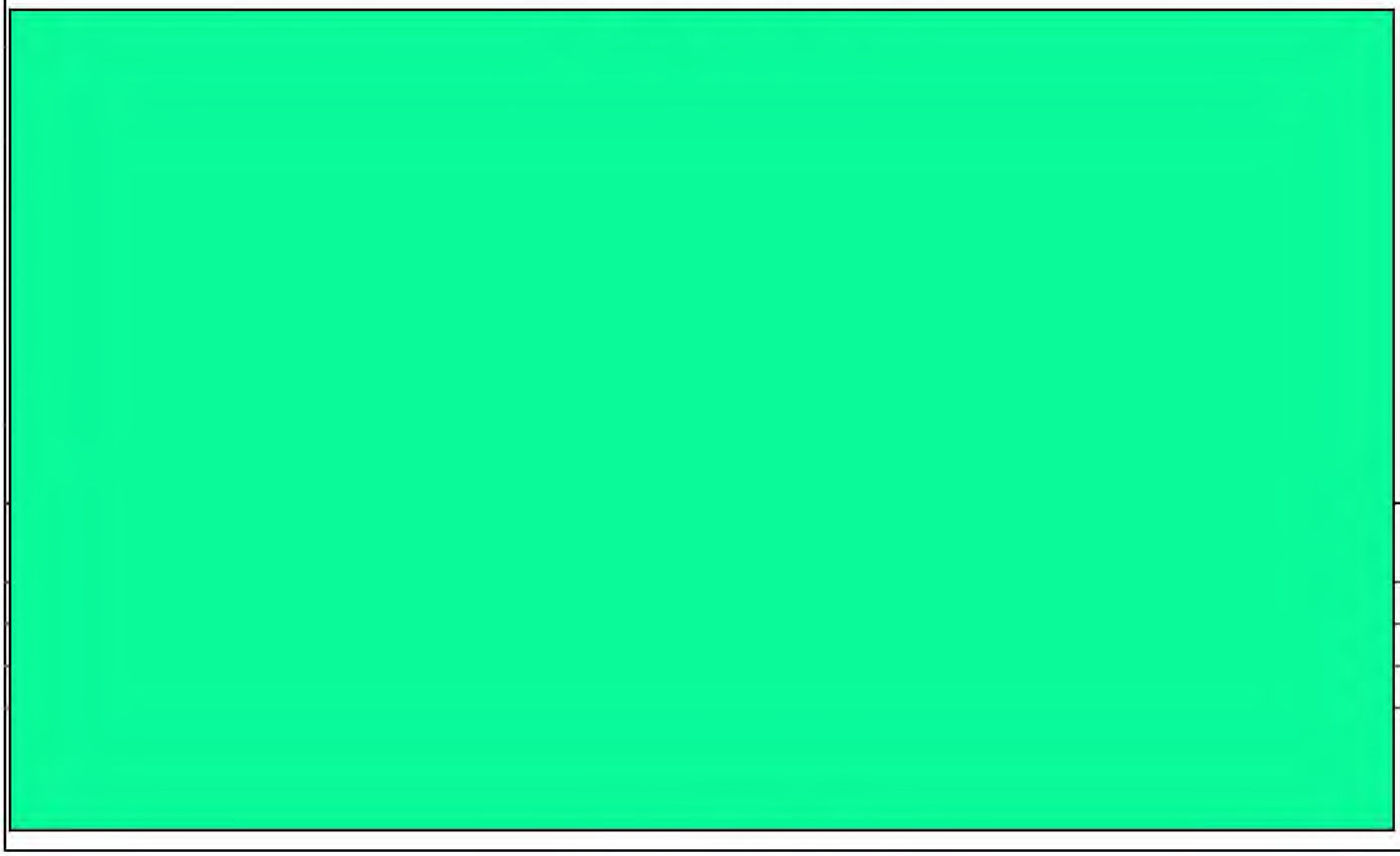
Credential, e.g., agency login: [redacted]

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

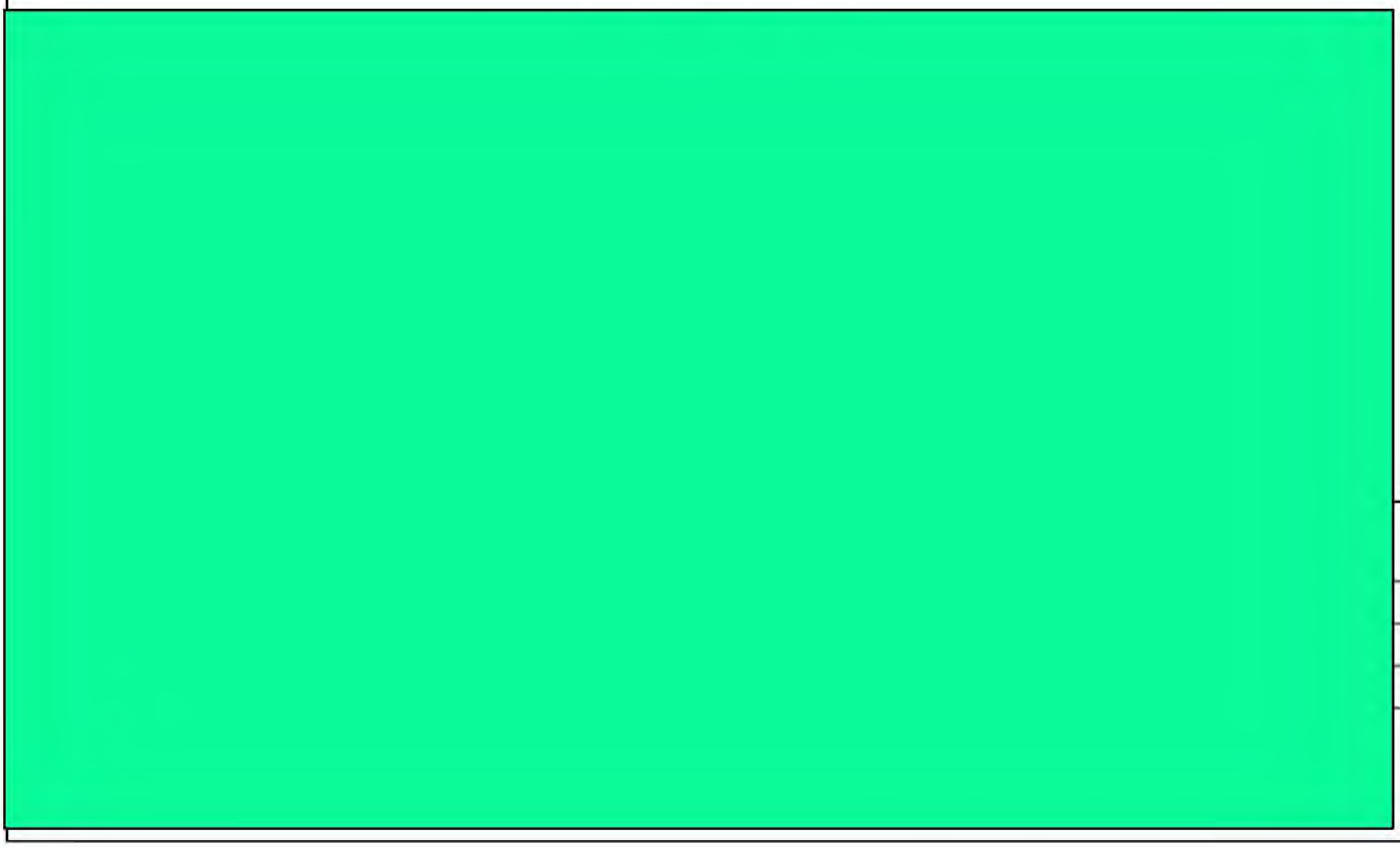
Degree Type: MD Degree Year: 2002

	File Name
Attach Biographical Sketch*:	1234-Biosketch r01 Ciolino 2016 (2).pdf
Attach Current & Pending Support:	

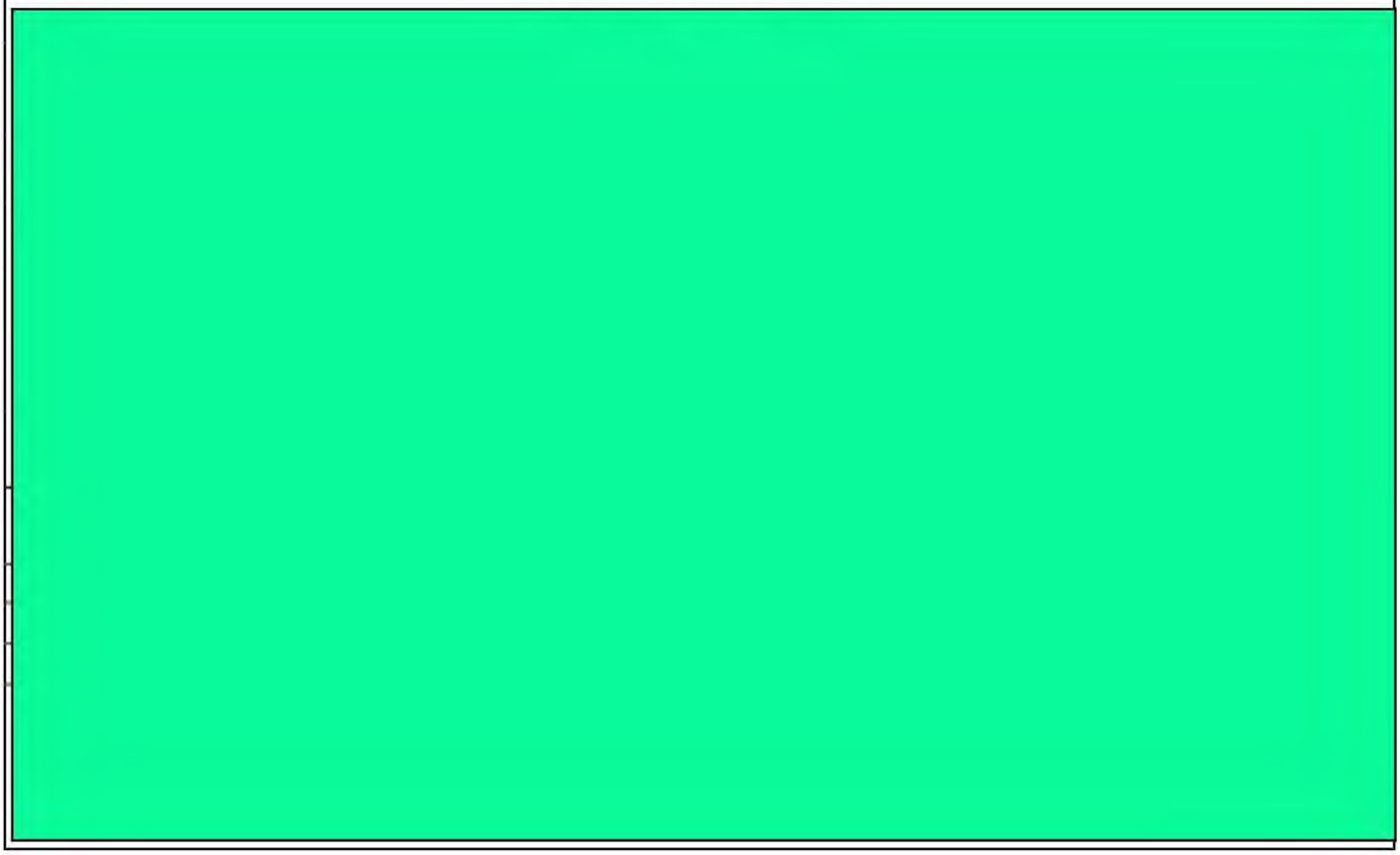
PROFILE - Senior/Key Person



PROFILE - Senior/Key Person



PROFILE - Senior/Key Person



Page 022 of 195 to Page 036 of 195

Personnel

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

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

Prefix:	Dr.
First Name*:	Joseph
Middle Name:	Bowers
Last Name*:	Ciolino
Suffix:	M.D.

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 Modular Budget

OMB Number: 0925-0001
Expiration Date: 10/31/2018

Budget Period: 1

Start Date: 10/01/2016 End Date: 09/30/2017

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium Indirect (F&A)*	250,000.00
Consortium Indirect (F&A)	4,920.00
Total Direct Costs*	254,920.00

B. Indirect (F&A) Costs

	Indirect (F&A) Type	Indirect (F&A) Rate (%)	Indirect (F&A) Base ($)	Funds Requested ($)
1.	MTDC	97.00	254,920.00	247,273.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): Department of Health and Human Services Council Moore Tel:212-264-2069

Indirect (F&A) Rate Agreement Date: 09/05/2014

Total Indirect (F&A) Costs: 247,273.00

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

Funds Requested ($): 502,193.00

Funding Opportunity Number: PA-13-302. Received Date: 2016-03-07T13:56:59.000-05:00

PHS 398 Modular Budget

Budget Period: 2

Start Date: 10/01/2017 End Date: 09/30/2018

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium Indirect (F&A)*	250,000.00
Consortium Indirect (F&A)	5,166.00
Total Direct Costs*	255,166.00

B. Indirect (F&A) Costs

	Indirect (F&A) Type	Indirect (F&A) Rate (%)	Indirect (F&A) Base ($)	Funds Requested ($)
1.	MTDC	97.00	251,835.00	244,280.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): Department of Health and Human Services Council Moore Tel:212-264-2069

Indirect (F&A) Rate Agreement Date: 09/05/2014

Total Indirect (F&A) Costs: 244,280.00

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

Funds Requested ($): 499,446.00

Funding Opportunity Number: PA-13-302. Received Date: 2016-03-07T13:56:59.000-05:00

PHS 398 Modular Budget

Budget Period: 3

Start Date: 10/01/2018 End Date: 09/30/2019

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium Indirect (F&A)*	250,000.00
Consortium Indirect (F&A)	5,356.00
Total Direct Costs*	255,356.00

B. Indirect (F&A) Costs

	Indirect (F&A) Type	Indirect (F&A) Rate (%)	Indirect (F&A) Base ($)	Funds Requested ($)
1.	MTDC	97.00	240,520.00	233,304.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): Department of Health and Human Services Council Moore Tel:212-264-2069

Indirect (F&A) Rate Agreement Date: 09/05/2014

Total Indirect (F&A) Costs: 233,304.00

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

Funds Requested ($): 488,660.00

Funding Opportunity Number: PA-13-302. Received Date: 2016-03-07T13:56:59.000-05:00

PHS 398 Modular Budget

Budget Period: 4

Start Date: 10/01/2019 End Date: 09/30/2020

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium Indirect (F&A)*	250,000.00
Consortium Indirect (F&A)	5,496.00
Total Direct Costs*	255,496.00

B. Indirect (F&A) Costs

	Indirect (F&A) Type	Indirect (F&A) Rate (%)	Indirect (F&A) Base ($)	Funds Requested ($)
1.	MTDC	97.00	240,271.00	233,063.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): Department of Health and Human Services Council Moore Tel:212-264-2069

Indirect (F&A) Rate Agreement Date: 09/05/2014

Total Indirect (F&A) Costs: 233,063.00

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

Funds Requested ($): 488,559.00

Funding Opportunity Number: PA-13-302. Received Date: 2016-03-07T13:56:59.000-05:00

PHS 398 Modular Budget

Budget Period: 5

Start Date: 10/01/2020 End Date: 09/30/2021

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium Indirect (F&A)*	250,000.00
Consortium Indirect (F&A)	5,641.00
Total Direct Costs*	255,641.00

B. Indirect (F&A) Costs

	Indirect (F&A) Type	Indirect (F&A) Rate (%)	Indirect (F&A) Base ($)	Funds Requested ($)
1.	MTDC	97.00	240,015.00	232,815.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): Department of Health and Human Services Council Moore Tel:212-264-2069

Indirect (F&A) Rate Agreement Date: 09/05/2014

Total Indirect (F&A) Costs: 232,815.00

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

Funds Requested ($): 488,456.00

Funding Opportunity Number: PA-13-302. Received Date: 2016-03-07T13:56:59.000-05:00

PHS 398 Modular Budget

Cumulative Budget Information

1. Total Costs, Entire Project Period

Section A, Total Direct Cost less Consortium Indirect (F&A) for Entire Project Period ($)	1,250,000.00
Section A, Total Consortium Indirect (F&A) for Entire Project Period ($)	26,579.00
Section A, Total Direct Costs for Entire Project Period ($)	1,276,579.00
Section B, Total Indirect (F&A) Costs for Entire Project Period ($)	1,190,735.00
Section C, Total Direct and Indirect (F&A) Costs (A+B) for Entire Project Period ($)	2,467,314.00

2. Budget Justifications

Personnel Justification	1248-Personnel_Justification_3 1 2016.pdf
Consortium Justification	1249-Consortium_Justification.pdf
Additional Narrative Justification	

Funding Opportunity Number: PA-13-302. Received Date: 2016-03-07T13:56:59.000-05:00

Schepens Eye Research Institute: Personnel Justification

Key Personnel

Dr. Joseph B. Ciolino, MD, Principal Investigator, [redacted] months (years 1 – 5):
Joseph Ciolino is an Assistant Professor of Ophthalmology at Harvard Medical School. He requests [redacted] salary support for years 1-5. He is a cornea trained-ophthalmologist and published multiple papers on the use of a novel contact lens to provide sustained drug delivery. As a K08 Mentored Clinical Scientist, he has had extensive exposure to polymeric drug delivery systems, methods of contact lens manufacturing and is familiar with all the techniques and methods proposed in this application. Dr. Ciolino will oversee daily laboratory activities; conduct brief daily and detailed weekly lab meetings. He will design and carry out experiments, write and edit manuscripts, and prepare research reports.

Non-key Personnel

[redacted], PhD, Postdoctural Fellow, [redacted] months (years 1 - 5):
[redacted] is a postdoctoral fellow in Dr. Ciolino's laboratory at Harvard Medical School. [redacted] salary support and effort is requested for years 1-5. [redacted] has a PhD in Chemical Engineering and has extensive experience in polymer drug delivery and contact lens drug delivery. He will oversee the day-to-day experimental activities, such as the fabrication of drug-eluting contact lens (for use in Aims 1- 3), *in vitro* drug kinetic studies, quantification of drug tissue levels, cytotoxicity studies, and contact lens characterization.

[redacted], Consultant, (years 1 – 5):
[redacted] is the Director of the Contact Lens Service and currently leads all contact lens related clinical trials at Mass. Eye and Ear. [redacted] will provide guidance on contact lens parameters and physical properties requirements needed for the successful completion of the study.

Consortium Justification:

Principal Investigator, University of Pittsburgh, months (years 1 - 5): will serve as site PD/PI at the University of Pittsburgh. has extensive knowledge of antimicriobial pharmacology, particularly with respect to antibiotics, which is the principle drug class studied in this application. He is one of the leading experts in animal models for bacterial keratitis and will assist in the study design and analysis. He and Dr. Ciolino will talk monthly to discuss experimental progress and results and he will assist with publications. salary support is requested.

MSc, Collaborator, University of Pittsburgh, months (years 1 - 5): is the research director of the Ophthalmic Microbiology Laboratory. He has over 30 years of experience using a variety of infectious keratitis eye models. will assist the PI with supplying clinical isolates, keratitis study designs and with practical experimental considerations regarding bench-top and animal bacteriologic studies in Aims 2 and 3 salary support is requested

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)	1250-Intro_resubmission 3 4 16_final.pdf
2. Specific Aims	1251-Ciolino R01 Aim Final.pdf
3. Research Strategy*	1252-Research Stategy 3 5 16_final.pdf
4. Progress Report Publication List	
Human Subjects Sections	
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Other Research Plan Sections	
8. Vertebrate Animals	1253-VAS_Ciolino.pdf
9. Select Agent Research	
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reviewers' comments

Bacterial keratitis is a leading cause of corneal blindness. The primary medical therapy involves an intensive regimen of antibiotic drops to eradicate the infection. In some cases, surgical approaches are used when the medical management has failed. Even when bacterial keratitis is successfully treated medically, the eye may be left with severe astigmatism or a blinding corneal scar. A major unmet need for the treatment of bacterial keratitis is a sustained method of corneal drug delivery in order to improve treatment efficacy and patient adherence. Contact lens (CL) drug delivery has the potential to achieve this goal; however, providing controlled CL drug release has historically proven challenging.

During the PI's K08, we developed an innovative drug-eluting therapeutic CL (TCL) that incorporates a thin drug-polymer film within the periphery of a standard CL (Fig. 1). In contrast to other TCL designs, our design enabled the release of large drug quantities in a controlled manner over the course of few weeks, while allowing unimpeded vision through the lens, and was composed of materials that are all FDA-approved for use on the eye. We have demonstrated sustained release of therapeutic amounts of an antibiotic (ciprofloxacin), an antifungal agent (econazole), a steroid (dexamethasone) and a glaucoma medication (latanoprost) for up to a month in bench-top studies. In rabbits, the latanoprost-eluting TCL demonstrated safety and sustained drug release for a month. While worn continuously for a week on the eyes of glaucomatous monkeys, the latanoprost-eluting TCLs effectively reduced intraocular pressure as much as latanoprost drops. With a dexamethasone-eluting TCL, we found that the lenses can safely maintain drug concentrations in the cornea that greatly exceeded that of hourly dexamethasone drops. To demonstrate the feasibility of a TCL for the treatment of bacterial keratitis, we separately incorporated three different 4th generation fluoroquinolone (FQ) antibiotics into our TCL design. Of the these lenses, besifloxacin-eluting TCLs provided the most consistent drug levels each day for 2 days *in vitro* (Fig. 2).

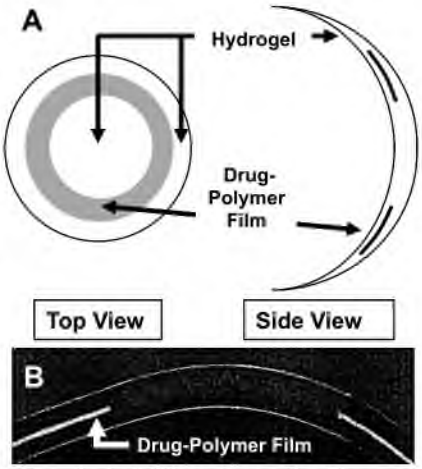

Fig. 1. A. TCL schematic; B. Ocular coherence tomography (OCT) image of TCL.

The underlying hypothesis of this application is that because of continuous administration at doses above the minimum inhibitory concentration (MIC), an antibiotic-eluting TCL can safely and more effectively treat bacterial keratitis than the current standard of care, topical antibiotic drops.

Aim 1. Characterize drug flux, biocompatibility, and physical properties. We will confirm *in vitro* antibacterial effectiveness, perform cytotoxicity studies, evaluate drug flux and biocompatibility in normal rabbit eyes, and characterize the physical properties required for a safe and functional use. Finally, we will work within the framework of our current formulation to extend delivery to 7 days.

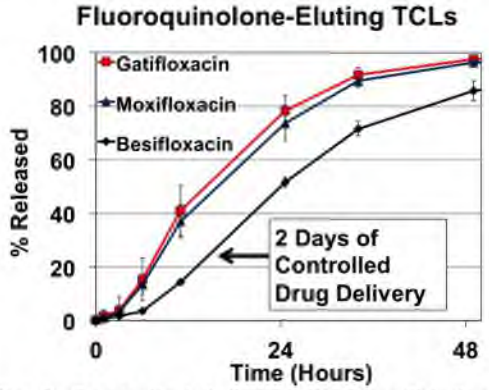

Fig. 2. Controlled delivery of FQ antibiotics.

Aim 2. Evaluation of efficacy against *susceptible* bacteria. We will compare the efficacy of TCLs with the same drug administered as its commercially-available drop formulation for the treatment of keratitis resulting from FQ-susceptible *S. aureus*, cytotoxic *P. aeruginosa*, and invasive *P. aeruginosa.*

Aim 3. Evaluation of efficacy against *resistant* bacteria. When locally delivered at high enough concentrations, FQs can effectively treat "FQ-resistant" keratitis, as defined by current Clinical & Laboratory Standards Institute (CLSI) criteria. To test the hypothesis that TCLs can provide enough antibiotic to overcome resistance, we will compare the treatment efficacy of TCLs, hourly commercial FQ drops, and hourly fortified vancomycin or tobramycin drops for the treatment of bacterial keratitis induced by FQ-resistant *S. aureus* and FQ-resistant *P. aeruginosa,* respectively.

This proposed approach is both feasible and valuable. The TCL can deliver high drug concentrations to the target tissue (cornea) and has been shown to be safe and effective in non-human primates. The TCL addresses a major compliance challenge with the current approach that requires hourly drops day and night. Finally, a TCL may overcome antibiotic resistance by maintaining supra-therapeutic corneal drug levels.

B. Background and Significance

B.1. Bacterial keratitis. Microbial keratitis is a potentially serious infection of the cornea that can result in corneal scarring, perforations, and blindness.[1,10] The treatment of severe infections can lead to hospitalization and surgical interventions.[10] Bacterial keratitis is estimated to cause approximately 30,000 bacterial ulcers in the United States per year[11] and the incidence has been found to be far higher in developing countries.[12]

B.2. Antibiotic drops–An imperfect solution. Eye drops are the mainstay of bacterial keratitis treatment.[13-15] Unfortunately, eye drops are an inefficient delivery system, and the eye absorbs only 1–7% of the medication in a solution and the cornea only receives a fraction of the total ocular absorption.[16] Pharmacokinetic studies suggest that drops only transiently achieve therapeutic drug levels at the ocular surface.[16] Because of their inherent inefficiency, antibiotic drops are initially administered **every hour for at least 48 hours** in an effort to obtain therapeutic drug concentrations within the infected cornea.[1]

Understandably, adherence with such intensive treatment regimens can be difficult. Also contributing to non-adherence, eye drops frequently sting, burn, and cause a transient blurring of vision upon application.[17] These symptoms often worsen with fortified antibiotic formulations.[1] In addition to the intensive regimen and side effects, self-administration of eye drops has been found to be problematic–even in experienced patients who take drops for chronic conditions, such as glaucoma. For example, only 21% percent of glaucoma patients properly instill their eye drops,[18] 29% miss their eye entirely,[19] and up to 38% require assistance.[19,20] Less experienced users of eye drops may have more difficulty with self-administration. Because of patient noncompliance, hospitalization can be required, significantly increasing treatment costs.[21]

Due to the limitations associated with antibacterial drops as the current standard of care, **a method of sustained ocular drug delivery remains a major unmet need for the treatment of bacterial keratitis.**

B.3. Overcoming resistance through elevated drug concentrations. Antibiotic resistance is a global health problem and the evolution of antibiotic resistant bacteria is accelerating faster than the development of new antibiotic compounds.[14,22] Therefore, it has been proposed that we limit the use of the antibiotics of last resort (e.g. vancomycin, linezolid) and better utilize 1st or 2nd line antibiotics, such as fluoroquinolones (FQ), through **more effective dosing regimens that increase drug tissue concentrations**.[23] Importantly, the risk of bacteria evolving new resistance to FQs **decreases** as the antibiotic tissue concentration **increases**.[3] Unfortunately, drops result in a peak and trough drug concentration profile.[24] The likelihood of bacteria evolving new antibiotic resistance increases when the trough levels dip below the concentration required to inhibit the growth of "first-step" resistant mutants (Mutant Prevention Concentration).[3] In terms of the treatment of microbial keratitis due to resistant bacteria, it has been demonstrated that *in vitro* antibiotic resistance, based on CLSI standards, can be overcome *in vivo* by aggressive topical treatment regimens (FQ drops every 15 minutes for 5 hours).[5] Therefore, a method of sustained drug delivery can help overcome resistance by providing the cornea with sustained antibiotic delivery at concentrations that far exceed bactericidal levels.

B.4. Drug-eluting TCLs. Compared to drops, antibiotic-eluting TCLs may be a more effective treatment for bacterial keratitis by continuously providing therapeutic drug concentrations to the cornea and by improving compliance by eliminating the need for frequent drop administration, avoiding the discomfort of drops, and reducing the side effects related to drop overflow and preservatives. Similar to a therapeutic bandage CL used to promote ocular surface healing, an antibiotic-eluting TCL could be inserted and removed by the eye care provider–thus replacing the need for hourly antibiotic drops. Indeed, there is evidence that drug-eluting TCLs would be widely accepted by practitioners. A survey of eye care providers demonstrated that practitioners found that 93% of eye care providers would use a CL to deliver therapeutics, if such a treatment existed.[25]

The concept of drug-eluting CLs dates back to the 1960s; however, sustained and controlled delivery has historically been a significant challenge.[26] CLs have demonstrated the ability to absorb and release drugs, such as antibiotics, but the release is very rapid with most of the drug release occurring in the first several hours.[27] Unfortunately, when CLs provide only a rapid antibiotic burst, there is no reduction in the bacterial load as demonstrated in bacterial keratitis animal models.[28] The duration of antibiotic exposure also appears to be important for the treatment of bacterial corneal ulcers.[28] In an effort to achieve sustained drug delivery, several CL designs propose to either modify the CL hydrogel[29] or incorporate particulate formulations (e.g. nanoparticles, microelmusions,[30] liposomes[31]). While these CLs have demonstrated sustained release for a month or longer in benchtop studies that purportedly mimic ocular surface conditions, they have only demonstrated sustained antibiotic release for **several hours** in animal models.[32]

C. Innovation. Innovative design. In contrast to other drug-eluting CL designs, we have taken a macroscopic approach and have demonstrated sustained ocular drug delivery for one month in animals.[8] Our

TCL design consists of a thin drug-polymer film encapsulated within the periphery of a typical hydrogel CL (Fig. 1),[33] which maintains a clear central aperture that allows unimpeded light and oxygen transmission. Both the drug-polymer film and the encapsulating hydrogel help to modulate drug release.[33]

Since the same drug-polymer film formulation can be incorporated into a wide variety of TCL shapes, drug loading will be uniform for TCLs with nearsighted correction (thin center/ thick periphery), farsighted correction (thick center/ thin periphery), or no refractive correction. This feature distinguishes our approach from other drug-eluting CLs that contains drug dispersed throughout the entire matrix of the hydrogel. Moreover, our TCL design surpasses others in drug-delivery performance: the drug-polymer film enables the loading and controlled release of large amounts of drug for extended periods of time. In benchtop[33,34] and animal studies,[8] this innovative design has shown ≥1 month of sustained release.

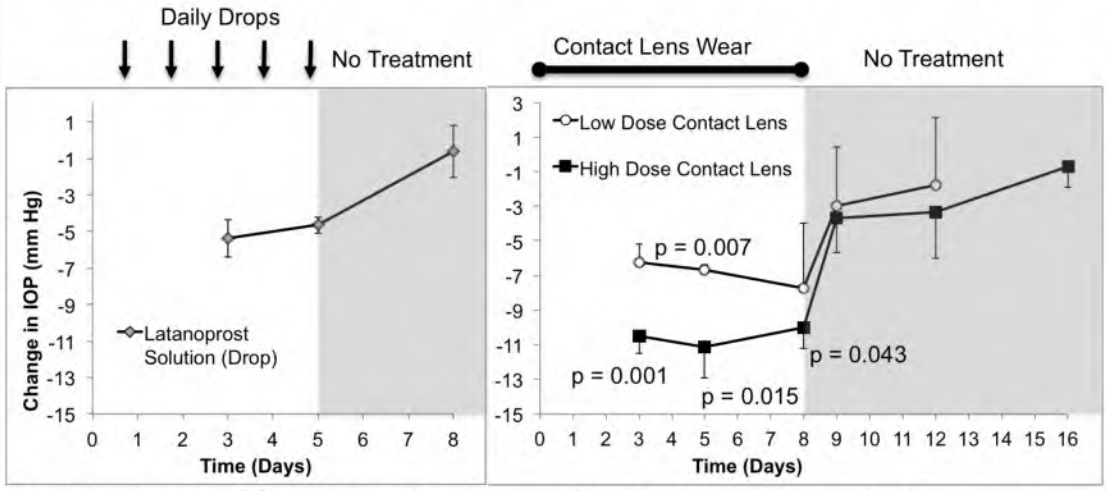

Fig. 3. Latanoprost-TCLs achieved sustained IOP reduction equivalent to or greater than daily latanoprost drops in glaucomatous monkeys (n =4). P value = comparison to drops.

D. Approach

D.0.1. Preliminary data supporting research approach.

D.0.1.a. Drug delivery from a TCL. Using a prototype TCL, we initially demonstrated sustained release of fluorescein (as a model drug) for over one month *in vitro*.[33] We learned that the drug-polymer film ([poly(lactic-co-glycolic] acid [PLGA]) and the encapsulating hydrogel (poly(hydroxyethylmethacrylate) [pHEMA]) affected the release rate, which could be further tailored by altering the composition of the drug-polymer film (e.g., ratio of drug to polymer).[33] Using prototype TCL designs, sustained release was also demonstrated with ciprofloxacin, which maintained its bactericidal properties throughout one month of drug elution,[33] as well as with econazole, which demonstrated dose-dependent fungicidal activity for up to 3 weeks *in vitro*.[34]

For glaucoma treatment, we developed a latanoprost-TCL with a thinner, curved drug polymer film (formed by solvent casting a solution of latanoprost and PLGA with a 1:15 ratio in ethyl acetate) encapsulated in methafilcon, a higher-water-content co-polymer of pHEMA and methacrylic acid.[8] The TCLs were professionally lathed by a contracted commercial contact lens company (Kontur Kontact Lens). The thinner drug-polymer film and precision lathing enabled manufacturing of TCLs that could be safely worn on rabbits. Latanoprost-TCLs demonstrated steady-state concentration in the aqueous humor for **one month—**comparable to the average hourly concentration (C_{AVE}) delivered from a drop of commercial latanoprost solution (50 μg/mL). Drug stability was analyzed by liquid chromatography–tandem mass spectrometry (LC-MS/MS), and the active ingredient eluted from the TCLs was unaltered from its initial form.[8] Smaller-diameter TCLs were fabricated, using similar drug loading, for the eyes of glaucomatous cynomolgus monkeys housed at Mount Sinai School of Medicine. During a week of continuous wear, TCLs demonstrated IOP reduction that was equal to or greater than that of latanoprost drops (50 μg/mL) (Fig. 3; presented at Glaucoma 360 New Horizons Forum, San Francisco, 2016).

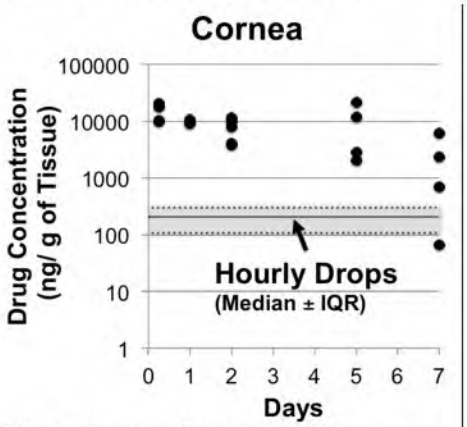

Fig. 4. Dexamethasone-TCLs provided elevated drug levels for 7 days (n=5). IQR = Interquartile Range

We also developed dexamethasone-eluting TCLs and compared their efficacy and drug flux to that of hourly dexamethasone drops (0.1%). Importantly, the continuously worn TCL achieved **significantly higher** corneal drug concentrations than hourly dexamethasone drops (Fig. 4). Not surprisingly, in rabbits, the dexamethasone-TCLs were as effective as hourly drops for the treatment of endotoxin-induced anterior uveitis.

D.0.1.b. Fluoroquinolone (FQ)-Eluting TCLs. To demonstrate the feasibility of TCLs to treat bacterial keratitis, we developed TCLs that separately eluted moxifloxacin, gatifloxacin, or besifloxacin. Drug flux was studied under infinite sink conditions as previously published[8,33] and drug concentrations were quantified using HPLC. Using TCL formulations composed of PLGA 85:15 and a 1:15 ratio of drug to polymer, we found that besifloxacin-TCLs eluted more consistent daily drug levels for 2 days (Fig. 2) at concentrations thought to be therapeutic (as defined in Sec. D.1.1.1.). Aims 2 and 3 will confirm TCL efficacy.

D.0.1.c. Cytotoxicity, Ocular Surface Biocompatibility, and Comfort. We exposed immortalized human corneal limbal epithelial (HCLE) cell lines to latanoprost-TCLs and commercial CLs (Kontur Kontact Lens) also composed of methafilcon but lacking a drug-polymer film. The viability of cells exposed to TCLs was indistinguishable from that of cells exposed to media or commercial CLs.[8] The biocompatibility of latanoprost- and dexamethasone-TCLs was evaluated in rabbits using fluorescein-assisted biomicroscopy. While the intraocular drug levels were greater than drops, there was NO evidence of ocular surface toxicity such as punctate epithelial erosions or fluorescein staining. None of the rabbits demonstrated any evidence of discomfort (eyelid twitching). Latanoprost-TCLs were well tolerated and retained in the eyes of monkeys, which also did not show evidence of discomfort that could have motivated them to remove the TCLs.

Contact Lens Type	Light Transmission (%)	Oxygen Permeability (Dk) (cm^2/s)/(mL O_2/mm Hg)	Elastic Modulus (MPa)*	Water Content (%)	Contact Angle (°)
Commercial CL	96.2 ± 1.3	19.6×10^{-11}	0.24 ± 0.07	50.4 ± 1.1	37.3 ± 6.1
TCL	95.5 ± 1.4	17.1×10^{-11}	0.26 ± 0.09	50.7 ± 0.3	37.2 ± 2.0

Table 1. Physical characteristics (n = 4). * = performed by contracted vendor (Contamac, Inc.)

D.0.1.d. Physical Property Characterization. Physical characteristics that are associated with CL wear and comfort were studied.[35] We compared latanoprost-TCLs to commercial methafilcon CLs (Kontur Kontact Lens) that contained the same monomers as our TCL. Using published methods that are described in D.1.2.4., we found that the inclusion of a drug-polymer film had a negligible effect on the measured values (Table 1), which were consistent with published reports.[36,37]

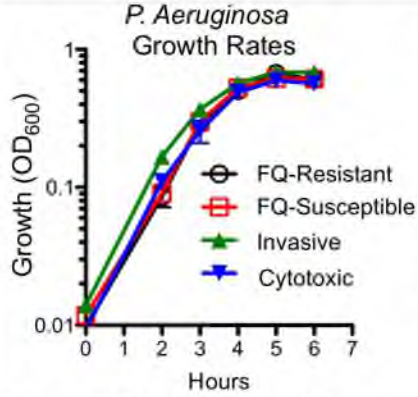

Fig. 5. *In Vitro* Growth Rates.

D.0.1.e. Bacterial Growth Rates. Testing the TCL's antibacterial effectiveness is an important component of this application. Therefore, we have completed the growth curve for the *P. aeruginosa* strains to be studied in this application. Bacteria were diluted in growth medium at 37°C *in vitro*, and culture optical density was used as a measure of bacterial growth. Each *P. aeruginosa* strain demonstrated a similar growth pattern (Fig. 5), which indicates that no one strain should have a growth advantage or disadvantage compared to the other strains. Following corneal intrastromal inoculation in rabbits, the growth kinetics were similar between FQ-resistant (FQ-R) *S. aureus* (K950) and FQ-susceptible (FQ-S) *S. aureus* (K1181) and also between FQ-R *P. aeruginosa* (PA-E) and FQ-S *P. aeruginosa* (K900).[6] Corneal colony counts were similar between *S. aureus* isolates at 4 hour post-inoculation and at 25 hours isolates.[6] The similar fitness demonstrated by FQ-R and FQ-S strains indicates that the isolates are appropriate for use in the proposed studies in this application.

D.0.2. Drug-eluting devices to prevent and treat infections. It may seem counterintuitive to use devices as vehicles for preventing or treating infections because medical devices can serve as a nidus for infection. This concern may be particularly true for CLs, which are a risk factor for bacterial keratitis. However, the concept of using devices and implants to treat infections is not new. In fact, many devices have been proposed and successfully used to treat local infections that are poorly responsive to systemic antibiotics; these devices have been able to achieve a high local antibiotic concentration over an extended period of time.[38] For example, antibiotic impregnated bone cements, fillers and coatings have been used for orthopedic applications for over 40 years.[38,39] A range of sustained drug delivery products are FDA-approved to treat periodontal infections.[38] Wound dressings containing antibiotic-loaded polymers have been studied clinically[38] and Band-Aid® Plus Antibiotic Adhesive Bandages (Johnson & Johnson) are now ubiquitously used throughout the world. Within the eye, Vitrasert® (Ganciclovir) is a sustained-release implant that is FDA-approved for the treatment of cytomegalovirus (CMV) retinitis. There has also been considerable interest in using CLs as a means of antibiotic delivery, but, as noted earlier, sustained drug delivery has been a historical challenge.[26,28,40]

D.0.3. Beyond bacterial keratitis. If successful, TCLs could be used to treat other ocular conditions such as fungal keratitis, uveitis, and glaucoma. Because TCLs may promote greater ocular drug absorption, they may enable the treatment of conditions (e.g., acanthamoeba keratitis) that are difficult to treat with topical solutions. Moreover, should our biodistribution studies demonstrate appreciable drug penetration to the posterior segment of the eye, this device may find application as an adjunctive therapy to intravitreal injections for the treatment of endophthalmitis.

D.0.4. Overview and timeline (Fig. 6). In general, Aim 1 will run concurrently with Aims 2 /3. In Aim 1 we will use our current formulation to study cytotoxicity, drug flux and biocompatibility of FQ-TCLs in normal rabbit eyes, and characterize important physical properties. We will also optimize our current formulation to extend the duration of drug delivery from 2 days to 7 days. In Aim 2, we will test the efficacy of the TCL vs. commercial FQ drops in FQ-S bacterial keratitis animal models. In Aim 3, we will test TCL efficacy against FQ-R *S. aureus* (MRSA) and FQ-R *P. aeruginosa*. In Aims 2 and 3, we will use besifloxacin-TCLs because they demonstrated the most controlled delivery with a similar daily drug flux on study day 1 and 2 (Fig. 2). Importantly, we have demonstrated the feasibility of sustained delivery with moxifloxacin, gatifloxacin, and besifloxacin; and in Aim 1 will we will study all 3 of these FQ-TCLs given that it will allow us to learn more about FQ formulation parameters, drug flux and biocompatibility. Moreover, should concerns arise over the use of besifloxacin-TCLs in Aims 1-3, moxifloxacin- or gatifloxacin-TCLs could serve as contingency TCLs for Aims 2 and 3.

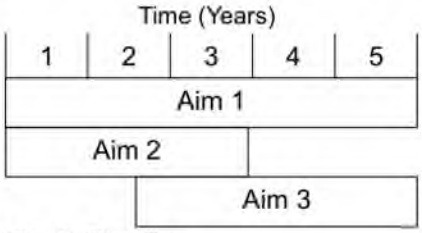

Fig. 6. Timeline.

The Vertebrate Animal Section describes in detail the proposed methods of anesthesia and euthanasia. We will use New Zealand white (NZW) rabbits, which have similar ocular dimensions and drug fluxes to the cornea and aqueous humor as humans.[41] We will perform a permanent lateral tarsorrhaphy (surgically approximating the outer 1/3 of the upper and lower lids) on all study eyes. We found that this procedure does improves CL retention, enabling up to 1 month of TCL wear.[8] Statistical methods are described in Sec. E.

D.0.5. Advisors and Co-investigators. To ensure successful completion of these goals, we have elicited the support of experts in their respective fields. [redacted] are well-respected leaders in material engineering and polymer-drug delivery, and are available to advise on questions related to polymer science. We have also secured support from leaders in the field of ocular bacteriology and bacterial keratitis animal models. Microbiologist [redacted] is internationally recognized for work on antibacterial resistance; if needed, he will provide bacterial isolates from human cases of infectious keratitis, and will advise on experimental design and bacterial growth and MIC studies. Clinical ophthalmic microbiologists [redacted] are experts in the fields of clinical antibiotic testing; they will advise on IACUC protocols, supply clinical bacterial isolates, travel to Boston to assist with the initial animal studies, and return yearly to confirm the validity of the keratitis models. For data analysis, they will meet with the PI at least 3 times a year (in Boston and at the Ocular Microbiology and Immunology Group and ARVO annual meetings). Optometrist [redacted] has advised the PI on CL design for rabbits and monkeys; as a consultant, she will advise on questions related to CL properties.

D.1. Aim 1: Characterize drug flux, biocompatibility, and physical properties.

We have demonstrated that a TCL provides controlled release of FQs at levels thought to be therapeutic for 2 days. **We hypothesize that the TCL can safely and continuously administer an antibiotic to the cornea at doses that are above the MIC for at least 2 days.** Based on our in vitro studies and our prior experience with the TCL as a platform, we anticipate that our TCL can safely provide continuous delivery of FQs for at least 2 days, if not longer, which will allow for sustained tissue drug concentrations that exceeds the MIC for Gram (+) and Gram (-) bacteria. We will also work within the framework of our current formulation to develop a TCL that has extended drug release for up to 7 days.

Aim 1.a. Study the cytotoxicity, bactericidal properties, and long-term stability of the eluted antibiotic.
Aim 1.b. Assess the biocompatibility and drug flux in healthy rabbits.
Aim 1.c. Characterize the physical properties of the TCL.
Aim 1.d. Formulate a TCL capable of 7 days of drug release.

D.1.1. Aim 1. Rationale, design considerations, and experimental strategy. Because FQs are "concentration-dependent" antibiotics, the parameters that correlate most closely with bacteriological success are the peak serum (or tissue) concentration (C_{max}) and the ratio of the 24-hour area under the tissue concentration curve

(AUC_{24}) to the MIC (AUC_{24}:MIC; Fig. 7).[4] Given that the TCL has achieved high ocular drug concentrations with other drugs (e.g., dexamethasone in Sec. C), we anticipate that the FQ-TCL will demonstrate a high C_{max} and a high AUC_{24}:MIC, which will translate into improved clinical effectiveness. We will elute a 4th generation FQ, which have enhanced activity against Gram-positive bacteria, such as MRSA.[42] We have demonstrated controlled release of moxifloxacin, gatifloxacin, and besifloxacin for 2 days; all of these 4th generation FQs have excellent tissue penetration and broad-spectrum coverage that includes Gram (+) and Gram (-) bacteria.[24] For Aim 1, we will individually study moxifloxacin-, gatifloxacin-, and besifloxacin-TCLs.

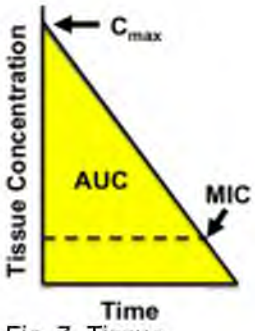

Fig. 7. Tissue Concentration vs. Time Curve.

D.1.1.1. Overview of adequacy. A wide variability of antibiotic drop dosing schedules have been described in both humans and animals for the treatment of bacterial keratitis.[1,43,44] However, most controlled clinical trials administer antibiotic drops intensively **hourly (day and night) for the first 48 hours**.[1,44] This application aims to demonstrate that a single TCL worn for 2 days will be comparable, if not more efficacious, to hourly drops. We achieved our goal of releasing 0.3 mg besifloxacin each day for the first 2 days in benchtop studies under infinite sink conditions. This goal was based on calculations that considered the following: 1) 24 drops (50 μl) of 0.6% besifloxacin (Besivance™) would provide 7.2 mg of antibiotic daily, and 2) we conservatively estimated that the CL will provide 25 times greater corneal tissue concentrations that hourly drops. Our animal studies have demonstrated that a dexamethasone-eluting CL results in 51 times greater corneal tissue concentrations over 48 hours than hourly dexamethasone drops. Diffusion models have estimated that TCLs are 35 times more efficient than drops.[45] Aim 1.b. will help us to better answer the question of adequacy when comparing *in vivo* drug delivery between hourly drops and TCLs.

D.1.1.2. Aim 1.a. Study the cytotoxicity, bactericidal properties, and long-term stability of the eluted antibiotic. Using LC-MS/MS, we previously demonstrated that the TCL manufacturing process did not alter the molecular structures of latanoprost or dexamethasone.[8] Moreover, eluted drugs such as ciprofloxacin,[33] dexamethasone, and latanoprost maintained their effectiveness (Sec. D.0.1.a.). We will confirm that the same is true of FQs, which appeared unchanged from the reference standard drug using HPLC (Sec. D.0.1.a.). Using LC-MS/MS analysis of the release samples from new lenses and those stored at room temperature for **3 months, 6 months, 1 year, and 2 years, we will be able to assess the short- and long-term stability of FQs** and detect the presence of impurities that may result from the manufacturing process. We will also study FQ efficacy against *S. aureus* and *P. aeruginosa*, which are the most commonly encountered gram (+) and gram (-) bacteria, respectively, in industrialized countries.[46] Using known antibiotic concentrations in bacteriology studies, we will study the eluted drug from days 1 and 2. As in our previous study,[33] we expect that the eluted antibiotic will kill drug-susceptible bacteria, and that the eluted antibiotic will not kill drug-resistant bacteria at concentrations that conform to CLSI criteria. We will include FQ-R bacteria to ensure that the killing is due to bactericidal properties of the drug and not due to residual solvent or polymer breakdown products. While we have NOT observed evidence of cytotoxicity using latanoprost- TCLs that were made using the same solvents, polymers, and hydrogels as FQ-TCLs,[8] we will assess FQ-TCLs for cytotoxicity using mammalian cell culture studies.

D.1.1.3. Aim 1.b. Biocompatibility, ocular surface analysis, and drug flux in healthy rabbits. In general, the correlation between *in vitro* and *in vivo* findings is notoriously poor. While we have demonstrated a high level of correlation between our benchtop and animal drug flux studies using a latanoprost-eluting CL,[8] this level of correlation may not be the same for TCLs that elute FQs. Therefore, we aim to demonstrate that the TCL can provide drug concentrations to the aqueous humor that are greater than hourly drops and that maintain these drug levels for at least 2 days. While the cornea is the target tissue for the treatment of bacterial keratitis, we will initially use aqueous humor fluid as a surrogate for tissue concentrations given that aqueous humor can easily be sampled in non-terminal studies. Our laboratory has extensive experience with aqueous humor drug flux studies using other small molecules (e.g., dexamethasone and latanoprost).[8]

It is also important to assess the biocompatibility and health of the ocular surface. Toxicities, such as chemical conjunctivitis and punctate erosions, have been reported with frequent FQ drop use and with chronic use of latanoprost.[9,47,48] Importantly, these side effects have been attributed to the preservatives (e.g., benzalkonium chloride) in the solution[49] and our **TCL are preservative-free**. Therefore, it is not surprising that we have not detected any evidence of ocular surface toxicity resulting from dexamethasone- or latanoprost-eluting TCLs in rabbits.[8] **This is particularly important given that the corneas of rabbits are more prone**

to develop drug toxicity than human corneas.[16] We anticipate that FQ-TCLs will not result in ocular surface toxicity and will study the ocular surface health using fluorescein-assisted biomicroscopy.

D.1.1.4. Aim 1.c. Physical property characterization. The physical properties of a CL can affect the physiological impact, fitting characteristics, and ocular compatibility of a CL.[35] When we previously compared the physical properties of latanoprost- TCLs and commercial CLs made of the same hydrogel (methafilcon), we did not note any differences (see Table 1). We anticipate that the physical characteristics of our TCL will not change with the substitution of FQs. Here, we will characterize the physical properties and compare them to commercial CLs. If we find that any of the measurements are outside of the range of those found in commercial CLs, then we will modify the TCL formulation in a way that address the parameter of concern (see D.1.3.).

D.1.1.5. Aim 1.d. Formulation for 7 days of drug release. Our current FQ-TCL formulation consists of a drug-polymer film containing a 1:15 ratio of drug to PLGA (85:15) that is approximately 50 microns thick and encapsulated in a methafilcon hydrogel. The TCL was constructed using our published methods.[8] This formulation has demonstrated therapeutic delivery of FQ for 2 days (Fig. 2). We will work within the framework of this formulation to increase duration of drug flux to 7 days, as recommended by the reviewers. An advantage of our TCL design is that we can modulate the drug release profile by changing the composition of the drug-polymer film.[33] We anticipate that we can increase the duration of drug release for up to 7 days by increasing the drug loading and by modified the TCL formulation. Below is a sequence of interventions and contingency plans that will be used singly or in combination to allow for 7 days of therapeutic drug release:

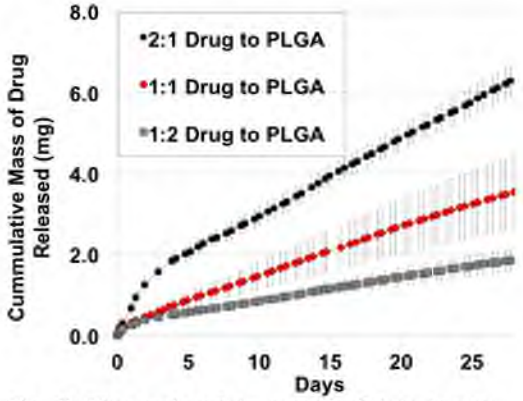

Fig. 8. The ratio of drug to PLGA helps to modulate the release rate.

i. Increase loading of both the drug and polymer. Keeping the ratio of the drug to polymer the same, we will increase the thickness of the drug polymer film by increasing the loading of the drug and polymer within the solvent that is used to make the drug-polymer film through solvent casting. Clearly, there are limits to this approach and the drug-polymer film should remain thinner than approximately 90 microns so that it does not interfere with the physical properties of the CL.

ii. Drug to polymer ratio. As noted previously, the release rate is influenced by the drug to polymer ratio (Fig 8).[33] As the percentage of polymer increases, drug diffusion from the TCL is slowed and the opposite is also true. Therefore, we can slow the release rate by increasing the polymer to drug ratio.

iii. Change polymer. Drug release from polymeric films has been shown to vary depending on polymer characteristics such as the polymer molecular weight, crystallinity, and copolymer ratio.[50] However, the most important characteristic for slowing the release rate could be difficult to predict and may depend on drug-polymer interactions.[51] If we need to change the release profile, then we will continue to work with PLGA since we have been able to demonstrate a range of sustained drug-release profiles using the copolymer. Within a PLGA system, increasing the ratio of lactide to glycolide increases the copolymer hydrophobicity and decreases the degradation rate. Biodegradability is not a primary consideration except to the extent that the degradation affects the release profile. Therefore, we could also use non-degradable hydrophobic polymers such as nylon, polyurethanes (whose hydrophobicity can be readily tuned to match the drug based on the length of the hydrocarbon chains between the urethane bonds) and poly (ethylene co-vinyl acetate). In fact, non-degradable polymers might be advantageous because they could potentially be stored in a hydrated state, which could offer additional convenience and a longer shelf life.

D.1.2. Aim 1. Methods.

D.1.2.1. TCL fabrication. While we have demonstrated that we can fabricate TCLs using either mold[34] or lathe construction,[8] we will employ the lathe technique here since it allows for greater design flexibility. This fabrication method is similar to the laminate construction technique used by the CL industry for the production of colored cosmetic CLs.[52] Our laboratory has over 5 years of experience using this process with the assistance of contracted commercial CL companies.

Drug-polymer films will be formed by solvent casting and then encapsulated in hydrogel. First, a solution of drug and polymer in organic solvent is pipetted onto a concavity lathed into a cylinder of dry polymerized hydrogel. The drug-polymer film is formed through solvent evaporation during approximately 6 minutes of rotation on a spin-coater. A 6-mm central aperture is then created within the drug-polymer film. Next, the

hydrogel containing the drug-polymer films is desiccated under vacuum and lyophilized to remove residual solvent. The side of the films not yet in contact with hydrogel will be encapsulated in hydrogel by UV photopolymerization. The hydrogel block is lathed (Kontur Kontact Lens) into a TCL that contains a fully encapsulated drug-polymer film. In a temperature-controlled container, the TCL is then terminally sterilized by a Gamma Cell 220E Cobalt 60 Irradiation Unit (Atomic Energy of Canada Ltd) with a total dose of 25 kGy. Gamma irradiation has been shown to have minimal influence on the properties of FQs or PLGA.[53-55]

D.1.2.2. Aim 1.a. Benchtop performance evaluation. i. Determination of drug content and morphology. Each film will be completely dissolved in 1 mL ethyl acetate and dialyzed for 48 hours against 750 mL deionized water using a Spectra Por® regenerated cellulose membrane with molecular weight cut off 1000 Da (Spectrum Labs). From the buffer, we will identify and quantify drug metabolites using LC-MS/MS. We will examine TCL morphology by OCT (Optovue Inc.) to determine uniformity and stability of the hydrogel and drug-polymer film (as shown in Fig.1.b).

ii. Benchtop release kinetics and analysis of long-term drug stability. Given that the shelf-life of most drugs is 2 years,[56] we will study the long-term stability of the drug within TCL over this duration. TCLs will be stored at room temperature for **3 months, 6 months, 1 year, and 2 years.** Stored and naïve TLCs will be submerged in 5 mL PBS solution, incubated at 37°C, and placed on a rotational shaker at 65 rpm. In order to maintain infinite sink conditions that ensure that the release of the drug is not influenced by elevated drug levels in the release media, the TCLs will be placed in fresh 5 mL of PBS and changed at least twice a day. Aliquots of the PBS release media will be sampled and stored at 4°C until quantification by HPLC and/or LC-MS/MS.

iii. Antimicrobial Assay. After the concentration is quantified, FQ-containing samples from the release study will tested for antibacterial effectiveness against common corneal ulcer pathogens: *S. aureus* (Gram-positive) and *P. aeruginosa* (Gram-negative). For each *S. aureus* and *P. aeruginosa,* bacterial killing will be tested against 3 clinical test isolates of FQ-S bacteria and one isolate of FQ-R bacteria. The resistant strain will serve as a positive control to assure that the bacterial killing is due to the eluted antibiotic and not to an unknown inhibitory excipient. Release medium without bacteria will be used as a negative control. The clinical ocular isolates will be obtained from the Massachusetts Eye and Ear Clinical Laboratory or the University of Pittsburgh Medical School (see Gilmore and Romanowski letters), where they were recovered from human ocular infections. Examples of clinical isolates to be used include the following FQ-S bacteria: A) *S. aureus* (K1181), B) cytotoxic *P. aeruginosa* (PA14), and C) invasive *P. aeruginosa* (PA01) that have a (MIC < 1 μg/mL). We will also include following FQ-R bacteria: *S. aureus* (E277; MIC > 12 μg/mL) and *P. aeruginosa* (K1278, MIC > 64 μg/mL). As described in our previous work, all samples will be tested in triplicate.[33] The MICs for all bacterial isolates will be determined by CLSI methods.

iv. Cytotoxicity. TCLs contain PLGA, which is FDA-approved for both ocular and systemic drug delivery devices. PLGA is also well known for its biocompatibility and ability to control drug release kinetics.[57] However, it is possible that the antibiotic, residual solvent, or polymer breakdown products could potentially cause local toxicity.[58] Thus, we will conduct cell culture studies to investigate this possibility. As previously described,[8,59] we will plate human corneal limbal epithelial cells on the bottom surface of 3.0-μm pore size Transwell® plates (Corning Life Sciences) and incubate them with a Transwell® insert holding either 1) CL with no drug or polymer, 2) TCL, 3) vehicle CL (polymer, no drug), or 4) media only. Cell viability will be assessed using a commercially available colorimetric assay (MTT viability assay kit, CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay, Promega) after 24 hours of exposure. Results will be reported as mean ± standard deviation of measured absorbance normalized to absorbance for non-treated control cells (% normalized cell viability = 100 X absorbance for CL-treated cells / absorbance for non-treated cells) as previously described.[8]

D.1.2.3. Aim 1.b. Biocompatibility and drug flux in healthy rabbits. i. Biocompatibility on a normal ocular surface. The eyes of NZW rabbits are large enough to wear a human-sized CL and are frequently used to evaluate the biocompatibility of CLs,[60] ocular drug flux of pharmaceuticals,[41] and models of bacterial keratitis.[43] We will study biocompatibility in rabbits using: 1) one week of continuous TCL wear, and 2) hourly commercial antibiotic solution (for humane and practical reasons, we will only administer 8 drops for consecutive 8 hours a day for 5 days). We will monitor for evidence of toxicity by gross observation of animal behavior (e.g., blepharospasm), fluorescein-assisted biomicroscopy, and cornea thickness measurement on normal eyes. We will perform slit lamp photography before the study (baseline) and then every other day until the last day of the study. We will record signs of conjunctival inflammation using a 4-point scale and assess cornea staining with fluorescein (a sign of ocular surface toxicity) using the NEI dry eye scale. Corneal thickness (a surrogate for cornea endothelial health) will be measured by ultrasound pachymetry on day 0 and the last treatment day.

Treatment arms will be compared. If the rabbits show signs of discomfort, the TCLs will be removed. Under anesthesia, the eyes and TCLs will be examined to determine the cause of the irritation and how the TCL design can be modified to eliminate irritation. If toxicity is observed, then we will also study the biocompatibility of a CL that contains a polymer film and no drug to determine if the toxicity is from the polymers, excipients, or the drug. If the drug is suspected as the cause of toxicity, then we will correlate these findings with the aqueous humor drug concentrations obtained during the ocular drug flux study.

ii. Comparative study of ocular drug flux. We will compare the ocular drug flux into the tears, aqueous humor, and serum from TCLs to the commercially available antibiotic drops in rabbit eyes. While we aim to deliver the same amount of drug as hourly drops for at least 2 days, the TCLs may demonstrate drug delivery for a longer duration. Therefore, we will compare the drug flux during the first 7 days of release from TCLs to the C_{AVE} of hourly drops. 5 μl of undiluted tears will be collected from inferior fornix using 10 μl capillary tubes. To collect the aqueous humor from anesthetized rabbits, the TCL will remain on the eye and will be gently slid superiorly in order to perform the anterior paracentesis with a 31-gauge needle along the superior cornea after the application of 1% proparacaine as described.[8] Serum will be collected from the marginal ear vein. All collected fluids will be stored at -80ºC until quantified by LC-MS/MS.

ii.a. Antibiotic drop drug flux. To calculate the $AUC_{0\text{-}60\ minutes}$ from the hourly topical antibiotic regimen, we will apply antibiotic drops on the rabbit's eye every hour for 8 consecutive hours (8 drops total) and then sample the aqueous humor, tears, and serum in anesthetized rabbits at various times (20, 40, and 60 minutes) after administration of the 8th drop. It is important to note that a rabbit will only have one aqueous humor sample taken per day and separated by at least 72 hours. We will calculate the $AUC_{0\text{-}60\ minutes}$ using the trapezoid rule[61] and then calculate the average concentration per minute (C_{AVE}) by dividing the $AUC_{0\text{-}60\ minutes}$ by 60. We are applying multiple doses because the intraocular absorption of topical drops has been shown to increase with repeated administration[62] and plateau after 3-4 doses.[63] Because the exact number of doses needed to reach a plateau concentration may vary between drugs, we will administer 8 consecutive hourly drops prior to sampling the aqueous humor (ii.b) or assessing drug tissue concentrations (ii.c).

ii.b. TCL drug flux. TCLs will be inserted on one eye of an anesthetized rabbit and kept in place for 1 week. At specified times after TCL insertion (2, 4, 8, 24 hours; and 2, 3, 5, and 7 days) we will collect tears (10 μL), aqueous humor (100 μl), and serum (1 mL). As in our previous studies with latanoprost[8] and dexamethasone, a rabbit will only have one aqueous humor sample taken per day and that each aqueous humor sampling will occur on different days that are separated by at least 72 hours. Therefore, multiple TCLs will need to be studied in order to obtain samples for all the time points. As in our previous studies,[8] the $AUC_{0\text{-}7\ Days}$ will also be calculated using the trapezoid rule.[61] **Importantly, we will also compare the drug flux between healthy eyes and infected eyes (aqueous humor collection from Aims 2 and 3) to determine if the drug flux is altered by the tearing and inflammation associated with microbial keratitis.**

ii.c. Tissue distribution in healthy eyes. We will apply the TCL on one eye and then the rabbits will be euthanized at 4, 8, 24, and 72 hours post-administration. Samples will be collected from the tears, serum, cornea, conjunctiva, sclera, aqueous humor, iris/ciliary body, lens, vitreous, and retina of both eyes to allow us to evaluate the contribution from any systemic absorption. In addition, antibiotic drops will be applied to the rabbit eyes every hour for 8 hours and then the same tissues will be collected. Thawed vitreous and solid tissue will be homogenized with 2.8 mm ceramic beads for two 30 sec intervals at the speed of 6.30m/s (Bead Ruptor). Solid tissue will be placed in a solution of 10% methanol in water prior to homogenization. Samples will be centrifuge at 14,000 RPM, at 4°C for 5 minutes to separate the tissue from the sample. Liquid samples will be collected and drug will be separated using liquid-liquid extraction. Samples will be dried, reconstituted with 10% methanol and stored at 4°C until drug concentration measurement using LC-MS/MS.

D.1.2.4. Aim 1.c. Physical property characterization. i. Light transmittance. As in our pilot studies (Table 1), we will measure light transmittance of TCL formulations using a Cary 500i UV-Vis/NIR spectrophotometer with diffuse reflectance accessory (Agilent Technologies). Hydrated TCLs will be placed in a 5x20mm quartz cuvette (Lambda X, Nivelles) filled with PBS. A 6mm aperture will be positioned between the cuvette and the beam entrance port to reduce the beam size to 6mm per ISO 18369-3 guidelines.[64] We will calculate average light transmission by averaging the transmission over the visible light spectrum (390-700nm). Here, we will aim for light transmission of ≥96%, which is found in commercial CLs (Table 1). ii. O_2 transmissibility will be obtained with a polarographic sensor having a 4mm-diameter cathode with a 7.80mm radius of curvature (Rehder Development Company) connected electronically to an O_2 Permeometer Model 201T (Createch, Inc.).[65] The preliminary amperages are achieved at 35°C in a water-saturated humid atmosphere containing

20.9% O_2. iii. Equilibrium water content will be determined by the standard gravimetric method, [64] which is the calculated ratio of the weight of the hydrated CL relative to the dehydrated CL. i.v. Water contact angle will be measured using the sessile drop technique, which is a commonly used method to assess the wettability of CLs.[66] A sessile drop of de-ionized water will be placed on a hydrated TCL and the contact angle the drop makes with the CL surface will be measured with a Drop Shape Analyzer (DSA100, Kruss GmbH). v. Elastic modulus is considered one of the most important quantifiable mechanical properties of a CL[35] and will be measured by a contract vendor (Contamac, Inc.) using a tensometer equipped for CL testing. Cross-section strips (2mm) of hydrated CLs will be placed in a tensometer and held under tension, which will be gradually increased until the sample breaks.[37] Each of the above studies will be performed on TCLs and on commercial CLs composed of the same hydrogel (lacking a drug-polymer film). As with latanoprost-TCLs (Table 1), we will anticipate that the values will be the same as commercial CLs. We will compare the physical properties of naïve TCLs to those of TCLs that have been worn on infected eyes (Aims 2 and 3).

D. 1.2.5. TCL formulated for 7-day release. We will work within the framework of our current besifloxacin-TCL formulation that demonstrated 2 days of controlled release and is composed of 1:15 (drug: PLGA [8515]). 1) Drug / polymer loading: we will simultaneously study the release rate of TCLs composed of 50% and 100% greater drug and polymer loading. 2) Decreased drug to polymer ratio: we will both increase the drug loading and add proportionally more polymer to decrease the drug: polymer ratio (1:20 and 1:30). 3) Change polymer: we will formulate TCLs composed of PLGA 50:50, PLGA 95:5, PLA, polyurethane, and nylon. After fabricating the TCL, we will study drug flux using the methods that we previously described.[8] We will later compare the daily in vitro release of the 7-day TCLC to those obtained with our current 2-day TCL formulation. For besifloxacin, we aim to release 0.30 mg of drug a day for 7 days in benchtop studies. If we find that the drug elution rate is too low or does not elute for long enough, then there are formulation components (e.g. antibiotic and hydrogel) that we can alter to modulate the release kinetics. (see below)

Hydrogel	Water Content	Ionic Character
LotrafilconA	24%	Non-ionic
Balafilcon A	36%	Ionic
Polymacon	38%	Non-ionic
Methafilcon	55%	Ionic

Table 2. Candidate hydrogels.

D.1.3. Pitfalls, limitations, and solutions. **(1)** We have demonstrated our TCL to deliver large quantities of medication. While we expect to achieve similar results with besifloxacin, it is possible that its release profile could differ from our anticipated goal of at least 0.3 mg of daily drug release for 7 days. There are several ways that we can modify our TCL formulation to change the release profile:

i. Change hydrogel. If we are unable to demonstrate optimal delivery profiles, then we will change the encapsulating hydrogel with the primary goal of improving the release kinetics. Using pHEMA-based hydrogels, we have shown that the surrounding hydrogel helps to modulate the drug release.[33] We hypothesize that we can slow drug release from the TCL by incorporating a low water content hydrogel into our TCL design. Ionic character has been shown to modulate ciprofloxacin release from a CL.[67] Therefore, we will evaluate the effect of a hydrogel's ionic character on release kinetics by using Lotrafilcon A or Polymacon, a non-ionic hydrogel (Table 2).

ii. Change antibiotic (Table 3). While single physiochemical properties may not dramatically affect release kinetics, hydrophobicity and molecular weight in combination could significantly alter TCL release rate. Drugs that have higher water solubility (e.g. gatifloxacin) may enable greater drug loading into the drug-polymer solvent system that is used to create the drug polymer film and higher drug loading may result in prolonged durations of drug release.

Drug	Molecular Weight (g/ mol)	Water solubility (mg/mL)
Moxifloxacin	401	19.6
Gatifloxacin	375	40.0
Besifloxacin	430	0.143

Table 3. Candidate antibiotics

2) If residual organic solvents are eluted and prove to be cytotoxic, then we can change solvents or make films using methods that avoid organic polymers, such as compression of polymer with drug crystals (i.e., using a press). **(3)** If there are signs of physical irritation (e.g. linear corneal staining) we will modify the TCL design, such as the thickness, diameter, or shape. Similarly, if there are signs of drug toxicity (conjunctival hyperemia, anterior chamber cells, and diffuse cornea epithelial staining) and the amount of drug eluted per day is greater than the drop $AUC_{0\text{-}60\ minutes}$, then we will reduce the drug elution rate using the principles outlined above. **(4)** If we find that the light transmittance is inadequate, we can potentially increase it by enlarging the diameter of the central aperture. **(5)** If hypoxia (corneal edema) occurs, we will retest the TCL O_2 transmissibility. If we find that the O_2 transmissibility is suspected to be the etiology, then we will change to a hydrogel that has a greater O_2 transmissibility or we could also reduce the thickness of the TCL, which has been shown to increase O_2 flow through a CL.[68] Both approaches could be successfully employed without changing the drug-polymer film and the properties of the film that affect drug delivery.

D.2. Aim 2: Evaluate TCL efficacy against *susceptible* bacteria. We hypothesize that TCLs can treat bacterial keratitis more effectively than topical antibiotic drops. Because TCLs can provide sustained drug delivery to the cornea at levels that exceeds that of drops, we anticipate that TCLs will be more effective than hourly antibiotic drops for the treatment of bacterial keratitis in well-established rabbit models.

Aim 2.a. To compare antibacterial effectiveness between TCLs and hourly antibiotic drops.
Aim 2.b. To determine if the antibacterial effectiveness is sustained through 2 days of continuous release.
Aim 2.c. To study drug flux into infected (inflamed) eyes.

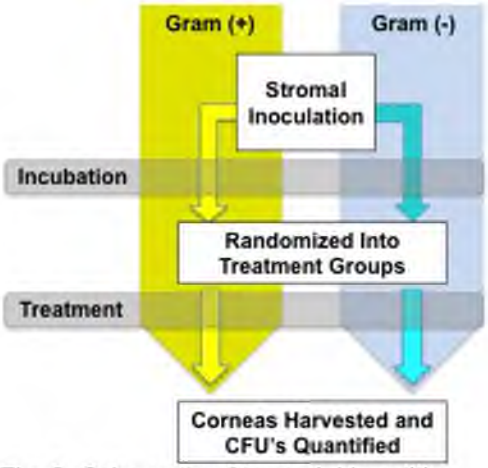

Fig. 9. Schematic of bacterial keratitis study design. CFU = colony forming units.

D.2.1. Aim 2. Rationale, design considerations, and experimental strategy.

D.2.1.1. Bacterial keratitis animal model. To test the TCL's bactericidal effects, we will use a well-established rabbit bacterial keratitis model and perform the following steps (Fig. 9): 1) Inject bacteria into the corneal stroma; 2) incubate to promote bacterial proliferation; 3) determine colony-forming units (CFU) at the end of the incubation period for one group of animals and initiate the other treatment groups; 4) harvest, homogenize, and serially plate corneas; and 5) quantify CFUs. We will study TCL efficacy against keratitis induced by the following FQ-S bacteria: A) *S. aureus* (K1181), B) cytotoxic *P. aeruginosa* (PA14), and C) invasive *P. aeruginosa* (PA01).

Rabbit bacterial keratitis models were developed to determine the efficacy of antibiotics to eradicate bacteria in the cornea.[6] The incubation period (or duration of infection prior to treatment) is important, and varies between bacteria. For *S. aureus*, a 4-hour incubation period allows the bacteria to be eradicated, whereas bacterial eradication becomes problematic with longer incubation periods.[6] For the same reasons, we will use a 16-hour incubation period for *P. aeruginosa* prior to initiating the treatment arms.

The experimental design includes two non-treatment experimental groups (Fig. 10). Group 1 (baseline) will determine the colony count in the inoculum at the end of the incubation period and at the onset of the other treatment groups. Group 2 (2nd non-treatment group) will allow us to confirm that the bacteria are replicating and that the severity of the condition will worsen over the 8-hour period. Group 3 animals will receive one drop of besifloxacin solution (0.6%) hourly for 8 hours, which is a regimen commonly used in bacterial keratitis clinical trials.[1] Group 4 will receive a new (naïve) TCL in the affected eye, and Group 5 will receive a TCL that has already released drug for one day in a normal rabbit's eye (see Sec. D.2.1.3). Group 6 will receive vehicle CLs with the same hydrogel and polymer film as the TCL, but lack drug; this will allow us to ensure that antibacterial effects are from the eluted FQ, not from excipients that may be released by the polymers or hydrogels. At end of the study, the colony counts from Groups 3-6 will be compared to the Baseline Group to determine whether there is a bactericidal effect (i.e. a decrease in colony counts contained in the inoculum) and not just an inhibition of bacterial replication between the onset of therapy and the time of euthanization.

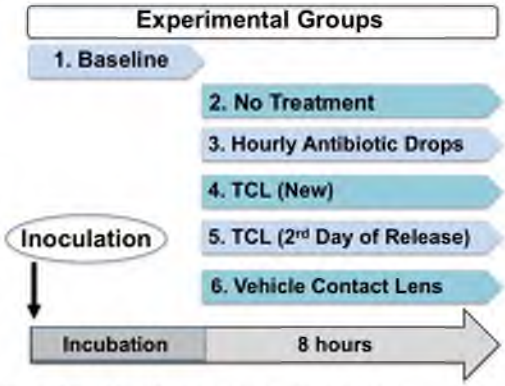

Fig. 10. Aim 2 experimental groups.

D.2.1.2. Corneal epithelial barrier. The corneal epithelium acts as a barrier to penetration for drugs and most bacteria (e.g., *S. aureus* and classical cytotoxic *P. aeruginosa*).[69,70] In fact, bacterial keratitis is rare in corneas that do not have a compromised epithelium.[71] Using bacteria that cannot penetrate an intact epithelium (*S. aureus* and cytotoxic *P. aeruginosa*), **we will study effectiveness in keratitis models that have an intact epithelia and, in separate studies, an overlying epithelial defect.** The intrastromal keratitis model that features an intact epithelium provides a maximum impediment to treatment, and if a TCL can treat a deep stromal infection with the epithelial barrier intact, it should also be able to treat a superficial infection with an epithelial defect. It is important to also study the effectiveness in keratitis models that have an epithelial defect in an effort to reproduce the most commonly encountered clinical presentation of bacterial keratitis.[10] We will also study effectiveness against invasive *P. aeruginosa*, which has the unique ability to invade an intact epithelium; for these studies **we will use a keratitis model has an intact epithelium.**

D.2.1.3. Tissue drug distribution in inflamed eyes. Due to the inflammatory response caused by bacterial keratitis, the drug flux from the TCL may be different in infected eyes compared to healthy eyes.[72] Similarly, ocular inflammation could alter the TCL and the release kinetics. Therefore, at the conclusion of the efficacy studies, we will harvest the ocular tissues and quantify drug tissue levels in the manner described in Aim 1.b. As part of the analysis, we will compare drug tissue concentrations between days 1 and 2 of TCL wear on inflamed (infected) eyes to days 1 and 2 of TCL wear on normal eyes from Aim 1.b.

D.2.1.4. Sustained bactericidal effects for 2 days. If we only study efficacy of a new (naïve) TCL, we will not know if the TCL provides the same anti-bacterial effectiveness on the second day of use. Importantly, this cannot be studied by simply extending the keratitis experiment for 2 days because of ethical considerations to prevent pain and distress to the animals. Therefore, we will quantify the bactericidal effects of the 2nd day of lens wear (Group 5) by initially placing the TCLs on the eyes of healthy NZW rabbits, removing the TCLs after 24 hours of wear, and placing them on recently infected eyes. We understand that there are limitations to this study design since the inflamed eye could influence drug delivery on the 2nd day. As noted above (Sec. D.2.1.2), we will compare the drug flux between infected eyes and normal eyes in order to better understand the influence of inflammation on drug flux. If we find that there is more than a 10% difference, then we will remove the TCLs from the rabbits after one day of wear (from Group 4) and place them on a recently infected eyes of a different rabbit (Group 5). By doing so, the TCL will have been worn for 2 days by 2 separate animals with infectious keratitis. One potential concern with this approach is that the TCL could conceivably carry bacteria from one eye to the next. Conversely, the pre-release could take place *in vitro*, however this would not perfectly replicate the environmental exposures (e.g. tear turnover and lid movement) that exist on a living eye.

D.2.2. Specific Aim 2. Methods.

D.2.2.1. Bacterial keratitis. For studies involving *S. aureus* or cytotoxic *P. aeruginosa*, we will study effectiveness in separate keratitis models that either have an intact or denuded epithelium. For the latter studies, we will create a 5mm corneal epithelial defect using an Amoils epithelial scrubber, which will only remove the epithelial layer of the cornea. Using a 100-μl Hamilton syringe and a 30-gauge 0.5-in needle, we will inject 25 μl containing 1000 CFU of *S. aureus* or *P. aeruginosa* into the corneal stroma (under the epithelial defect, if present). The first group of rabbits will be euthanized after the post-inoculation incubation period (4 hours for *S. aureus* and 16 hours for *P. aeruginosa*), and the remaining animals will be randomized into a non-treatment arm and 4 treatment groups: commercial antibiotic drops administered hourly for 8 hours, a new TCL, a TCL that has been pre-worn for at least one day, and a vehicle CL (as shown in Fig. 10). Following the 8-hour treatment period, we will remove the TCL from the animals in groups 4-6, and examine the eyes of the remaining rabbits using a portable slit-lamp. At the conclusion of the treatment periods, a masked examiner will grade the signs of infection (blepharitis, iritis, conjunctivitis, corneal edema, and corneal infiltrates) using the McDonald-Shadduck score slit lamp scoring system.[73] The TCL will be removed and the physical properties examined as in Aim 1.c. A 31-gauge needle will be inserted at the corneal limbus of anesthetized rabbits to collect 100 μl of aqueous humor, which will be frozen at -80°C. After euthanasia, a 9.5mm corneal button that includes the inoculation site will be excised from the cornea and added to 1 ml PBS. The remaining eyes will be stored in -80°C for quantification of drug tissue concentrations. The fresh corneal buttons will then be homogenized using a Pro Scientific motorized homogenizer under a laminar flow hood. We will make 10-fold dilutions from these homogenates, and plate 0.1 ml of the dilutions in duplicate on trypticase soy agar supplemented with 5% sheep blood. As noted earlier, we will compare the colony counts (CFU/ml) of the treatment arms to Group 1 (baseline), which represents the colony count at the initiation of treatment. Frozen eyes will be thawed and dissected so that the tissue drug concentrations can be quantified by LC-MS/MS. We will compare drug tissue levels between infected and normal eyes (Aim 1.b.)

D.2.3. Pitfalls, limitations, and solutions. **(1)** If the TCL fails to eradicate bacteria as well as topical drops on Day 1, then we will increase the drug loading of the TCL or attempt to increase the release rate over the first day (potentially by changing FQ to moxifloxacin) **(2)** If the new TCL (Group 4) eradicates bacteria, but the Day 2 TCL (Group 5) is not effective, then we will change the TCL formulation to increase drug loading and decrease the release rate, thus allowing more drug to be eluted on Day 2. **(3)** If severe symptoms are noted during the exams, then the drug treatments will be discontinued and the rabbit will be euthanized.

D.3. Aim 3: Evaluate TCL efficacy against *resistant* bacteria.

We hypothesize that TCLs can safely and effectively treat FQ-R *S. aureus* (MRSA) and FQ-R *P. aeruginosa* keratitis as well as their current standards of care (fortified vancomycin and tobramycin

drops, respectively). Due to continuous administration to the cornea at local doses above the MIC, a TCL may effectively treat FQ-R keratitis.

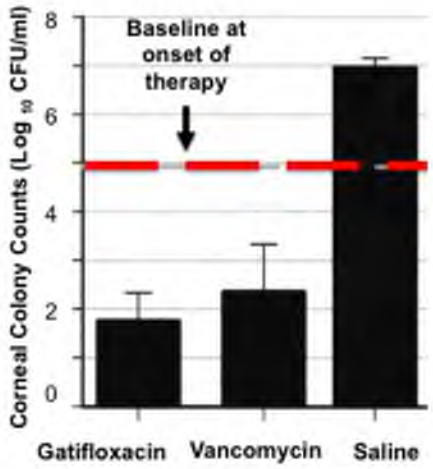

Fig. 11. Colony counts for gatifloxacin were similar to that of vancomycin.[5]

D.3.1. Aim 3. Rationale, design considerations, and experimental strategy. Additional clinical rationale for studying TCL efficacy against FQ-R bacteria are outlined in Sec. B.3. In accordance with the CLSI, *in vitro* susceptibility and resistance are based on safe achievable **serum concentrations** after systemic administration. However, when FQ drops administered in an intensive manner, **corneal drug concentrations** have been shown to be higher than the levels obtained in the serum with systemic administration. In rabbit models of bacterial keratitis, our collaborators have shown that intensive administration of topical FQs (every 15 minutes for 5 hours) can effectively treat FQ-R *S. aureus* as effectively as highly concentrated vancomycin (Fig. 11).[5] However, in clinical practice **adherence with this very intensive drop regimen is extremely difficult to maintain for days or even hours.** In contrast, a single TCL could potentially achieve the same outcome with considerably less frequent administration. In this aim, we will compare the treatment efficacy against FQ-R *S. aureus* (E277) and FQ-R *P. aeruginosa* (K1278).

D.3.2. Aim 3. Methods. We will study the antibacterial effectiveness of the experimental groups (Table 4) in separate keratitis models that either have an intact or denuded epithelium. The methods used for the FQ-R keratitis models will be the same to the ones described in Aim 2 for FQ-S *S. aureus* and cytotoxic *P. aeruginosa*, with the following changes: a treatment group with hourly fortified vancomycin (25 mg/ml) or hourly tobramycin (14 mg/ml) will be respectively be added to the FQ-R *S. aureus* or FQ-R *P. aeruginosa* studies.[6] Colony counts for each group will be compared to that of the baseline colony count and the clinical scores will be compared to that of Group 4 (vancomycin for *S. aureus* or tobramycin for *P. aeruginosa*).

Group #	Treatment Group Description
1	Baseline (euthanized at the end of incubation)
2	No treatment (negative control)
3	Hourly besifloxacin (to confirm resistance)
4	Hourly vancomycin or tobramycin (positive control)
5	TCL (naïve lenses)
6	TCL (2nd day of release)
7	Vehicle contact lens (polymer film, no drug)

Table 4. Aim 3 experimental groups.

D.3.3. Aim 3. Pitfalls, limitations, and solutions. **1)** If TCLs are equivalent to vancomycin drops during day 1 of release, but have decreased effectiveness on the day 2 (Group 6), then we will change the TCL formulation, as described in Aim 1, so we can deliver a greater amount of drug over the 2-day period, **2)** If the TCLs are not as effective on day 1 as fortified antibiotics, then we will consider reformulating the TCL so it releases more FQ; this can be accomplished by using a different FQ (e.g. moxifloxacin), by increasing drug loading and decreasing polymer % in the drug-polymer film. Alternatively, the TCL could elute vancomycin or tobramycin.

E. Power Calculations and Statistical Analysis. Statistical handling of data will proceed according to fairly standard approaches. For continuous real-number data exhibiting few outliers or without excessive clustering (suggesting a normal distribution, which can be verified formally), will be described with means and standard deviations, and analyzed with ANOVAs, t-tests, etc. For data that do not meet those criteria, we will use medians with IQRs and nonparametric tests (Kruskal-Wallis and Mann-Whitney U tests). Proportions will be compared with Fisher's exact test. Multiple comparisons will be treated with the Bonferroni correction—the most conservative approach. It is assumed that sample sizes will be adequate for the tests used. The number of animals to be used was determined with sample size calculations with power=0.8, α=0.5, and variance=1. One limitation of these calculations is the unknown real variance within the various experimental groups, so these calculated estimates may have to be revised once we have empirical data. When these calculations indicate that less than four animals would be necessary due to a large anticipated effect, we will use n = 4 to be able to power the statistical tests noted above. We calculated the sample size for Aims 2 and 3 as follows. Data from previous experiments were used to perform a power analysis, which demonstrated that a minimum of 6 animals per group is needed to achieve sufficient power. The analysis assumed 80% power, a beta of 0.05 and a sigma of 0.95 using Minitab Statistical Software. Given this information, a one-way ANOVA analysis should detect an effect size of a 1-log decrease in colony counts between the antibiotic and control groups if the data from 6 animals are used.

VERTEBRATE ANIMAL SECTION (VAS)

Performance site: This proposal includes animal studies in rabbits that will all be conducted at Schepens Eye Research Institute (SERI).

This section has been organized according to the VAS Required Five Points.

1. Description of the Animals and their proposed use:

New Zealand white rabbits will be used in Aims 1, 2, and 3. All studies will receive approval through the SERI IACUC. We will use healthy mature New Zealand white rabbits that weigh 2-4 kg. In our experience, rabbits of this weight typically are at least 3 months of age. Although there is not any evidence that the sex of the animals will influence the outcome of ocular drug flux or bacterial keratitis studies, we will plan on using an even ratio of male and female rabbits for each study arm. The study will require 348 rabbits.

The proposed procedures involving rabbits are as follows: a) Comparative study of the biocompatibility and drug flux in healthy rabbits drug flux, b) Evaluate the drug tissue distribution resulting from a drug-eluting therapeutic contact lens (TCL), c) Comparative study of bacterial keratitis treatments in animal models using susceptible and resistant bacteria. Prior to each study arm, we will perform fluorescein-assisted slit lamp biomicroscopy to ensure the health of the eyes. Only rabbits with healthy eyes will be used. Only one eye will be used for this study and the eye will be chosen at random. Because retention of the contact lenses is crucial for the success of this project, all animals will receive a partial lateral tarsorrhpaphy in the study eye. Regardless of whether animals in the treatment arms receive the TCL, all animals across the treatment arms will receive the tarsorrhpaphy so that the procedure does not result in a confounding effect.

Anesthetics. We will anesthetize the animals with subcutaneous injection of 10mg/kg xylazine and intramuscular injection of 35 mg/kg ketamine hydrochloride. A drop of proparacaine hydrochloride 0.5% eye drops would be used for topical anesthesia. Immediately post-operatively, yobine 0.1 mg/ kg will be given IV to reverse anesthesia following all procedures.

Analgesics. For the tarsorrhaphy, a subcutaneous injection of Buprenex 0.3 mg/Kg will be given immediately before procedure and the rabbit will also be given a fentanyl patch (12 mcg/ hr) for 3 days of wear. For the bacterial keratitis study, we will also use a subcutaneous injection of Buprenex as noted above.

Partial Lateral Tarsorrhaphy. Under general anesthesia (intramuscular injection of Ketamine [35 mg/kg] combined with Xylazine [10 mg/kg]), we will perform a permanent lateral tarsorrhaphy (surgically approximating the outer 1/3 of the upper and lower lids) on the study eye, which we have found to improve TCL retention greatly and has enabled 1 month of TCL wear. Following the lateral tarsorrhaphy, daily topical antibiotic / steroid (tobradex) drops will be used 3 times a day for the first 3 days after procedure. The following studies will be performed only after the tarsorrhaphy is fully healed, which we anticipate will take approximately two weeks.

Biocompatibility on a normal ocular surface. We will study the biocompatibility in the following treatment groups: one week of continuous TCL wear and hourly antibiotic solution (for humane and practical reasons, we will only administer 8 drops for 8 hours a day for 5 days). We will monitor for evidence of toxicity by observation of animal behavior, fluorescein-assisted

biomicroscopy, and cornea thickness measurements before the study (baseline) and then every other day until the last day of the study (day 7 for the TCL and day 5 for the antibiotic solution). Gross observation of animal behavior will be monitored daily to see if the rabbits show behavioral signs of ocular irritation.

Comparative study of drug flux. TCLs will be inserted on one eye and kept in place for 1 week. At specified times after TCL insertion (2, 4, 8, 24 hours; and 2, 3, 5, and 7 days) we will collect tears (10 μL), aqueous humor (100 μl), and serum (1 mL) from systemically and locally anesthetized rabbits. A rabbit will only have one aqueous humor sample taken per day and that each aqueous humor sampling will occur on different days that are separated by at least 72 hours. Therefore, multiple TCLs will need to be studied in order to obtain samples for all the time points. We will also administer a drop of moxifloxacin solution (0.5%) to one eye every hour for 8 consecutive hours (8 drops total) and then sample the aqueous humor, tears, and serum in anesthetized rabbits at various times (20, 40, and 60 minutes) after administration of the 8th drop. For each of the three time points, we will collect samples on different days separated by at least 72 hours.

Aqueous humor will be collected by performing an anterior chamber paracentesis, which is accomplished by inserting a 31-gauge needle through the peripheral cornea and sampling 100 μL of aqueous humor. To collect the aqueous humor from eyes wearing the TCL, the device will remain on the eye, but will be gently slid superiorly in order to perform the paracentesis along the peripheral cornea. For the serum collection, we will collect 1 mL of blood from the marginal ear vein. Care will be taken to alternate blood sampling between the two blood vessels and between the right and left ears. Drug concentrations will be quantified by liquid chromatography–tandem mass spectrometry (LC-MS/MS).

Tissue distribution. We will apply the TCL on one eye and then the rabbits will be euthanized at 4, 8, 24, and 72 hours post-administration. In a separate group of rabbits, moxifloxacin solution will be topically applied to the rabbit eyes every hour consecutively for 8 hours (8 drops total) and the rabbits will be euthanized 30 minutes after the last drop. After the animals have been euthanized, the eyes will be enucleated and immediately placed in a -80 degree freezer until the time of dissection. At that time, samples will be collected from various ocular tissues (conjunctiva, sclera, aqueous humor, iris/ciliary body, lens, vitreous, and retina), homogenized, and then the drug concentration will be quantified by LC-MS/MS.

Bacterial keratitis study. The bacterial keratitis study will take place within dedicated BSL2 animal rooms. In Aim 2, we will study the effectiveness of TCLs in a bacterial keratitis model. We will inject into the corneal stroma of systemically and locally anesthetized rabbits 25 μl containing 1000 CFU of *S. aureus* or *P. aeruginosa* using a 100-μl Hamilton syringe and a 30-gauge 0.5-in needle. For the study that includes an overlying epithelial abrasion, we will create a 5mm corneal epithelial defect (over the site of the intrastromal injection) using an Amoils epithelial scrubber, which will only remove the epithelial layer of the cornea. For the study that includes an intact epithelium, the epithelial layer will not be disrupted. An incubation period (4 hours for *S. aureus* and 16 hours for *P. aeruginosa*) will allows the bacteria to proliferate, but not too much so that they cannot be eradicated by topical antibiotics. At the end of the incubation period a group of rabbits will be euthanized and treatment will be initiated on the remaining treatment groups: no treatment, hourly antibiotics for 8 consecutive hours, a newly hydrated TCL, a vehicle contact lens (no drug), and a TCL that has undergone release for one day in the eye of a healthy rabbit. 8 hours later the anesthetized animals will be examined by a masked observer, the aqueous humor will be collected in the manner described above, and then the animals will be euthanized. A 9.5mm corneal button that includes the inoculation site will be excised from the cornea and added to 1 ml PBS, homogenized, and then dilutions will be

plated on trypticase soy agar supplemented with 5% sheep blood. The colony counts will be compared between treatment groups and the baseline group. The remainder of the eyes will be stored in -80°C and used for tissue distribution studies in the same manner as noted above. In Aim 3, we will study the effectiveness of the TCL in a fluoroquinolone-resistant *S. aureus* and fluoroquinolone-resistant *P. aeruginosa*. For the fluoroquinolone-resistant *S. aureus* study, we will add hourly vancomycin drops (25 mg/ml) for 8 hours as an additional treatment arm. For the fluoroquinolone-resistant *P. aeruginosa* study, we will instead use a treatment arm that includes hourly tobramycin (14 mg/ml) drops for 8 hours.

2. Justification of Animal Use, Species Selected, and Numbers

Justification of animal/specie use

Unfortunately, the correlation between *in vitro* and *in vivo* findings is notoriously poor. Therefore, *in vivo* testing of drug flux into the eye is crucial to our evaluation of TCLs. We will use New Zealand white rabbits, which have similar ocular dimensions and drug fluxes to the aqueous humor as humans. Given that the ocular dimensions are similar to humans, contact lenses intended for human use can be placed on the corneas of rabbits. As a result, this specie of rabbit is frequently used for toxicology studies using contact lenses and contact lens products. In addition, animal models of bacterial keratitis have been well established. Reaction of rabbit ocular tissue to topical medications has proven to be predictive of their clinical efficacy in patients. This has not been demonstrated in phylogenetically lower species such as rats and mice. The rabbit is commercially available, docile, easy to work with, and has become the standard model of ophthalmic infectious keratitis research. There has been an extensive amount of research and publications by our collaborators (see letters by [redacted] on bacterial keratitis in rabbits and similar rabbit ocular model have been used by their group for more than 20 years.

Number of rabbits to be used for the study. Where possible, we will try to consolidate experiments (for example, biocompatibility and drug flux will be performed in the same animals). For experiments with a numeric read-out (e.g. antibiotic concentration in the aqueous humor), power analysis indicated that a sample size of 4 for each TCL formulation and concentration would provide 80% power to detect 50% differences between groups using the nonparametric Friedman test (version 7.0, nQuery Advisor, Statistical Solutions, Saugus, MA). We propose groups of n=6 in order to detect smaller differences between groups, which is more realistic for most of the endpoints we will be studying, and correlates well with our published experience. Therefore, we will use 6 rabbits for the biocompatibility and drug flux studies. In an effort to limit the number of rabbits used for the tissue distribution study, we will only use 4 rabbits per time point for a total of 40 rabbits.

For Aim 1, we anticipate using a total of 48 rabbits. We calculated the sample size for Aims 2 and 3 as follows. Data from previous experiments were used to perform a power analysis, which demonstrated that a minimum of 6 animals per group is needed to achieve sufficient power. The analysis assumed 80% power, a beta of 0.05 and a sigma of 0.95 using Minitab Statistical Software. Given this information, a one-way ANOVA analysis should detect an effect size of a 1-log decrease in colony counts between the antibiotic and control groups if the data from 6 animals are used. For Aim 2 (6 treatment groups with fluoroquinolone-susceptible *S. Aureus*, cytotoxic *P. aeruginosa*, and invasive *P. aeruginosa*) will anticipate using 108 animals for the epithelium intact study and 72 animals for the epithelial defect study (only studied for *S. Aureus*, cytotoxic *P. aeruginosa* since invasive *P. aeruginosa* can invade an intact epithelium). In Aim 3 (7 treatment groups with fluoroquinolone-resistant *S. Aureus* and 7 treatment groups with fluoroquinolone-resistant *P. aeruginosa*) we anticipate using 84 rabbits for the epithelium intact study and 84 rabbits for the epithelium denuded study.

3. Veterinary Care

[redacted] DVM, serves as the Attending Veterinarian at SERI. She is an ACLAM Diplomat with more than 20 years experience in laboratory animal medicine. Whenever Dr. [redacted] is unavailable, back-up veterinary assistance is provided by [redacted] DVM.The Animal Health Technicians, under supervision from the Veterinarian and Associate Director, perform health rounds throughout all the animal housing rooms and document findings at least once a day on weekdays. The Animal Health Technicians communicate their findings to the Veterinarian either by phone or email. The Animal Care Technicians are also part of our daily health monitoring process since they "sick-flag" and notify the Animal Health Technicians when they notice any health issues during routine cage changes. Finally, the Investigators are also responsible for performing health checks and drug administration. Investigators and animal facility staff document animal procedures/health issues by using the "Animal Procedure Form" and the "Clinical Cage Record" on the back of the rodent cage cards.

The health of the rabbits will be assessed by veterinary staff immediately after their arrival to our facility. The rabbits will arrive by courier in a climate-controlled vehicle from the vendor. Once they arrive crates are immediately checked for damage. Animals in crates are then moved into the rabbit housing area. The veterinary staff will have already made cage cards prior to their arrival. In addition, rabbits will have individual paper records prepared. Once in the housing area, the crates are opened one at a time. The animal's overall appearance is assessed. The rabbits are examined thoroughly, specifically looking for wounds, diarrhea, or discharge from nasal passages and eyes. The animal's food and water source within the transport box is also checked to make sure it is adequate and provided. Rabbits are than weighed and sex is checked. Rabbit are given a unique identification number, which is tattooed on the ear by the animal vendor prior to arrival. They are than placed in a new cage. The rabbit's clinical records are than updated accordingly.

Rabbits are housed individually in cages according to Guide standards, with each cage placed directly across from another for visualization. Music is played at a low volume to provide background noise and minimize startling. Rabbits are on a weekly enrichment schedule, which includes new interactive enrichment devices. The enrichment schedule includes the use of autoclaved cardboard towel rolls, rattles, fresh vegetables, 'bunny blocks' on a chain, jingle balls and timothy hay. The rabbits are also held and brushed by humans at this time at least once a week.

4. Procedures for Limiting Pain, Discomfort, and Injury

All the procedures will be performed in a manner that will limit pain, discomfort, and injury. All procedures will only be performed on one eye, chosen at random. Prior performing any procedures such as placement of the lateral tarsorrhaphy or collection of aqueous humor and other fluids, rabbits will be anesthetized with an intramuscular injection (IM) of Ketamine (35 mg/kg) combined with Xylazine (8 mg/kg) using a 1"-25 gauge needle. Proparacaine 0.5% ophthalmic solution (1-2 drops) will be applied to the cornea surface prior to procedure. The use of proparacaine has not been found to affect our drug delivery studies. The animals will be kept on a heat pad until full recovery from anesthesia. Yobine I.V. (0.2 mg/kg) would be used to reverse the effects of Xylazine and speed recovery after anesthesia. To treat discomfort following the lateral tarsorrhaphy, we will administer one subcutaneous injection of buprenorphine 0.03 mg/kg dose of buprenorphine followed by the application of a Fentanyl patch (applied on the inside of the ear which lacks hair), which will remain in place for 3 days.

Animals will be observed for signs of squinting, ocular discharge and blepharospasm, which are considered clinical symptoms associated with discomfort and/or side effects. The treated eye will be clinically assessed by an experienced ophthalmologist and also compared with the fellow, control eye. If a contact lens is in place, it will be removed if these signs of discomfort are present.

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Letters of Support

RESOURCE SHARING PLAN

The Schepens Eye Research Institute/Massachusetts Eye and Ear Infirmary are committed to open dissemination of research and medical findings. Accordingly, the investigator of this application will abide to the NIH guidelines as described in the "Principles and Guidelines for Recipients of NIH Research and Contracts on Obtaining and Disseminating Biomedical Research Programs". The results will be presented at relevant scientific meetings and published timely. Publications resulting from this work will be submitted to the digital archive in PubMed Central.

Intellectual and Material Property Plan.
Because the techniques under investigation have been previously described, this multi-institutional clinical trial is not anticipated to generate any proprietary information.

Should any intellectual property arise, then each Party will commit, as reasonable, sufficient technology transfer administration resources to manage its Intellectual Property resulting from the Award Work in a manner consistent with any laws, statutes, rules, regulations and guidelines promulgated by any government agency, authority or regulatory body having jurisdiction over the Party's conduct, including, but not limited to, the Bayh-Dole Act, institutional policies, and any relevant Award language. In the event of any dispute between the Parties relating to Project Intellectual Property management, Ojas P. Mehta, J.D., Director, Intellectual Property & Commercial Ventures, Massachusetts Eye and Ear, shall enter into good faith negotiations with the appropriate institutions to arrive at a equitable resolution within a reasonable period of time, taking into consideration the objectives of the Prime Award and any laws, statutes, rules, regulations or guidelines to which the involved Consortium Parties are subject.