AWARD NUMBER: W81XWH-19-1-0145

TITLE: Steroid-Eluting Therapeutic Contact Lens to Treat and Prevent Inflammation and Scarring Following Ocular Trauma

PRINCIPAL INVESTIGATOR: Joseph B. Ciolino, M.D.

CONTRACTING ORGANIZATION: Schepens Eye Research Institute, 20 Staniford St. Boston, MA 02114

REPORT DATE: AUGUST 2022

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
AUGUST 2022	Final	4/15/19 - 4/15/22

4. TITLE AND SUBTITLE

Steroid-Eluting Therapeutic Contact Lens to Treat and Prevent Inflammation and Scarring Following Ocular Trauma

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-19-1-0145

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Schepens Eye Research Institute
20 Staniford St
Boston MA 02114

Pro Med Pharma, LLC

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Steroids are frequently used in the care of ocular inflammation, for both post injury and postoperative care. In our previous work with the DOD, we developed a dexamethasone-eluting contact lens continuously worn on the eye which dispenses drug for seven days. Here, we seek to perform further efficacy testing, physicochemical characterization of the TCL, and transfer production to a GLP facility, followed by formal biocompatibility testing in a GLP lab. At this point, we have received IACUC approval for one animal protocol, and are awaiting ACURO approval before we begin animal work. Technology transfer to a GLP facility for fabrication has commenced.

15. SUBJECT TERMS

NONE LISTED

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** USAMRDC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified	**39**	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Steroids are frequently used to treat ocular inflammation, including post-traumatic and post-operative inflammation. In our previous work with Department of Defense funding, we developed a fully functional steroid-releasing therapeutic contact lens (TCL) to prevent trauma-related ocular inflammation and scarring. In animals, TCLs provided a week of sustained ocular dexamethasone delivery, suppressed inflammation in 2 different ocular models of anterior inflammation. Our pharmacokinetic data demonstrated high drug concentrations in the back of the eye. In order to prepare the TCL for clinical use, additional steps must be taken to translate the TCL from the laboratory to industrial production. In addition, we must do further testing of the TCL under ISO standards. In this project, we seek to characterize the TCL, investigate efficacy in rabbit models of vitreoretinal disease.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Inflammation, contact lens, sustained release, dexamethasone, proliferative vitreoretinopathy, uveitis

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

1. Perform *in vivo* efficacy testing for of the steroid eluting therapeutic contact lens (TCL) in back of the eye animal models
2. Complete in-vitro characterization of the TCL
3. Perform studies to establish comfortable, fitting lenses for human use

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Aim 1: Perform *in vivo* efficacy testing for of the steroid eluting therapeutic contact lens (TCL) in back of the eye animal models

1. Retinal vascular leakage. We demonstrated the TCL can prevent retinal vascular leakage in a rabbit model. Retinal vascular leakage is a component of diabetic macular edema and can cause compromised vision. Intravitreal injections of anti-angiogenic drugs (such as bevacizumab) have traditionally been used, though in recent years, steroid intravitreal injections have gained popularity to treat DME. As rabbits lack a macula, inducing retinal vascular leakage via an intravitreal VEGF injection was the closest approximation in a rabbit model.

New Zealand Pigmented rabbits (NZPs) received a 500 ng intravitreal injection of VEGF-165 in 50 µL sterile PBS to induce retinal vascular leakage. The NZPs were assigned to one of five treatment groups: 1) TCL for two days, 2) 0.1% dexamethasone (DEX) eye drops hourly for 8 hours for two days, 3) one 400 µg intravitreal injection of dexamethasone, 4) a contact lens with polymer but no drug worn for two days, or 5) no treatment. Eye drops were limited to 8 hours per day for practical and humane reasons. Intravitreal steroid injections commonly used for treating diabetic macular edema, thus group 3 was used as a positive control. Groups 4 and 5, with no drug provided, served as negative controls.

Fluorescence angiography to visualize the retinal vessels was performed prior to VEGF injection and at 48 hours. Sodium fluorescein was injected IV, while early phase and late phase images of the retina were obtained for up to ten minutes after injection. The amount of vessel leakage was quantified by scanning ocular fluorometry at baseline and 48 hours after injection.. The affected eye was scanned in a completely darkened room using a Fluorotron™ Master Ocular fluorometer. Three scans were taken for each rabbit.

The no treatment groups demonstrated florid vascular leakage post-injection, the TCL group demonstrates little no leakage, similar to the intravitreal dexamethasone group, the standard of care (Figure 1).

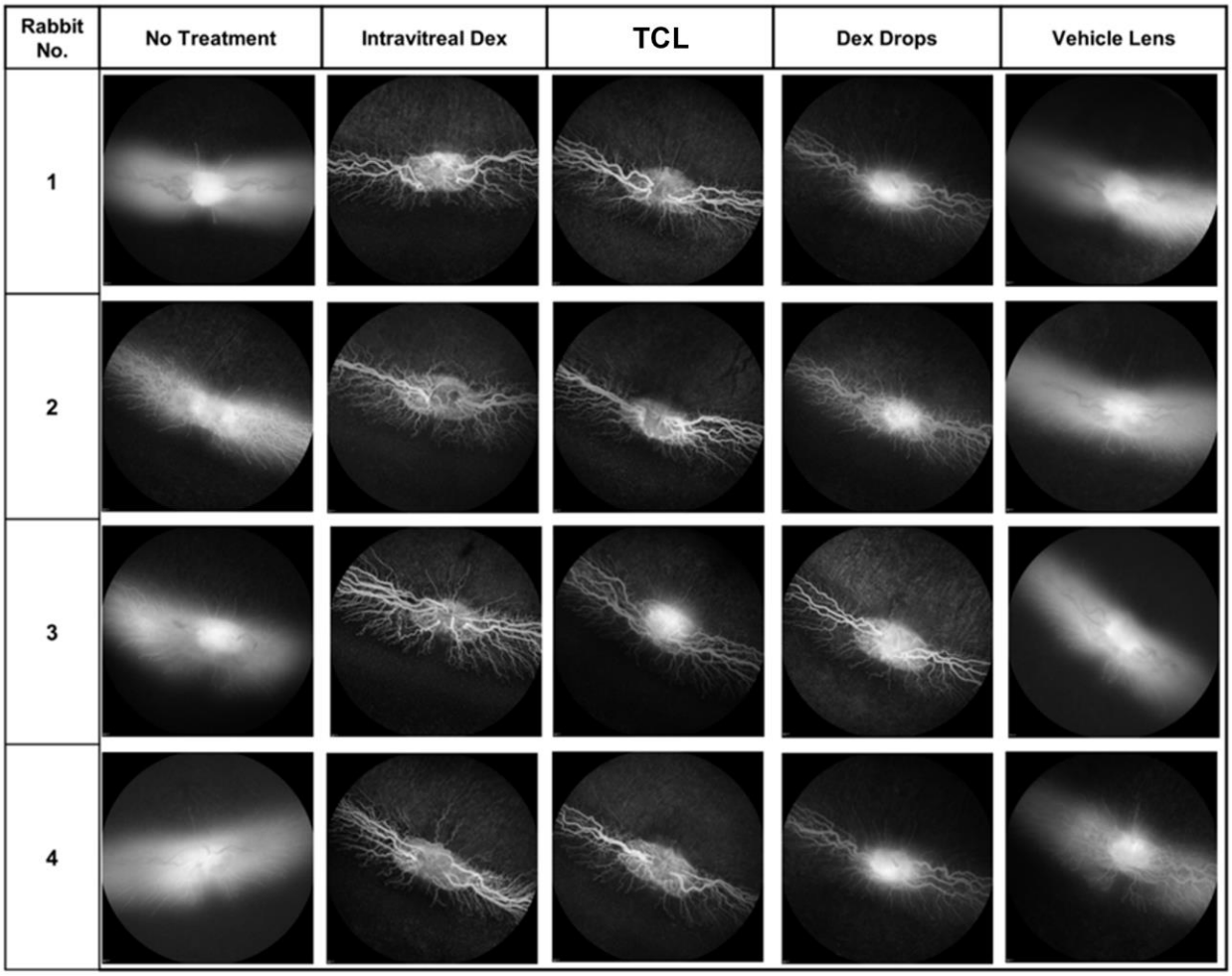


Figure. 1. Scanning ocular fluorometry was used to quantify fluorescein in the eye. 40 minutes after injection, readings were taken in the treated eye by Fluorotron Master Ocular fluorimeter. This was performed at baseline and 48 hours after VEGF injection. The results were obtained by subtracting baseline fluorescence from fluorescence at day 2.

In addition, vitreous humor protein was measured by Bradford assay as a marker of inflammation.

The TCL showed significantly lower fluorescein compared to the no treatment and vehicle contact lens groups (Figure 2a). Differences in vitreous humor protein levels were not significant (Figure 2b).

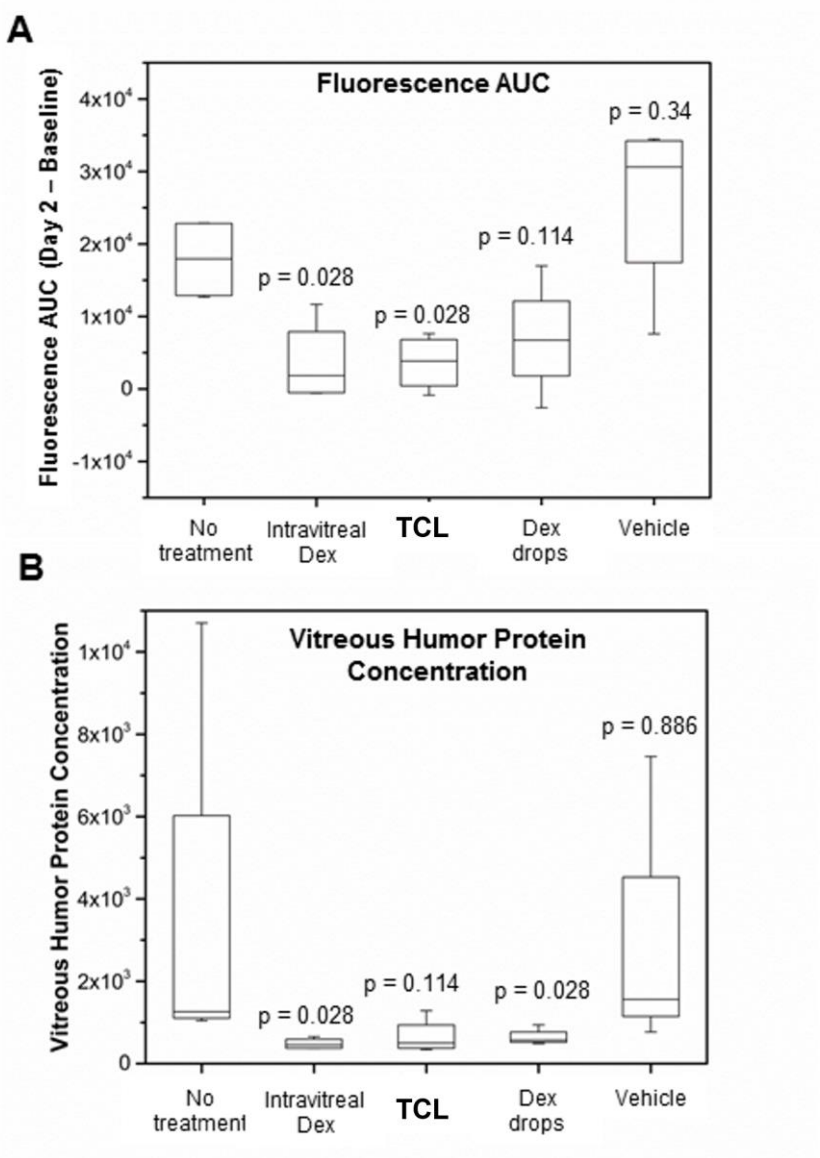


Figure 2. A) Fluorescence AUC for retinal vascular leakage study and B) vitreous humor protein concentrations. Statistical comparisons performed by Mann-Whitney U. n=4 for all groups. Dex drops=0.1% dexamethasone eye drops given hourly for eight hours for two days, Intravitreal Dex=one time 500 ng intravitreal injection of dexamethasone.

2. Traumatic proliferative vitreoretinopathy in a rabbit model.

Proliferative vitreoretinopathy (PVR) is a complication of retinal reattachment surgery or ocular injury. In PVR, retinal cells form contractile membranes in the vitreous and retina, causing vision loss. The standard of care for PVR is surgery to reattach the retina, remove scar tissue, and remove vitreous humor. Nonsurgical treatments are under investigation for PVR. Some researchers have proposed dexamethasone as a potential PVR treatment, and we wanted to investigate if the TCL could be used in this fashion.

Dexamethasone was applied to the C-PVR cells in concentration of 0.02, 0.2, and 2.0 mg/mL and incubated. Cell proliferation was measured by Ki67 staining. Results were compared to untreated PVR cells.

The *in vitro* assay found no differences between the untreated PVR cells and any of the dexamethasone concentrations (Figure 3). Since dexamethasone failed to demonstrate an antiproliferative effect, no animal studies were performed using the TCL because the drug itself did not appear to be effective in treating PVR using our proposed PVR cells.

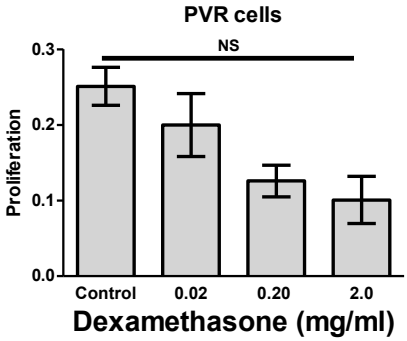


Figure 3. Dexamethasone proliferation in PVR cells measured by Ki67 staining showed no significant differences among the groups. Error bars represent standard error of the mean.

Aim 2: Complete *in-vitro* characterization of the TCL

Characterization of the TCL was classified into three sections: 1) physicochemical characteristics of the TCL and 2) stability of dexamethasone 3) residual solvent removal.

1. Physicochemical characteristics-we hypothesized TCLs would have properties similar to those of a commercial (Kontur) contact lens

1A. Equilibrium water content (EWC)

EWC is a measure of contact lens comfort, and in HEMA-based contact lenses, oxygen permeability, as water acts as a carrier for oxygen through the contact lens. EWC was measured according to ISO 18369-1 guidelines. In brief, TCLs were hydrated and weighed until they reached equilibrium mass (<5% change in mass). Equilibrium mass was measured three times. Then TCLs were dried overnight at 60°C and re-weighed three times. Percent water content was calculated by the following formula:

$$\frac{W_S - W_D}{W_S} X\ 100\%$$

Where W_s = weight at equilibrium swelling and W_d = dry weight. Results were compared to those from commercial methafilcon contact lenses (Kontur).

TCLs had a water content of 55.5±0.9%, comparable to Kontur lenses (Figure 4).

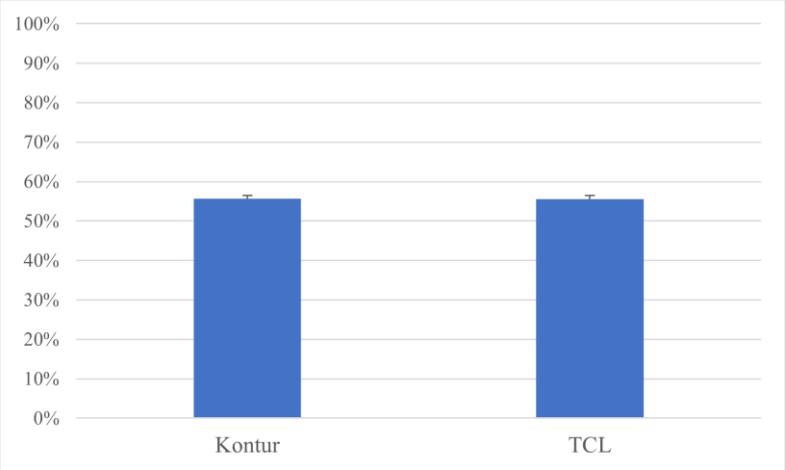


Figure 4. Equilibrium water content in TCLs. n=4.

1B. Contact angle

Contact angle is a measure of surface wettability. In general, greater comfort is associated with lower contact angles. Poor wettability of a contact lens can break up the post-lens tear film, leading to dry eye. Contact angle was measured by sessile drop technique. A drop of distilled water (about 5 µL) was applied onto the surface of hydrated TCLs via a syringe. Then, a high-resolution image was captured from the side using the Dino-Lite Edge camera. The contact angle for each formulation (n = 4 and 5 measurements per specimen) was determined using ImageJ software (NIH, Bethesda, Maryland) as a function of time.

Contact angle was measured at 54.0 ±3.8°, which was higher than a commercial contact lenses that we measured which was made of the same methafilcon hydrogel (26.2±4.0°). The contact angle we measured was well within the range of many commercial contact lenses and lower than most silicone hydrogel contact lenses. Therefore, it is not clear whether the contact angle we

measured would adversely affect comfort. Therefore, our team will continue to evaluate the TCL, including the need for surface modifications, for a more wettable contact lens.

1C. Elastic modulus

The elastic modulus of a contact lens determines its stiffness, which also contributes to comfort. The mechanical features of the TCLs were determined at room temperature using a Mark-10 ESM 303 motorized test stand (Mark-10 Corporation, NY, USA). Before analysis, samples were hydrated by immersing them in DI water for 2 days. The compression test was conducted on rings with 7 mm and 5 mm external and internal diameter, respectively and with a crosshead speed of 1 mm/min. The Compression Modulus was obtained from the linear derivative of the stress-strain curve in the low stiffness range. Three independent measurements were conducted for each group. Compression modulus (n=5) was 250 kPa, not significantly different than Kontur values of 491 +/- 171 kPa.

2. Stability

Active pharmaceutical ingredient (API) is essential to determine in a drug delivery system, as an unstable API will result in less effective treatment. We reviewed the dexamethasone stability under a) fabrication, b) sterilization, and c) long-term storage. We hypothesized dexamethasone would remain stable through all forms of processing.

2A. Stability through fabrication

During fabrication, dexamethasone was exposed to organic solvents and intense UV light. We released TCLs and compared the HPLC chromatogram (Figure 5a) to a neat dexamethasone standard (Figure 5b). Two peaks were seen on the TCL chromatogram, one at six minutes, and one at twelve. The second peak matched that of pristine dexamethasone. The first peak was determined to be Irgacure ®2959, the photoinitiator used to polymerize liquid methafilcon (Figure 5c). Irgacure® 2959 was only detected throughout the first 24 hours of TCL *in vitro* release (30 µg cumulative release). These concentrations and higher concentrations of up to 5 mg/mL have been reported to be safe in cytotoxicity studies. We performed cytotoxicity testing of Irgacure® 2959 at the maximum concentration (30 µg) and three times the maximum (90 µg) of cumulative amount of Irgacure® 2959 that was released during in vitro release of TCL and observed no cytotoxicity, with cell survival of 97±10% at the highest does studied.

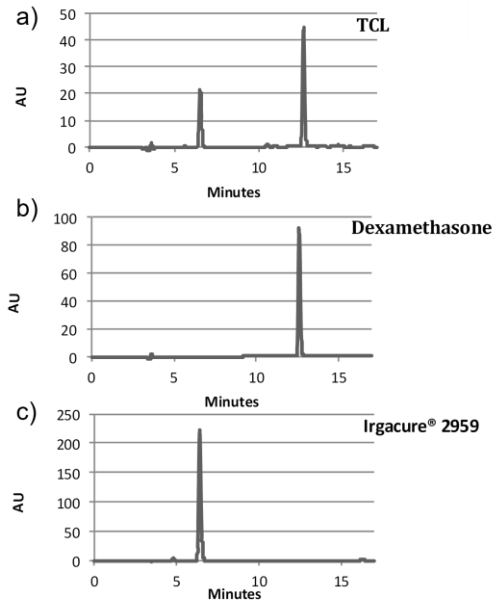


Figure 5. Representative chromatograms of: a) TCL, b) dexamethasone standard and c) Irgacure®2959. Cytotoxicity testing of Irgacure® 2959 indicating no cytotoxicity at the release levels observed with the TCL.

2B. Stability through sterilization

Although most commercial contact lenses are sterilized by autoclaving, the inner PLGA film would be compromised by the high temperatures in autoclaving. Instead, TCLs are sterilized by gamma radiation, during which TCLs are placed in sealed vials and packed in dry ice to for temperature control. However, the gamma particles can cause chain scission in polymers, altering release kinetics.

We irradiated TCLs at 25 kGy and released, comparing the results to non-irradiated TCLs. The release profile showed no difference between the two groups (Figure 6).

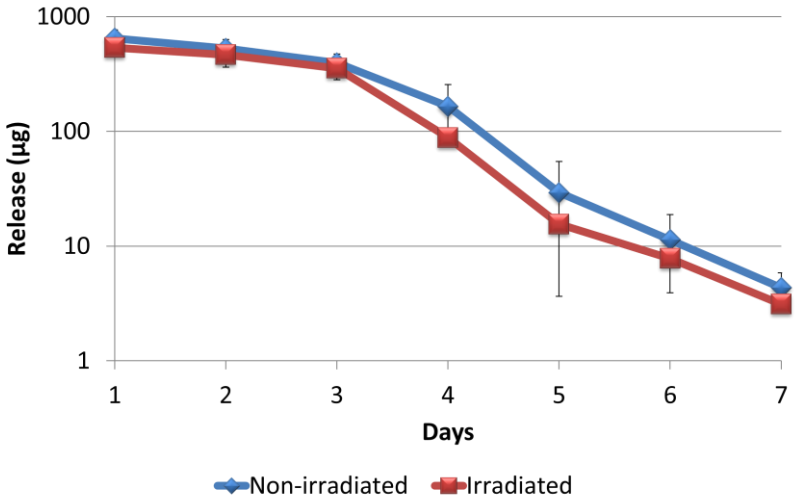


Figure 6. Daily dexamethasone release from irradiated and non-irradiated TCLs (n=4)

C. Stability through storage

To make the TCL a useful and commercially viable product, it must be capable of long term storage We stored TCLs at 4°C for one year and compared the results to newly released TCLs (n=4). There was no significant difference between the groups (Figure 7).

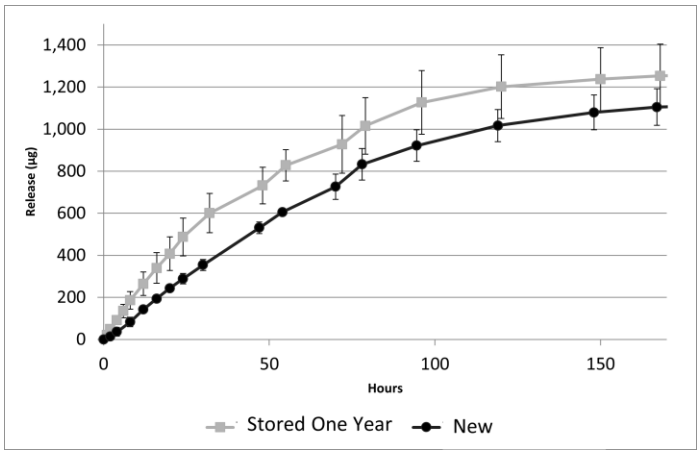


Figure 7. Cumulative release for TCLs after one year of storage. n=4.

3. HFIP level in the release medium from the completed lens (residual solvent)

Fabrication of the TCL uses hexafluoroisopropanol (HFIP) to dissolve the dexamethasone and PLGA in sufficient quantities. Although most of the highly volatile solvent evaporates quickly, some residual solvent can remain in the film and elute from the TCL. We evaluated different ways of removing HFIP after film casting by vacuum desiccation at room temperature, using a vacuum oven (at 43°C), lyophilizing, and vacuum centrifuging (Speedvac). The solvent removal was performed after film casting and before encapsulation and lathing. After lathing, TCL were released in PBS on an incubator shaker at 37°C. At 24 hours, the lenses were removed and placed in fresh PBS. The release buffer was collected and analyzed by HPLC.

Speed vacuum with higher temperature (43 °C) drying method resulted in significantly lower levels of HFIP released from Dex-Lens at 24 hour than our standard method (desiccated 1d and lyophilize 1d, Fig. 8). We further increased the vacuum levels and drying time (Fig.9). Desiccation for 30 days led to the lowest HFIP level.

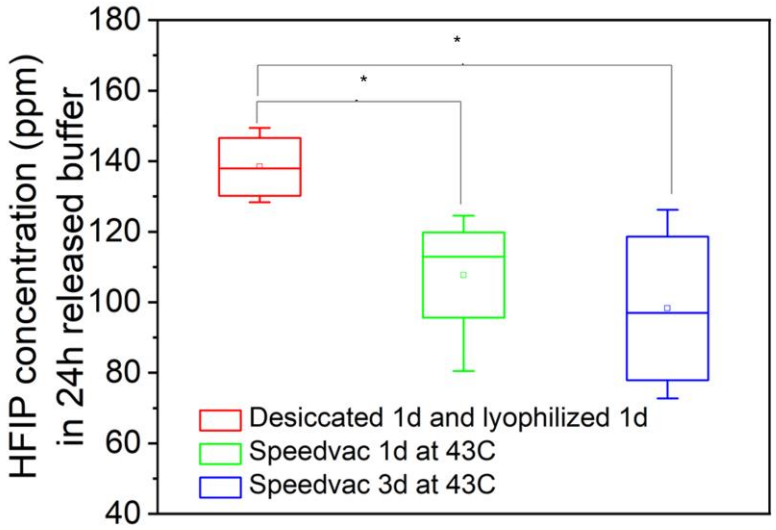


Figure 8. HFIP level in 24 h release medium after drying using our standard method (desiccated for 1 day and lyophilized for 1 day), speed vacuum with higher temperature (43°C) for 1 day, desiccated 7 days, and desiccated 30 days. P valued calculated by ANOVA one-way unpaired t-test. n=4

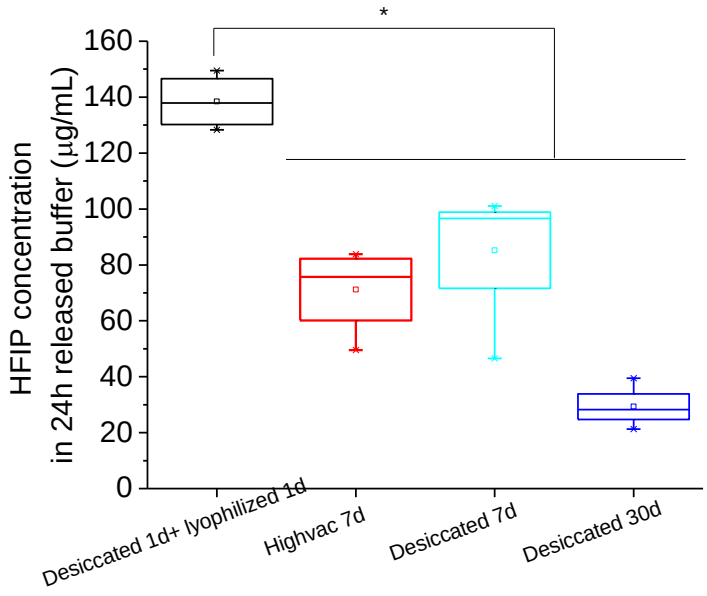


Figure 9. HFIP level in 24 h release medium after drying using our standard method (desiccation for 1 day and lyophilization for 1 day), high vacuum for 7days, desiccation for 7 days, and desiccation for 30 days. *p<0.05 by one-way ANOVA followed by unpaired T-test. n=4

Aim 3. Perform studies to establish comfortable, fitting lenses for human use.

Using data gathered from our previous Translational Research Award and Aims 1 and 2 of this award, we prepared an Investigational New Drug (IND) application for a Phase I/II clinical trial. The trial indication was patients with recurrent cystoid macular edema (CME). CME can occur under multiple circumstances but is most often a complication of cataract surgery. Although a steroid-eluting contact lens could be used to treat a variety of indications, CME was chosen as the indication as 1) improvement could be detected in a small number of patients and 2) demonstrates dexamethasone could reach the back of the eye. A two-phase clinical trial was proposed. In Phase A (3 patients), the TCL would be worn for one week. The patient would undergo intense follow up and regular exams of both the front and back of the eye. In Phase B, patients would be randomized to one of three groups) 1) wearing the TCL for one week and a vehicle contact lens for two weeks, 2) wearing the TCL for three weeks, and 3) wearing a vehicle contact lens for three weeks. For both phases, the primary outcome measure is comfort and fit of the TCL, with reduction in macula thickness (at least 50 µm) as a secondary outcome measure.

The IND received approval from the FDA in 2019. The clinical trial was then submitted to the Mass General Brigham Institutional Review Board, which granted approval in 2020.

Although we had initially contracted with ProMed LLC for GMP manufacturing, we found it would be less expensive to fabricate the TCLs for human use ourselves under GMP-like conditions. Prior to fabrication, we developed SOPs and quality control standards standardized TCL

fabrication. Working in the Mass Eye and Ear Pharmacy cleanroom, we fabricated over 60 TCLs and 40 vehicle contact lenses for the clinical trial in aseptic conditions. TCLs and vehicle contact lenses were lathed by Kontur, and sterilized by gamma radiation by Sterigenics (Mentor, OH). These lenses were analyzed and reported as a Certificate of Analysis (CoA) to the FDA, who determined that our study was safe to proceed. The first-in-human pilot study has IRB approval and has enrolled begun enrolling patients.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

We presented preliminary results of our "Dexamethasone-Eluting Contact Lenses Inhibit VEGF-Induced Retinal Vascular Leakage in a Rabbit Model" at the Military Health Services Research Symposium in 2019. Dr. Ciolino presented the results at the Innovations session at ASCRS in 2022.

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

N/A (Final Report)

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

We demonstrated topically applied drug is able to reach the back of the eye when applied in a contact lens. For many diseases of the retina, intravitreal injections (into the eye) must be used. Drug continuously diffused out of a contact lens can overcome those barriers to reach the retina and vitreous humor in therapeutic amounts.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

The drug eluting contact lens patent has been licensed to a company, TherOptix, which was founded to commercialize the technology.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*

- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

N/A (final report)

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Not applicable

Significant changes in use of biohazards and/or select agents

Not applicable

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**

Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Published: Ross AE, Bengani LC, Tulsan R, Maidana DE, Salvador-Culla B, Kobashi H, Kolovou PE, Zhai H, Taghizadeh K, Kuang L, Mehta M, Vavvas DG, Kohane DS, Ciolino JB. Topical sustained drug delivery to the retina with a drug-eluting contact lens. Biomaterials. 2019 Oct;217:119285. doi: 10.1016/j.biomaterials.2019.119285. DOD support acknowledged

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Oral presentation: “Dexamethasone-Eluting Contact Lenses Inhibit VEGF-Induced Retinal Vascular Leakage in a Rabbit Model”, Military Health Services Research Symposium in 2019.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

In progress.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding,

prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name: Joseph B Ciolino
Project Role: PI
Nearest person month worked: 3.00
Contribution: Oversight, experimental design

Name: Leo Kim
Project Role: Co-investigator
Nearest person month worked: 0.6
Contribution: Study of dexamethasone in PVR cells

Name: Amy Ross
Project Role: Technician
Nearest person month worked:
Contribution: fabrication of TCLs, development of SOPs, performing animal studies, performing characterization studies.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
<u>Organization Name:</u>
<u>Location of Organization: (if foreign location list country)</u>
<u>Partner's contribution to the project</u> (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Organization: ProMed LLC
Location: Minneapolis, MN
Contribution: Prototype of scaled up TCLs in a GMP environment

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

Steroid-eluting contact lens to treat and prevent inflammation and scarring following ocular trauma

MR130201

PI: Joseph B. Ciolino **Org:** Schepens Eye Research Institute **Award Amount:** $2,100,000

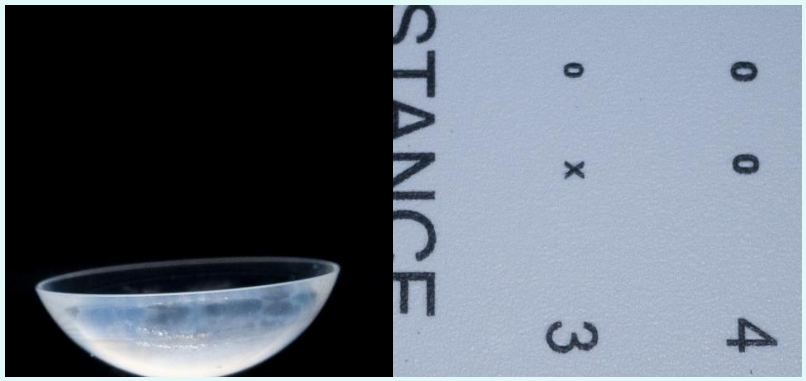
Study Aims

- Aim 1: *In vivo* evaluation of TCLs for prevent of inflammation in the back of the eye.
- Aim 2: Characterize TCL physical properties and release products.
- Aim 3: Optimize TCL fit and comfort for human use

Approach

We will assess the steroid eluting TCL in three animal models of vitreoretinal diseases (Aim 1). We will examine characteristics such as drug loading, light transmission, and residual solvent (Aim 2). We will perform base curve measurements of the TCls and ensure they provide a comfortable fit for clinical use (Aim 3).

Steroid-eluting therapeutic contact lens



We will use our innovative approach to develop a steroid-eluting contact lens for the treatment of ocular inflammation and cornea neovascularization.

Timeline and Cost

Activities CY	20	21	22	
Aim 1: *In vivo* efficacy testing				
Aim 2:Characterization				
Aim 3: Base curve/fit testing				
Estimated Budget ($)	**$700**	**$700**	**$700**	

Updated: (8/13/2021)

Goals/Milestones

CY19 Goal –
X Characterize TCL properties and drug release products
X Quantification of drug loading

CY20 Goals –
X Demonstrate TCL efficacy in vitreous and retinal diseases
X Prevention of VEGF-induced retinal vascular leakage using TCL

CY21 Goal –
X Fabricate TCLs in a cleanroom environment
☐Identify hydrated lens with base curve between 8.5-9.0

Budget Expenditure to Date
Projected Expenditure: $2,098,765
Actual Expenditure: $2,098,765

Biomaterials 217 (2019) 119285

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Topical sustained drug delivery to the retina with a drug-eluting contact lens☆

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HIGHLIGHTS

- Contact lens Dexamethasone Delivery System (Dex-DS) released dexamethasone for 7 days *in vitro* and *in vivo* in rabbits
- Dex-DS topically delivered therapeutic amount of dexamethasone to back of the eye
- Dex-DS inhibited VEGF-induced retinal vascular leakage in rabbits
- Dex-DS was demonstrated to be safe in a 4 week repeated use biocompatibility study
- Des-DS offers a therapeutic alternative to intraocular injections

ARTICLE INFO

Keywords:
Dexamethasone
Retinal edema
Topical drug delivery
Contact lens
Pharmacokinetics
VEGF

ABSTRACT

Intravitreal injections and implants are used to deliver drugs to the retina because therapeutic levels of these medications cannot be provided by topical administration (i.e. eye drops). In order to reach the retina, a topically applied drug encounters tear dilution, reflex blinking, and rapid fluid drainage that collectively reduce the drug's residence time on the ocular surface. Residing under the tears, the cornea is the primary gateway into the eye for many topical ophthalmic drugs. We hypothesized that a drug-eluting contact lens that rests on the cornea would therefore be well-suited for delivering drugs to the eye including the retina. We developed a contact lens based dexamethasone delivery system (Dex-DS) that achieved sustained drug delivery to the retina at therapeutic levels. Dex-DS consists of a dexamethasone-polymer film encapsulated inside a contact lens. Rabbits wearing Dex-DS achieved retinal drug concentrations that were 200 times greater than those from intensive (hourly) dexamethasone drops. Conversely, Dex-DS demonstrated lower systemic (blood serum) dexamethasone concentrations. In an efficacy study in rabbits, Dex-DS successfully inhibited retinal vascular leakage induced by intravitreal injection of vascular endothelial growth factor (VEGF). Dex-DS was found to be safe in a four-week repeated dose biocompatibility study in healthy rabbits.

1. Introduction

Sustained topical delivery of therapeutics to the retina remains a major unmet need. Retinal diseases, such as diabetic macular edema, are the leading causes of blindness in the industrialized world [1]. Most retinal diseases are treated by intravitreal injections or therapeutic-

☆ The data was presented in part at the Glaucoma 360: 5th Annual New Horizons Forum (2017), San Francisco, CA and at the Association for Research in Vision and Ophthalmology, Honolulu, HI (2018).

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https://doi.org/10.1016/j.biomaterials.2019.119285
Received 26 March 2019; Received in revised form 7 June 2019; Accepted 13 June 2019
Available online 21 June 2019
0142-9612/

eluting implants, which risk potential side effects such as elevated intraocular pressure, retinal detachment, intraocular bleeding, and infection [1,2]. Patients are not uniformly enthusiastic about the insertion of needles into their eyes. In fact, 22–25% of patients who receive intravitreal injections do not return for their follow up appointment [3,4].

Another major drawback of the intravitreal approach is that the drug cannot be easily removed from the eye if drug-related side effects occur. For instance, many patients that receive steroid-eluting intravitreal implants eventually require glaucoma medications and glaucoma surgery to treat steroid-induced increases in intraocular pressure [5–7]. In contrast to intravitreal injections and implants, a topically applied treatment could be easily discontinued if drug-related side effects occur. Unfortunately, most drugs that treat retinal conditions are not currently administered topically. Moreover, a topical method of sustained drug delivery to the retina is not available.

In order for a topically applied drug to reach the retina, it has to overcome many of the barriers that the eye has evolved to protect itself from toxins and infections [8–12]. Foreign substances are rapidly washed away from the ocular surface through reflex tearing and blinking. Similarly, topically applied drugs typically have reduced ocular surface residence time due to reflex tearing, tear dilution, and unidirectional fluid drainage through the nasolacrimal duct [13]. Reflex blinking further pushes a drug away from the ocular surface [13]. Furthermore, the periodic application of most topically applied drugs, usually in the form of eye drops, does not allow for sustained drug concentrations on the ocular surface thus limiting drug flux [14,15]. More recently, there have been increased efforts focused on increasing the drug residence time as a means of improving ocular drug flux [16–18].

Drugs on the ocular surface enter the eye by passing through either the conjunctiva or the cornea, which have anatomical differences that influence drug flux [13]. The conjunctiva covers nearly 95% of the ocular surface (covering the sclera and the inside of the eyelids) providing ample surface area for drug absorption [13,19]. However, the conjunctiva has a dense vascular network that can cause significant drug loss to the systemic circulation. In contrast, the cornea is avascular and in general, provides a more efficient conduit for ocular drug penetration, particularly for lipophilic molecules [13]. However, directing drugs to the cornea is challenging since it accounts for a small percentage (about 5%) of the ocular surface [19].

The cornea has anatomical barriers, such as the epithelial tight junctions that provide additional barriers to infection and drug penetration. It has been shown that preservatives (e.g. benzalkonium chloride [BAK]) in ophthalmic solutions can disrupt the ocular surface and can act as permeation enhancers to improve ocular drug flux [20]. However, because preservatives, such as BAK, can also lead to ocular toxicity, there is an increasing demand for drugs that can enter the eye in a preservative-free manner. There are additional barriers to drug delivery within the eye: aqueous humor turnover, choroidal circulation, Bruch's membrane and retinal pigmented epithelium, blood-aqueous barrier, blood-retinal barrier and retinal layers that severely limit drug penetration to the retina [8–12].

We hypothesize that ocular drug flux to the retina can be improved by providing sustained drug delivery directly to the cornea, increasing drug concentrations at the ocular surface, increasing drug residence time at the ocular surface, and by using mechanical forces as a permeation enhancer. Further to that hypothesis, a sustained drug-eluting contact lens would seem well-suited for drug delivery to the eye, including the retina, as it is positioned directly over the cornea. A contact lens also creates a potential space between the lens and cornea generally referred to as post-lens tear film (POLTF). The POLTF has a considerably slower turnover than tears [21] and thus can act to increase the drug residence time on the cornea [22,23]. This may be particularly true if the contact lens is a drug depot and is able to provide sustained release of the drug directly to the POLTF and increase the ocular drug bioavailability. Ultimately, providing higher drug concentrations in a sustained manner to the ocular surface could overcome many of the barriers to ocular drug delivery and thus increase the amount of drug that could permeate to the retina.

The concept of using contact lenses to deliver drugs to the eye is not new, but achieving controlled drug delivery has been a historical challenge [22,24–27]. To date, there are no commercially available drug-eluting contact lenses. Here, we describe a novel dexamethasone-eluting contact lens drug delivery system (Dex-DS) and investigate its safety and efficacy in preventing inflammation in the posterior segment of the eye [28–30]. Dexamethasone was incorporated into the contact lens because it is a corticosteroid with an established safety profile with a history of topical or intraocular administration for a wide variety of inflammatory conditions, and one of the most potent of the topically-applied corticosteroids used in ophthalmology.

2. Results

2.1. The dexamethasone delivery system design enables drug loading without compromising light transmission through the contact lens

The dexamethasone delivery system (Dex-DS) was prepared by encapsulating a dexamethasone-poly(lactide-co-glycolide)(PLGA) film within the periphery of a hydrogel contact lens composed of a commonly used soft contact lens material, methafilcon, as described in Methods [28,31]. PLGA has an established safety profile and is often used in drug delivery and specifically, ocular drug delivery [32]. PLGA has often been used as a delivery system for dexamethasone [33–35] and the interaction of PLGA with dexamethasone is well established [36–38]. Methafilcon is a co-polymer of 2-hydroxyethyl methacrylate and methacrylic acid. In the hydrated state, the Dex-DS had an outer diameter of 15.4 mm and contained the drug polymer film with an inner diameter of 7.4 mm and an outer diameter of 11.7 mm. When imaged by ocular coherence tomography (OCT), the drug-polymer film can be seen within the contact lens (Fig. 1A and B).

The drug-polymer film within the periphery of the Dex-DS maintains a clear central aperture that is analogous to cosmetic colored hydrogel contact lenses [39,40]. For colored contact lenses, the size of the aperture is limited in order to produce the desired cosmetic appearance; however the small diameter has been reported to interfere with light transmission [39,40]. To avoid this problem, the Dex-DS was designed with a larger aperture (7.8 mm diameter) than typical colored contact lenses (6.7 mm diameter for Air Optix® COLORS). In this study,

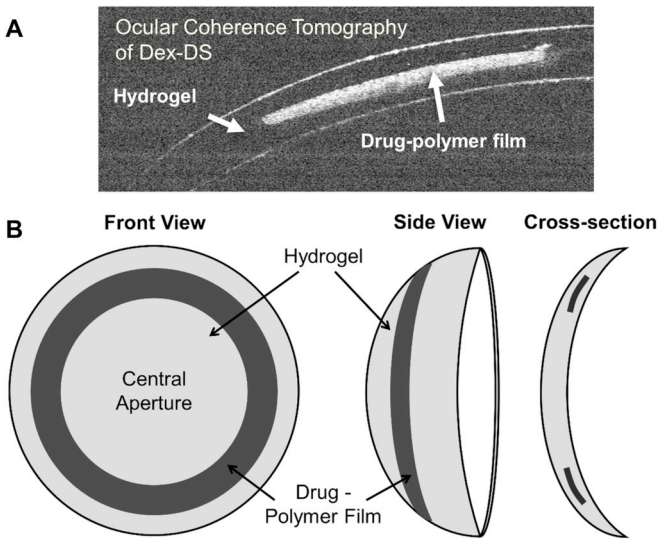

Fig. 1. Dexamethasone Delivery System (Dex-DS). (A) An ocular coherence tomography (OCT) image of a section of a (Dex-DS). (B) Schematic of the Dex-DS showing an encapsulated drug-polymer film and central aperture to allow for visual acuity.

we found that average light transmission in the visible light range (390–700 nm) through the Dex-DS was 98.1 ± 0.7%. The difference in the light transmission between Dex-DS (98.1 ± 0.7%), a commercial methafilcon lens (99.9 ± 0.8%), Air Optix® Blue (79.7 ± 0.5%) and Air Optix® brown (78 ± 2.1%) was found to be significant ($p < 0.001$, ANOVA). There was no significant difference between commercial contact lens and Dex-DS ($p = 0.31$, Multiple Comparison using Tukey's HSD post hoc test). Commercial contact lens and Dex-DS had significantly better transmission than both types of Air Optix® lenses ($p < 0.001$, Multiple Comparison).

2.2. Dexamethasone delivery system provided one week of sustained drug release

The total amount of dexamethasone in the Dex-DS was quantified by high-performance liquid chromatography (HPLC, see methods) and measured to be 1484 ± 118 μg (mean ± standard deviation) (n = 4). The loading amount was selected based on the maximum amount generally used in intensive eye drop therapy since the goal was to encapsulate and release a therapeutically meaningful amount of drug in a controlled manner for 1 week. Dexamethasone 0.1% ophthalmic solution is typically used clinically 4–8 drops a day. Assuming that the volume of 1 drop is 30 μL, the eye drops deliver about 900 to 1800 μg of dexamethasone per week. Thus, an equivalent amount of dexamethasone was incorporated in the Dex-DS.

Bench-top drug release from the Dex-DS was characterized by immersing the lenses in phosphate buffered saline (PBS) incubated at 37 °C under constant agitation at 64 rpm under infinite sink conditions. The Dex-DS maintained a release rate between 170 and 290 μg per day for the first 4 days (Fig. 2A), and continued to release dexamethasone for 7 days. Because PLGA 85:15 degrades in 5 – 6 months [41], the degradation of PLGA 85:15 did not seem to play a role in the release kinetics since the release from Dex-DS was almost completely over in 7 days. Thus, the most likely mechanism of release from Dex-DS would be diffusion of dexamethasone out of the PLGA 85:15 matrix and the methafilcon hydrogel.

High Performance Liquid Chromatography (HPLC) was performed on dexamethasone released from the Dex-DS after sterilization and compared to native dexamethasone spectra. The drug eluting from the Dex-DS was found to be unaltered from native dexamethasone indicating that gamma sterilization had no detectable effect on the steroid (Supplement fig. 1).

2.3. In vitro drug release from the Dex-DS exceeded that from commercially available hydrogel contact lenses soaked in dexamethasone solution.

In an effort to improve topical drug delivery to the eye, commercially available hydrogel contact lenses have been soaked in ophthalmic solutions (eye drops) before being inserted onto the eyes of patients [22,24–27]. Therefore, the release of dexamethasone from the Dex-DS was compared to that of commercially available contact lenses that can be used for uptake and release of the same drug from readily available 0.1% dexamethasone phosphate ophthalmic solution. Commercial contact lenses composed of the same methafilcon hydrogel material and with dimensions similar to the Dex-DS (thickness and diameter) were soaked in 0.1% dexamethasone phosphate solution for 24 h and then placed in PBS using the same bench-top release conditions and HPLC quantification noted above. The drug release profile from commercial contact lenses was consistent to that of previously published studies, which showed an early burst with the vast majority of the drug released within the first 4 h [25,42–44]: 92 μg (71%) of dexamethasone was released after the first hour, 121 μg (93%) was released after the first 2 h and no drug was detected after 4 h (Fig. 2A and B). Commercial contact lenses were also soaked in higher concentration dexamethasone solutions in an attempt to maximize drug absorption into the lens. When the commercial contact lenses were placed in dexamethasone

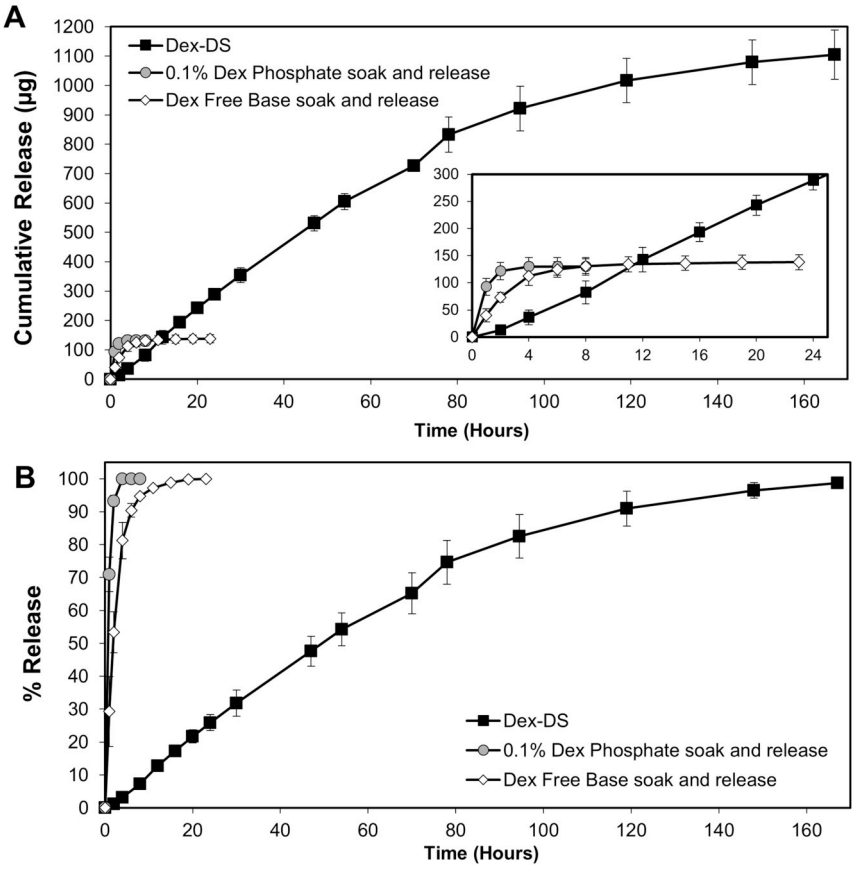

Fig. 2. In vitro Dexamethasone release. Cumulative mass (A) of dexamethasone released and % of dexamethasone mass (B) release respectively from the Dexamethasone Delivery System (Dex-DS) and commercial contact lenses soaked in different dexamethasone formulations. Insert represents the first 24 h of release. Data are mean ± standard deviation (n = 4).

phosphate solution in PBS, the lenses disintegrated into pieces.

Because the drug used in the Dex-DS is dexamethasone free-base, separate commercial contact lenses were soaked in 0.05% dexamethasone free base solution (above solubility limit of dexamethasone to ensure maximum absorption). The commercial lenses remained intact and released a cumulative amount of 139 ± 16 μg of dexamethasone, with approximately 74 μg (53%) released after the first 2 h and 114 μg (82%) released after the first 4 h (Fig. 2B). Commercial contact lenses soaked in various dexamethasone solutions released significantly less drug than the Dex-DS over the first day and in total ($p < 0.001$, Student t-test).

Compared to the commercial contact lenses tested under the conditions describe above (Fig. 2), the Dex-DS exhibited a more controlled release profile, delivered more drug over a longer duration of time (90% drug released in 5 days), and released significantly more total drug ($p < 0.001$, Student t-test).

2.4. Ocular tissue concentrations from Dex-DS exceeded those from hourly dexamethasone drops

We studied the drug flux into the eyes of rabbits at various time points over the course of one week of continuous lens wear. The Dex-DS provided sustained delivery of dexamethasone to ocular tissues in the front and back of the eye of New Zealand (NZ) white rabbits for one week (Fig. 3). Compared to other tissues, steroid levels in the cornea demonstrated the least variability throughout the week of lens wear. The cornea also demonstrated the highest drug levels of all the tissues studied. In the posterior segment of the eye, dexamethasone concentrations in the retina and choroid were significantly greater than those measured in the vitreous ($p = 0.026$ (retina), $p < 0.001$ (choroid), repeated measures ANOVA).

The tissue drug flux from dexamethasone eye drops was also assessed so that the tissue levels achieved with the Dex-DS could be compared with an intensive drop regimen that is currently used for treatment. While dexamethasone eye drops are approved for use 4 to 6 times a day, they are prescribed as frequently as every hour (while awake) to treat severe inflammatory conditions. Therefore, 1–2 drops of 0.1% dexamethasone phosphate ophthalmic solution were placed on the rabbit's eye every hour for 8 consecutive hours. The drops were given for 8 h in order to measure tissue levels after they have plateaued [45–47]. Compared to the intensive drop administration, we found that the Dex-DS provided significantly more dexamethasone to all the ocular tissues at 24 and 48 h (Fig. 3). The Dex-DS maintained significantly higher drug concentrations in the retina, aqueous humor, iris, and cornea than hourly drops through 5 days. Across the tissues and time points, the difference in tissue concentration between hourly drops and the Dex-DS was greatest in the retina at 48 h ($p = 0.004$, Mann-Whitney *U* test). The Dex-DS is placed next to the cornea, which is likely the reason that the dexamethasone concentrations in cornea peaked early and were the highest. In comparison, the retina and choroid are further from the Dex-DS and thus showed a more delayed peak drug concentration. Such a delay is consistent with pharmacokinetic modeling of topical drug delivery [48]. The differences between vascularity of the various ocular tissues also likely contributed to the difference in the drug concentration profiles. For instance, the cornea is avascular, whereas the retina and choroid are vascularized and an important function of the vasculature is to quickly remove heat and waste from the retina [49]. This vasculature can transport the drug away from the retina and choroid; these tissues have been known to efficiently and quickly transport drugs away from the retina and out of the eye [50].

2.5. Dex-DS inhibited retinal vascular leakage in the posterior segment of the eye

In general, topically applied medications are less effective for the treatment of diseases in the posterior segment of the eye than they are for the treatment of diseases in the anterior segment of the eye. Therefore, we used an animal model of retinal vascular leakage to

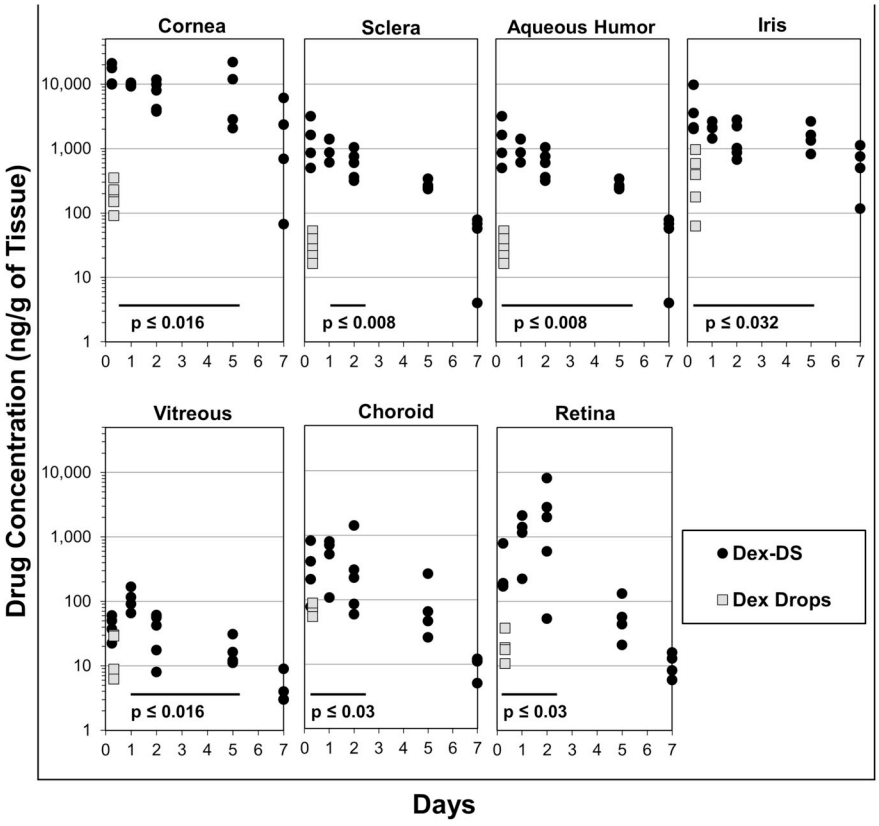

Fig. 3. Dexamethasone delivery system pharmacokinetics. Scatterplot demonstrates tissue concentrations at various time points after continuously wearing the Dexamethasone Delivery System (Dex-DS) for up to 7 days or received 0.1% dexamethasone drops every hour for 8 consecutive hours ($n > 4$ per time point). P-values calculated by comparing tissue concentration at different time points while wearing the Dex-DS to that of hourly drops using Mann Whitney *U* test.

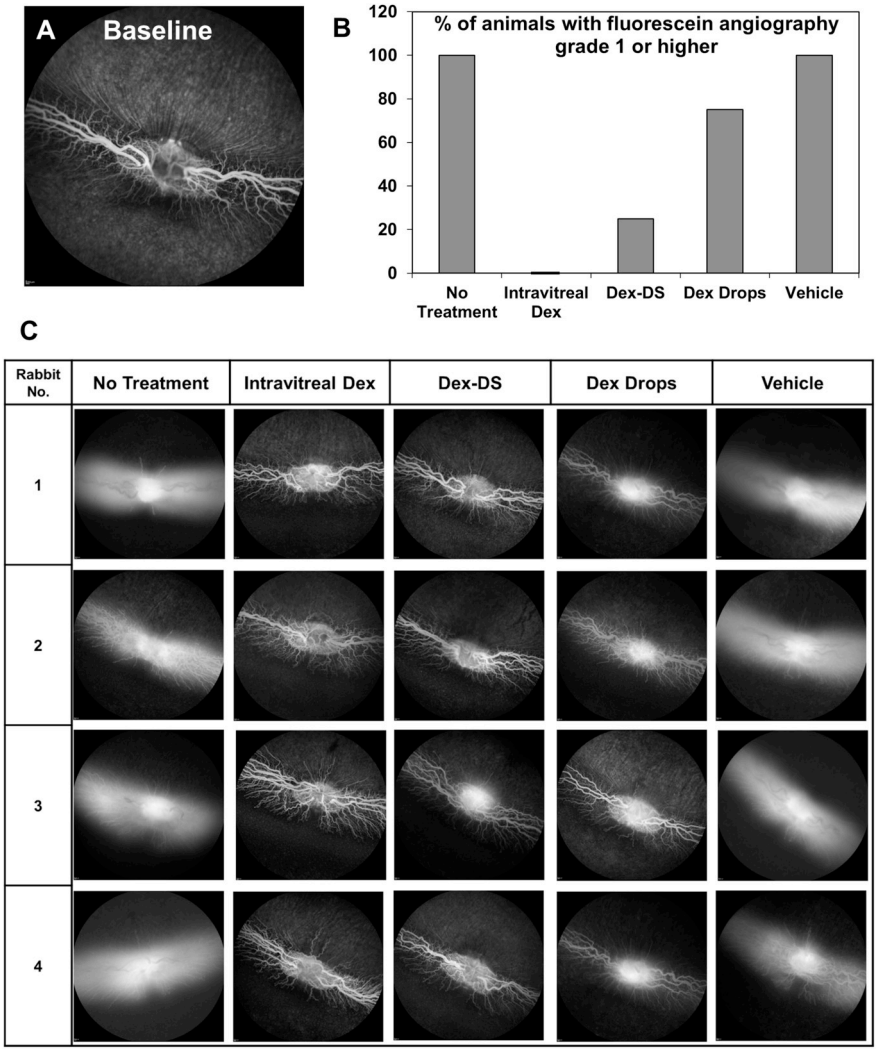

Fig. 4. VEGF-induced retinal vascular leakage study fluorescein angiography. (A) Late phase fluorescein angiography (FA) baseline image. (B) FA images graded by two masked retina specialists using the following scale: 0 = no evidence of leakage, 1 = mild to moderate leakage not obscuring the vascular pattern, 2 = severe leakage obscuring the individual capillaries. (C) FA images from each rabbit 2 days after receiving an intravitreal VEGF injection (n = 4 per treatment group). Dex = Dexamethasone, Dex-DS = Dexamethasone Delivery System, Vehicle = polymer (no dexamethasone) film encapsulated in methafilcon contact lens.

determine if the Dex-DS was effective for the treatment of conditions in the posterior segment of the eye. In this study, retinal vascular leakage was induced in NZ pigmented rabbits by an intravitreal injection of vascular endothelial growth factor (VEGF), which has been shown to have maximal vessel leakage 2 days following the VEGF injection [51]. Animals were examined before the VEGF injection to ensure that the baseline exam was free of retinal vascular leakage. All animals received a partial temporary tarsorrhaphy covering about 40% of the eye and then began 1 of 5 assigned regimens: 1) no treatment, 2) hourly 0.1% dexamethasone phosphate eye drops for 8 h a day for 2 days, 3) 400 μg intravitreal dexamethasone injection, 4) Dex-DS worn continuously for 2 days) or 5) vehicle contact lenses (no drug) worn continuously for 2 days. Two days following the VEGF injection, the animals were examined, euthanized, and the vitreous and aqueous humor collected. Fluorescein angiography (FA) was used to assess retinal leakage after a VEGF intravitreal injection (representative baseline FA shown in Fig. 4A). As shown in Fig. 4C, animals that received no treatment or vehicle demonstrated florid fluorescein leakage after 2 days. Animals treated by the Dex-DS, intravitreal dexamethasone, or hourly dexamethasone drops exhibited fluorescein leakage that was either minimal or undetectable. The fluorescein angiography images were graded by 2 masked retinal specialists using an established 0–2 point grading scale (Grade 0 = no evidence of leakage, Grade 1 = mild to moderate leakage not obscuring the vascular pattern, Grade 2 = severe leakage obscuring the individual capillaries) [52]. Significantly less leakage was observed in rabbits treated by Dex-DS, intravitreal dexamethasone or hourly dexamethasone drops when compared to either no treatment or vehicle (Fig. 4B). The amount of fluorescein leakage was objectively quantified using scanning ocular fluorophotometry [51], which found significantly less leakage in the animals treated with dexamethasone injection and the Dex-DS (Fig. 5A). Vitreous protein concentration, an indicator of retinal vascular leakage, was also lower in animals treated by hourly dexamethasone drops, intravitreal injection, or the Dex-DS compared to animals in the no treatment group or the vehicle group (Fig. 5B). We observed a transient elevation in IOP following VEGF injection, which lasted for less than an hour and resolved without further intervention. None of the animals in this study developed sustained elevated IOP (Supplement Table 1) or corneal complications as evident by fluorescein-assisted slit lamp examination.

2.6. Dex-DS did not result in toxicity during a 4-week repeated dose study

Steroids have been reported to cause ocular and systemic side effects such as increased intraocular pressure, tissue necrosis, and weight

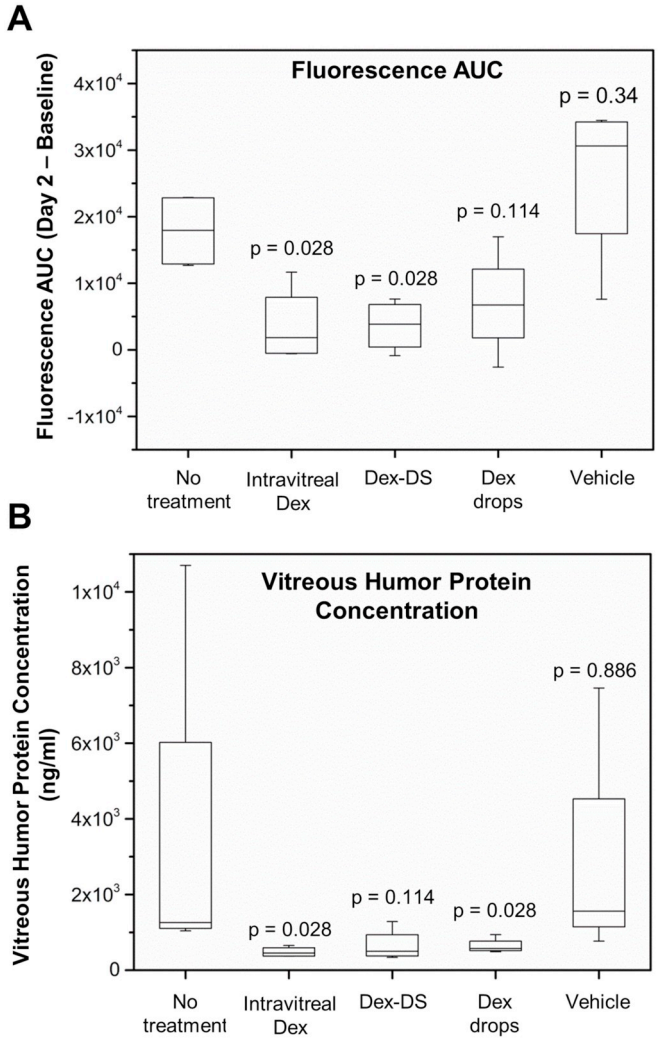

Fig. 5. VEGF-induced retinal vascular leakage quantification. Box plots demonstrating (A) fluorescence change from baseline and (B) vitreous protein levels. P-values were calculated by Mann Whitney *U* test comparing the treatment groups to No Treatment. AUC = area under the curve. n = 4 per treatment group. Dex = dexamethasone, Dex-DS = Dexamethasone Delivery System, Vehicle = polymer (no dexamethasone) film encapsulated in methafilcon contact lens.

Table 1
Ratio of peak tissue dexamethasone concentration to peak serum dexamethasone concentration. Ratios were calculated by taking the median value for each concentration.

Ratio of Drug Concentration in Tissue to Serum[a]		
	0.1% Dexamethasone Hourly Drops	Dex-DS
Cornea	7	758
Scleral	0.5	51
Aqueous Humor	1	98
Iris	13	165
Vitreous	0.3	8.3
Choroid	2	48
Retina	0.6	195

[a] For the Dexamethasone Delivery System (Dex-DS), peak tissue concentrations were measured at 6 h for the cornea, iris, and aqueous humor, 8 h for serum, 24 h for choroid and sclera, and vitreous, and 48 h for retina. For hourly drops, all measurements are within an hour of administering eight hourly drops.

loss in rabbits [45,53,54]. Therefore, the safety of Dex-DS was studied in normal NZ white rabbits that continuously wore a Dex-DS for one week for 4 continuous weeks. Using the same study design, commercial contact lenses composed of methafilcon were also evaluated and exam findings were compared to those from the Dex-DS. All of the Dex-DS were retained in each of the 4 animals at each of the visits during the study. Despite of presence of a partial temporary tarsorrhaphy, commercial contact lenses were retained in all four of the rabbits by the end of the first week, but were retained in only 2 of 4 rabbits by the end of the 2nd week, 1 by the end of the 3rd week, and 3 by the end of the 4th week. Only data from animals that retained the contact lens were used for data analysis for that week. The Dex-DS had a base curve of 8.7 as compared to 8.9 for the commercial lenses. A base curve of 8.7 is slightly steeper than 8.9 and it is possible that Dex-DS had a tighter fit on the rabbit eye. While the difference in the base curves is likely negligible and was not apparent on examination of the rabbit eyes, it could possibly explain why the commercial lenses were lost frequently in spite of the presences of a partial temporary tarsorrhaphy.

Contact lenses resting on the eye and topical steroids could potentially compromise the ocular surface, which can result in corneal abrasions and corneal tissue necrosis. Therefore the health of the ocular surface of the cornea was assessed by two masked ophthalmologists by grading the representative slit lamp digital photographs using the NIH 15-point staining grading scale [55]. Fluorescein is used to assess the integrity of the corneal epithelium because it stains the cornea where the epithelium has been injured or compromised. There was little to no fluorescein staining of the cornea with either commercial contact lenses or the Dex-DS at any time point (Supplement Table 2).

Steroids have been associated with an increase in IOP, which is more likely to occur with high dosing or long-term use, and has been reported in rabbits [53,54]. IOP remained within normal limits for animals wearing the Dex-DS and commercial contact lenses (Fig. 6A). For both treatment groups, IOP was not statistically significantly different from the baseline measurements and did not differ between the treatment groups.

Contact lens wear has been reported to temporarily increase corneal thickness [56,57]. Contact lens-associated corneal edema has been shown to increase with extended and overnight contact lens wear. In addition, the presence of partial tarsorrhaphy to retain the lenses along with the lens is also known to cause corneal edema [58,59]. Therefore, we measured the corneal thickness following each week of continuous lens wear (Fig. 6B). Compared to baseline measurements, rabbits wearing Dex-DS did not demonstrate an increase in corneal thickness at any of the time points (3.24 ± 8.4%, $p = 0.73$, ANOVA). However, eyes wearing commercial contact lenses had statistically significantly increased corneal thickness to eyes that wore the Dex-DS ($p < 0.001$, Student t-test). The cornea thickness of eyes that lost the commercial contact lenses was compared to eyes that retained the lenses. The % change in central corneal thickness of the eyes that retained the commercial contact lenses (28.1 ± 10.1%, n = 10, mean ± standard deviation) was significantly higher than those that lost the lenses (15.8 ± 10%, n = 6, $p = 0.02$ using Student t-test) indicating the reversible nature of the edema associated with contact lens wear.

Outside of the eye, steroids are also known to cause systemic side effects and rabbits are particularly sensitive to steroids [47,53,60–62]. Weight loss, an indicator of animal appetite and well-being, has been reported following dexamethasone drop use and weight measurements are part of many toxicology evaluations [53,61]. Compared to the baseline weight, there was no significant change in weight observed in either study group ($p = 0.99$, ANOVA) (Fig. 6C).

At the end of the study, the animals were euthanized and eyes enucleated. A masked ophthalmic pathologist evaluated and graded the histology slides that were stained with Periodic acid-Schiff (PAS) and hematoxylin and eosin (H&E). All the eyes treated with Dex-DS had normal histology (Fig. 7, Supplement Table 3) with non-keratinizing squamous epithelium resting on the Bowman's layer (basement

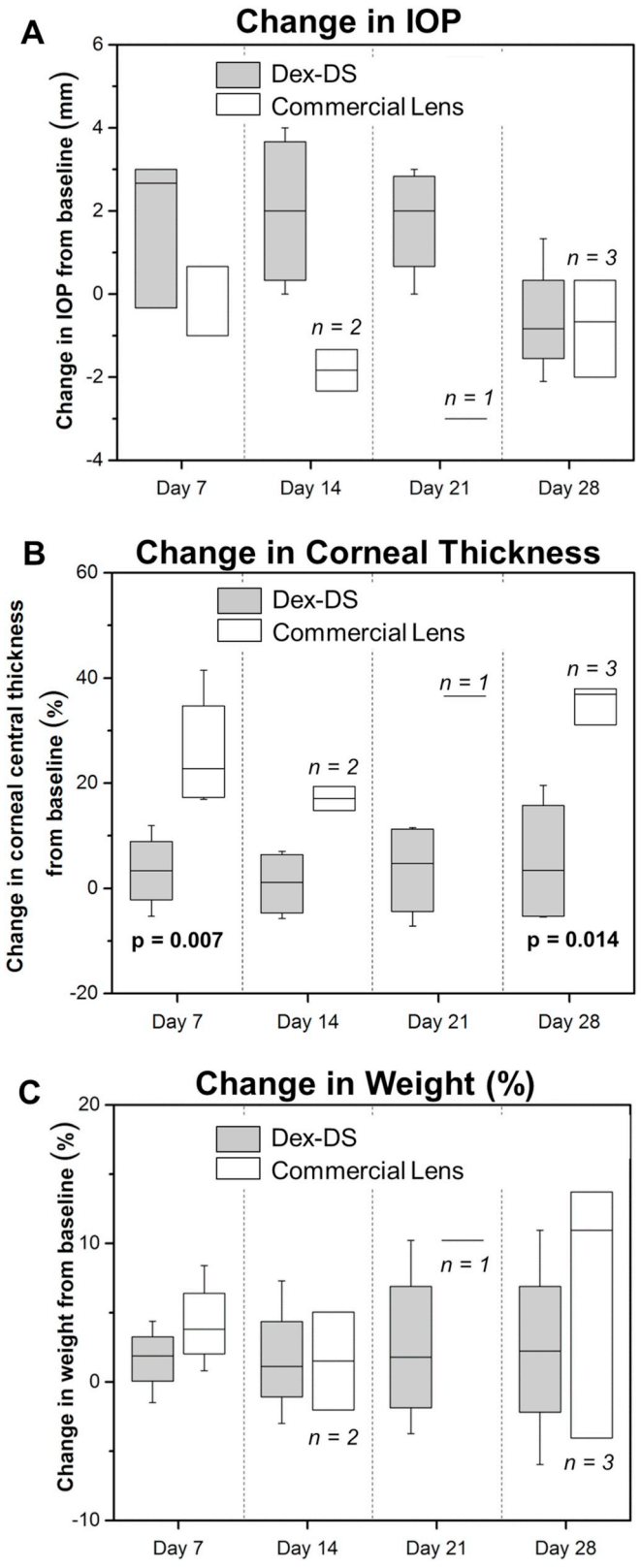

Fig. 6. Four week repeated dose biocompatibility study. Change from baseline (day 0) measurement of (A) Intraocular pressure, (B) Central corneal thickness and (C) Weight (%). There were no significant changes in IOP or weight compared to baseline. P-values comparing change from baseline using two tailed Student t-test. (n = 4 unless otherwise noted). Dex-DS = Dexamethasone Delivery System.

membrane), stromal lamellae separated by artifactitious clefts induced by dehydration during normal processing for paraffin embedding, Descemet's membrane and endothelium.

One out of the 4 eyes treated with the commercial contact lens showed mild (< 30% of cornea) superficial epithelial bullae and slight (< 5% of cornea) basal vacuolar changes (Fig. 7). The contralateral control eyes for both groups that did not wear either Dex-DS or commercial contact lens (n = 8) were also studied and one of these eyes had slight (< 5% of cornea) stromal edema. The differences in the mean severity scores between the various groups were not statistically significant by Mann-Whitney *U* test. Necropsies were performed and no pathology was observed. Kidneys, gall bladder, heart, lungs, liver, spleen, stomach, intestines, reproductive system and lymph nodes were found to be normal (size, shape, color and absence of nodules). For details, refer to the supplemental section.

2.7. Serum drug levels were lower with Dex-DS than with hourly dexamethasone drops

One of the advantages of local drug delivery is its ability to maximize drug delivery to the target tissue and minimize systemic drug exposure. This is important to avoid the systemic side effects of steroids [47,63]. Blood serum was collected during the Dex-DS repeated dose biocompatibility study (described above) from 4 rabbits at 5 different time points over the course of a week of lens wear.

For dexamethasone eye drop group, one drop of 0.1% dexamethasone ophthalmic eye drops was placed every hour for 8 consecutive hours. Blood was drawn from anesthetized animals approximately 1 h following administration of the 8th drop. Consistent with the literature, the maximum serum drug concentration occurred 1 h after administration of the last hourly drop (27.5 ± 8.04 ng/mL) [47,60,64,65], and then declined after 4 h (9.9 ± 3.7 ng/mL) and 24 h (undetectable) (Fig. 8). Following the insertion of the Dex-DS, serum dexamethasone concentration peaked after 8 h (13.9 ± 2.0, p = 0.07 compared to drops at 1 h) and then declined thereafter. For all other time points, the serum drug levels were lower with the Dex-DS than with hourly dexamethasone drops: 4 h (5.8 ± 3.1, p = 0.01), 1 day (12.1 ± 2.4, p = 0.046), 3 days (2.2 ± 1.8, p = 0.01), and 7 days (below level of detection in 3 of 4 animals). The ratio of tissue concentration to serum concentration was higher by 1-2 orders of magnitude in the Dex-DS compared to hourly drops (Table 1). On day 7, the serum drug level was very high for one animal wearing the Dex-DS at a single time point and appears to be an artifact for the reasons noted in the Supplement Information section.

3. Discussion

An ocular drug delivery platform has been developed that can provide sustained delivery of a steroid, dexamethasone, to the back of the eye for at least 7 days. The Dex-DS resulted in much higher drug concentrations in the anterior and posterior segments of the eye compared to hourly dexamethasone drops. The Dex-DS prevented VEGF-induced retinal vascular leakage in the posterior segment of the eye. Systemic distribution of dexamethasone was much lower from the Dex-DS than that from hourly eye drops. The Dex-DS was biocompatible *in vivo* and demonstrated no evidence of increased IOP over a period of repeated use for 4 weeks.

The Dex-DS, in contrast to currently available treatments, provides the unique combination of topical drug delivery and sustained medication release to the back of the eye for one week. This approach is well aligned with current physician practice pattern of seeing patients one week after surgery or after diagnosis of other inflammatory conditions. For example, patient self-administered steroid eye drops and physician-inserted therapeutic bandage contact lenses are already used together for approximately one week following several types of eye surgeries [27,66–68]. Under this clinical scenario, the Dex-DS would eliminate

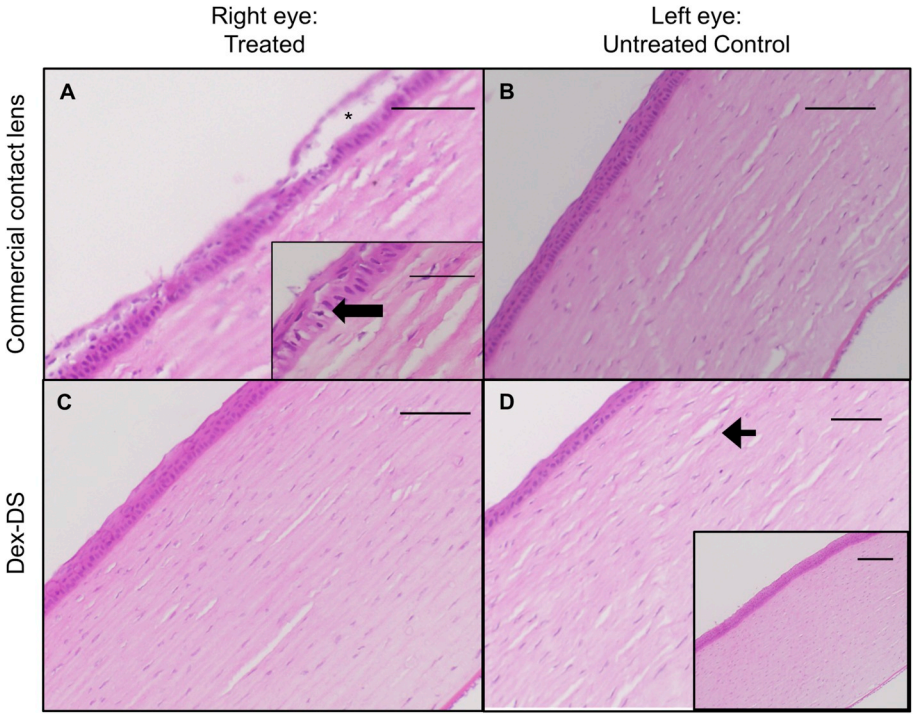

Fig. 7. Corneal histology after four week repeated dose biocompatibility study. Corneal histology after 4 weeks of wearing (A) commercial contact lenses or (C) Dexamethasone Delivery System (Dex-DS). (Hematoxylin-eosin, 100× magnification) (A) Commercial contact lens, right eye: Epithelial bullae (*) in < 30% of the cornea and basal vacuolar change (arrow) of the epithelium involving < 5% of the cornea (inset) in 1 of 4 eyes. (B) Contralateral eye (no contact lens) from animals in the commercial contact lens group, left eye: Normal histology (C) Dex-DS, right eye: Normal histology. (D) Contralateral control (no contact lens) eye for the Dex-DS, left eye: Normal histology seen in 3 out of 4 eyes (arrowhead indicating artifactitious clefts seen in normal stroma). Inset: 1 out of 4 cases with < 5% stromal edema indicated by loss of stromal artifactitious clefts.

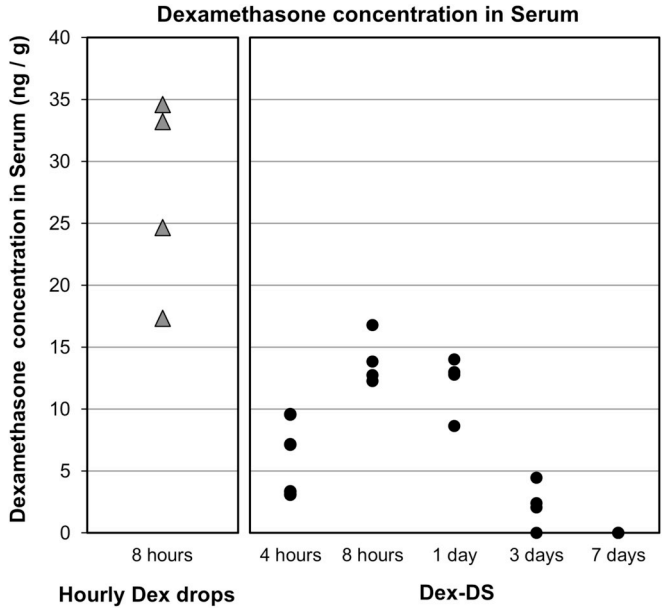

Fig. 8. Scatterplot demonstrating serum dexamethasone (Dex) concentrations at various time points. Serum concentrations after continuously wearing the Dexamethasone Delivery System (Dex-DS) for up to 7 days (right) or after receiving 0.1% dexamethasone drops every hour for 8 consecutive hours (n = 4 per time point) (left). For the Dex-DS, serum drug concentrations were below limit of detection for one animal on day 3 and for 3 of 4 animals at day 7. P-values compared to dexamethasone drops using two tailed Student t-test.

the numerous self–administered drops or intravitreal injections. An ophthalmologist would administer the Dex-DS and then remove or replace the lens a week later as indicated by the clinical response or the development of side effects, such as ocular hypertension. A key advantage of the Dex-DS is that it can be easily removed if ocular hypertension develops. It is worth noting that the Dex-DS did not result in an increase of IOP in the rabbits studied. In comparison, previously published studies reported that 1% dexamethasone drops given 3 times a day resulted in a mean IOP increase of 5 mm Hg after 1 week of treatment and an increase of 10 mm Hg after 2 weeks of treatment [53,54]. In a previous study with a dexamethasone intravitreal implant, a similar trend was noted where the IOP did not change when the rabbit eyes were exposed to dexamethasone for prolonged periods (8 weeks) [69]. This could indicate that localized delivery of dexamethasone may not be affecting IOP in rabbits. Additionally, the age of rabbits can also have an impact on whether dexamethasone can cause an increase in IOP [70].

The Dex-DS resulted in drug concentrations within the anterior segment (cornea) and posterior segment (retina) of the eye that were significantly higher than from dexamethasone eye drops. There are several significant differences between drug delivery with the Dex-DS and topical ophthalmic solutions (eye drops) that may explain these results. One important consideration is the residence time of the drug on the ocular surface. When a drop is instilled on the eye, most of it is washed away due to dilution and tear turnover, and it has previously been estimated that only 1–7% of the drug enters the eye [22,71]. In contrast, the drug-eluting contact lens itself may prevent the rapid turnover of the tear film between the lens and cornea (post-lens tear film), which thereby drastically increases the residence time of the drug on the ocular surface. This can lead to higher concentration of the drug in the post-lens tear film, which would increase the concentration gradient and increase the ocular drug influx. It has been estimated that the bioavailability of the drug released from hydrogel contact lenses could range from 35% to 50% [22,23].

It has been suggested that topical drugs may get to the back of the eye through a corneal (cornea – aqueous humor – eye tissues) route or a non-corneal (conjunctiva – scleral) route [8,10–12,50]. In this study, the Dex-DS resulted in significantly higher drug concentrations in the choroid and retina than the vitreous. Based on this data, it is unlikely that most of the drug that reached the retina did so by traveling through the vitreous. Further research is needed to determine the exact mechanism by which the drug reaches the retina from a contact lens or other topically applied device.

Our data demonstrated that the Dex-DS is highly efficient in terms of topical drug delivery to the eye relative to the systemic drug exposure as measured by serum drug concentration, meaning that the

lenses provide far higher drug concentrations to the ocular tissues than to the blood serum. For instance, the ratio of the peak drug concentration within the cornea vs. the serum is 758 with the Dex-DS. Similarly, the corresponding ratio within the retina is 195. In contrast, the same ratios for hourly dexamethasone drops are 7 and 0.6 in the cornea and retina, respectively (Table 1). Within the posterior segment of the eye, the Dex-DS was as effective as intravitreal dexamethasone injections for the prevention of VEGF-induced retinal vascular leakage. Given that the drug flux studies did show much greater drug delivery to the tissues of the front and back of the eye than hourly drops, it is possible that the Dex-DS could have been more effective than hourly dexamethasone drops using larger treatment groups or animal models that induce more robust inflammation.

Good correlation has been found between results obtained with rabbits and those from human studies using topically applied dexamethasone (a small molecule) [72–75]; thus this drug-animal model combination was used in our studies. For example, cyclodextrin has been used to modify dexamethasone eye drops in order to increase drug concentrations in the retina. The dexamethasone-cyclodextrin formulation resulted in retinal drug concentrations in rabbits that were significantly higher than unmodified dexamethasone eye drops [72–75], and was effective in treating macular edema in humans [73,75]. Compared to the dexamethasone-cyclodextrin eye drops, our Dex-DS resulted in much higher retinal drug concentrations in rabbits. For instance, average retinal dexamethasone concentrations in rabbits 120 min after topical administration of 1.5% dexamethasone-RMβ Cyclodextrin solution were reported to be 66 ng/g. In our study, the average retinal dexamethasone concentration was 383 ng/g after 6 h of wear and 4353 ng/g after 48 h of wear. This favorable drug flux comparison suggests that the Dex-DS may be used to treat some forms of macular edema in humans.

The Dex-DS was found to be safe throughout the repeated dose study 4-week biocompatibility study in normal rabbit eyes. It was surprising that Dex-DS prevented the occurrence of corneal edema that is observed with prolonged hydrogel contact lens wear in rabbits [57,76]. Contact lenses have been used in rabbits to induce and study corneal edema, which has been reported to develop about 2–3 h after lens placement and resolves about 3 h after lens removal. Consistent with these publications, we observed an average corneal thickening of 85.4 μm (28.1 ± 10.1% change from baseline) with commercial contact lenses. In contrast, corneal edema was not observed in eyes that wore the Dex-DS. Corneal edema is known to result from contact lens use by itself and under closed eyelids (tarsorrhaphy); this edema is typically attributed to corneal hypoxia [58,59,77,78]. The current understanding is that the contact lens impedes oxygen to the cornea epithelium, which becomes hypoxic and releases lactate into the stroma [79]. The endothelium, which is responsible for pumping lactate out of the corneal stroma, cannot keep up with the build-up of lactate. As a result of the increase in stromal lactate levels, water is retained due to the osmotic effect and the cornea becomes edematous [80–83]. The reduced corneal edema seen in the rabbits that wore the Dex-DS therefore seems to be an effect of dexamethasone. While there is some evidence in the literature to support the use of steroids to reduce corneal edema, there are conflicting reports as well and hence dexamethasone (and steroids in general) are not used clinically to reduce corneal edema in the absence of an inflammatory etiology [81,83–85]. There is supporting evidence for several mechanisms by which steroids may prevent or reduce corneal edema. One hypothesis is that steroids suppress lactate production by corneal epithelial cells [86]. An alternative hypothesis is that steroids accelerate endothelial cell pump efflux of lactate and water [87,88] Another hypothesis is that CLs cause subclinical inflammation that is suppressed by steroids [80,89] Further research is required to test these hypotheses. Steroid-induced ocular hypertension has been reported in rabbits, but there are many conflicting reports about its reproducibility [53,54,70,90,91]. It was not observed in our study or in a previous study with a dexamethasone intravitreal implant, which found a similar trend of no IOP change when rabbit eyes were exposed to intravitreal dexamethasone for prolonged periods (8 weeks) [69]. When steroid-induced ocular hypertension does occur, it has been reported to be dependent on dosage, duration of treatment as well as age of the rabbits [53,54,70,90,91]. The side effects that may occur, specifically with high dosages of steroids include elevated IOP, weight loss and systemic toxicity – these side effects were not observed in rabbits wearing the Dex-DS. Additionally, the systemic (serum) concentrations from Dex-DS were same as or lower than hourly 0.1% dexamethasone eye drops. These results point to another possible advantage of localized drug delivery; lower systemic drug exposure that could lead to fewer side effects.

We have developed a steroid-eluting contact lens to provide non-invasive sustained ocular drug delivery, and have demonstrated its efficacy in inflammatory-mediated model in the back of the eye in rabbits. All of the drugs and polymers used in the Dex-DS have been FDA-approved for ocular use, and its duration of release is compatible with clinical practice. Given that steroids are used to treat many eye conditions in the front and back of the eye, the Dex-DS could find broad application within ophthalmology. The Dex-DS reported here may eliminate the need for intensive eye drop self-administration or intraocular steroid injections, can improve adherence, and potentially provide a more effective treatment for inflammatory-mediated eye conditions. In contrast to intravitreal injections that are typically given by retina specialists, most eye care providers could administer the Dex-DS; this could increase treatment accessibility and potentially expedite patient care. Based on the safety and efficacy results on this study, we have taken steps to initiate a Phase I/II clinical trial for the treatment of recurrent cystoid macular edema, a condition often treated by steroids. Moreover, this contact lens drug delivery system can be used to deliver other steroids or drugs beyond steroids [28,29,31], and as a platform it can potentially be used to treat a broader range of ophthalmic diseases.

4. Materials and methods

4.1. Materials

High molecular weight (119 kDa) 85:15 PLGA (85 glycolide: 15 L-Lactide) was obtained from Lakeshore Biomaterials (Birmingham, AL). Dexamethasone free-base was obtained from Spectrum Chemical (New Brunswick, NJ). Commercially available dexamethasone solution (0.1%) was obtained from Bausch and Lomb (Wilmington, MA). Unpolymerized methafilcon was purchased in liquid form from Kontur Kontact Lens Company (Hercules, CA). Phosphate buffered saline (PBS, pH 7.4) was obtained from Invitrogen (Carlsbad, CA). Biopsy punches (6 mm) were obtained from Sklar Instruments (West Chester, PA). Human recombinant VEGF-165 was purchased from R&D Systems (Minneapolis, MN). All the other reagents were purchased from Sigma Aldrich (St. Louis, MO).

4.2. Fabrication of the dexamethasone delivery system

60 mg of dexamethasone powder and 60 mg of PLGA were dissolved in 1 mL of hexafluoroisopropanol (HFIP). Using a 1:1 drug:polymer ratio, 40 μL of the combined solution was then pipetted into a concavity lathed into a cylinder of dry polymerized methafilcon (Kontur Kontact Lens Company). After 6 min of rotation on a spin coater (Model SC100, Best Tools LLC, St. Louis, MO), the liquid HFIP evaporated, leaving a drug-polymer film. A 6 mm biopsy punch was used to incise and remove the central 6 mm diameter of the drug-polymer film to create a clear central aperture that lacked a drug-polymer film. In order to remove any residual organic solvent, the hydrogel blanks containing the films were placed on a desiccator under vacuum for 3 days and then lyophilized for a day. The side of the films that was not yet in contact with methafilcon was then encapsulated in methafilcon by ultraviolet (UV) photopolymerization. The methafilcon cylinder was then lathed into

thin contact lens that consisted of the drug-PLGA film fully encapsulated within methafilcon that were then stored in airtight glass vials sealed with a sealed plastic screw top. To sterilize the Dex-DS, the glass containers holding the lenses were placed in a temperature-controlled container and terminally sterilized by irradiation in a Gamma Cell 220E Cobalt 60 Irradiation Unit (Atomic Energy of Canada Ltd., Ottawa, Canada) with a total dose administration of 25 kGy. High Performance Liquid Chromatography (HPLC) was performed on dexamethasone released from Dex-DS before and after sterilization to ensure that sterilization did not affect the drug.

4.3. Optical coherence topography of the Dex-DS

The Dex-DS was imaged using anterior segment optical coherence topography (AS-OCT); RTVue, Optovue, Fremont, CA) to assess the morphology. Non-hydrated Dex-DS was positioned with the convex side of the lens facing the OCT camera. Raster scanning imaging was used in four segments for each contact lens to obtain cross sectional images of the contact lens and drug-polymer film.

4.4. Light transmission studies

The light transmission was measured through hydrated Dex-DS using the method previously described [92]. Light transmission was also measured through plano (no refractive power) commercial hydrogel (methafilcon) contact lenses (Kontur Contact Lenses, Hercules, CA) and plano Air Optix® colored contact lenses that were composed of lotrafilcon b (a co-polymer of pHEMA) and contained an opaque brown or a blue iris segment surrounding a 6.7 mm central aperture (n = 4). Hydrated contact lenses were placed in a 5 × 20 mm quartz cuvette (Lambda X, Nivelles) filled with PBS. Light transmittance was measured using a Lambda 1050 spectrophotometer with integrating sphere (PerkinElmer, Waltham MA). A 6 mm aperture was positioned between the center of the lens and the beam entrance port to reduce the beam size to 6 mm per ISO 18369-3 guidelines. Light transmission was calculated by averaging the transmission over the visible light spectrum (390–700 nm) and compared between the Dex-DS and different contact lens groups.

4.5. Quantification of dexamethasone in PBS by High Performance Liquid Chromatography (HPLC)

Bench-top release samples of dexamethasone were quantified using a Dionex™ ICS 5000 + HPLC (Thermo Fisher Scientific, Waltham, MA). The gradient mobile phase started at 20% Acetonitrile and 80% 0.1% trifluoroacetic acid (TFA) and ending at 50% acetonitrile and 50% TFA over 17 min. Samples were analyzed on a C18 column and detected by a UV detector at 250 nm.

4.6. Quantification of Dex-DS drug loading

Using a pestle, the Dex-DS was ground into a fine powder and submerged in dimethyl sulfoxide to dissolve dexamethasone and precipitate PLGA. Samples were centrifuged, and the supernatant was further diluted by PBS and then filtered for HPLC analysis using gradient elution and UV detection at 250 nm.

4.7. In vitro drug release

Bench-top release of the Dex-DS was conducted by immersing Dex-DS (n = 4) in 10 mL PBS and incubating at 37 °C. At predetermined time-points, the Dex-DS was removed from PBS and immersed in fresh PBS. The volume of PBS and time-points were chosen to maintain near-infinite sink conditions. Aliquots of the PBS release media were sampled and stored at 4 °C until drug concentration was quantified using HPLC.

The ability of commercial contact lenses to absorb and release dexamethasone was analyzed and compared to the release profile of the Dex-DS. Commercial contact lenses composed of the same methafilcon hydrogel (Kontur) with the same diameter and thickness as the Dex-DS were placed in 1.5 mL of the following solutions of dexamethasone for 24 h at room temperature (n = 4 in each group):

1) PBS with dexamethasone (free base) at supersaturated drug concentration (0.5 mg/mL)
2) 2) PBS with dexamethasone phosphate at supersaturated drug concentrations (5 mg/mL)
3) Commercial dexamethasone 0.1% ophthalmic solution

After 1 day, the commercial contact lenses were removed from the dexamethasone solution, submerged in 5 mL of fresh PBS solution incubated at 37 °C, and placed on a rotational shaker at 64 rpm. Separately, the Dex-DS was also placed in PBS under the same environmental conditions. At predetermined time points, the contact lenses were removed from the release media and placed in a fresh 5 mL solution of PBS. Aliquots of the PBS release media were sampled and stored at 4 °C until drug concentration was quantified using HPLC.

4.8. Animals

The study protocols were approved by the Institutional Animal Care and Utilization Committees at Massachusetts Eye and Ear Infirmary and Schepens Eye Research Institute (Boston, MA). All animals were treated according to the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research (ARVO Handbook, 1993). New Zealand (NZ) white (Charles River Laboratories, Boston, MA) and NZ pigmented rabbits (Robinson Services, Inc., Mocksville, NC) were separately included in studies, age 2–4 months each weighing 3–5 kg. To improve lens retention, eyes with contact lenses received a temporary tarsorrhaphy in which the eyelids were closed with one to two 5-0 Nylon sutures. The tarsorrhaphy was removed for imaging and procedures. The contralateral eye remained untreated. In each of the studies, intramuscular injection of 30 mg/kg ketamine, 5 mg/kg xylazine, and 1 mg/kg acepromazine were used for anesthesia and 120 mg/kg pentobarbital was used for euthanasia. When dilation was required, it was achieved using one drop of 0.1% Tropicamide and repeated in 20 min if the pupil was not sufficiently dilated.

4.9. Ocular drug flux

Drug flux into the eye was compared between hourly administration of commercial 0.1% dexamethasone eye drops and the Dex-DS. Female New Zealand (NZ) white rabbits received one drop of 0.1% dexamethasone solution in one eye every hour for eight consecutive hours and then were euthanized within 30 min after the last drop. Separate rabbits received a Dex-DS in one eye and were then euthanized after 6 h and 1, 3, 5, and 7 days (n = 5 per time point). Following euthanization of the rabbits, the eyes were enucleated, immediately placed on dry ice, and then stored at −80 °C until tissue dissection, when the aqueous humor, cornea, sclera, iris/ciliary body, vitreous, retina, and choroid. The iris and ciliary body were collected together as one continuous tissue specimen. The central 6 mm of the cornea was excised with a 6 mm biopsy punch.

Each tissue was placed in 100 μL 10% methanol and 300 μL 100% methanol and homogenized using an Omni Bead Ruptor (Omni International, Kennesaw, GA). Ice cold 100% methanol was added to precipitate proteins. Samples were centrifuged at 14,000 rpm at 4 °C for 25 min. Then the supernatant was collected, filtered and stored for dexamethasone quantification.

4.10. Dexamethasone quantification in ocular tissues

Drug levels were quantified using liquid chromatography-tandem mass spectrometry (LC/MS-MS) with an Agilent 6410 triple quad LC/MS-MS using electrospray ionization (ESI) as the source. The gradient mobile phase was 0.1% acetic acid and 0.1% acetic acid in acetonitrile. The column was an Agilent C18 2.1 × 150 mm (3 μM), with a flow rate of 0.25 mL/min. The polarity was positive and scan type was multiple reaction monitoring with dwell energy of 200 ms. Collision energy was 4eV and fragmentation voltage was 100 V.

Select samples were quantified using an Agilent Technologies 6510 quadrupole time of flight liquid chromatography–tandem mass spectrometry (LC/MS/MS). Mobile phases consisted of 5 mM ammonium acetate and methanol. Chromatographic separation was achieved with a Phenomenex (Torrance, CA) Luna (C18) LC column (150 × 2 mm). Several samples were run on both machines and produced similar results. The lower limit of drug detection was 0.5 ng/mL and lower limit of drug quantification was 1 ng/mL.

4.11. Efficacy assessment in a rabbit model of retinal vascular leakage

The right eye of female NZ pigmented rabbits were dilated followed by ocular examination (slit-lamp and retinal OCT) and baseline imaging (described below). Pigmented rabbits were used for this study, as using albino rabbits would have resulted in inaccurate quantification of vascular leakage due to light scattering. To induce retinal vascular leakage, 500 ng of VEGF-165 in 50 μL PBS were injected into the vitreous humor of the inferotemporal quadrant of the right eye [51]. Rabbits were randomized to receive one of 5 treatment groups (n = 4 per group) that were initiated after the VEGF-165 injection: 1) 400 μg intravitreal injection of dexamethasone sodium phosphate, 2) 1 drop of 0.1% dexamethasone sodium phosphate solution hourly for 8 h per day over two days, 3) Dex-DS worn continuously for 2 days, 4) vehicle that contained a PLGA film and no dexamethasone worn continuously for 2 days, or 5) no treatment. In rabbits receiving the 50 μL intravitreal dexamethasone injection, 50 μL of aqueous humor was removed before the intravitreal injection; this was done to prevent transient IOP elevation secondary to the injection itself in eyes treated by intravitreal injection and therefore to maintain similar IOP across the treatment groups. After 2 days, the right eye of anesthetized animals was examined under an operating microscope, re-imaged, and the IOP was measured. The animals were then euthanized and the eye tissues were removed and collected. Vitreous humor protein concentration was analyzed by Bradford Assay.

Fluorescein angiography (FA) of the right eye was performed to assess the extent of retinal vascular leakage. Anesthetized animals received an intravenous injection of 50 mg/kg sodium fluorescein. FA images of the retinal vessels were acquired using a Heidelberg Eye Explorer. Early FA images were acquired immediately after the injection and then repeated every minute for 10 min after the injection. Late phase images (approximately 10 min after the injection) were randomized and independently graded by two masked retina specialists on a standardized scale from 0 to 2 (0 = no evidence of leakage; 1 = mild to moderate leakage not obscuring the vascular pattern; 2 = severe leakage obscuring the individual capillaries) [52].

Scanning Ocular Fluorometry was performed to provide objective and quantitative measurement of fluorescein leakage [51]. Forty minutes after fluorescein injection, fluorometry was performed on the rabbit using a Fluorotron™ Master Ocular Fluorometer (Ocumetrics, Inc., Mountain View, CA). Three scans were performed for each time point (baseline and 48 h after VEGF-165 injection). Pupil dilation was confirmed prior to the scan and tropicamide reapplied if necessary. Fluorometry gives the fluorescein concentration along the optical axis from retina to cornea while the area under the curve (AUC) gives the total fluorescein concentration. Retinal vessel leakage was determined by subtracting the AUC at 48 h from the AUC at baseline.

4.12. 4-Week biocompatibility study

NZ white rabbits wore either the Dex-DS or a commercial hydrogel (methafilcon) contact lens (Kontur, Hercules CA) in the right eye that was changed weekly for four consecutive weeks (n = 4 per group, 2 males and 2 females). On day 0, the right eye of the rabbit was imaged by slit-lamp photography and OCT to establish baseline measurements. Every week, the tarsorrhaphy and the Dex-DS or contact lens were removed. The eyes of the rabbits were imaged, IOP was measured using a Tono-pen®, a new Dex-DS or lens was inserted and partial lateral tarsorrhaphy was redone. Slit lamp photography with fluorescein staining of the ocular surface was performed to evaluate the cornea for epithelial defects or evidence of toxicity. Slit lamp images were masked, randomized and graded on the NEI Cornea Staining Scale by two masked cornea specialists.

AS-OCT was performed using Heidelberg Eye Explorer (Heidelberg Engineering, Franklin, MA) to measure central corneal thickness (CCT) as previously described [93]. Twenty-one line raster images were taken horizontally. The image closest to the center of the eye without artifact was selected for CCT measurement. Measurements were taken in the center of the cornea. Three measurements were made at each time point and averaged. After 28 days, the rabbits were euthanized and the eyes were enucleated. Gross necropsy was performed on the rabbits wearing the Dex-DS to examine for systemic pathology.

The eyes were fixed in 10% formalin, embedded in paraffin and cut into 10 μm sections in an anterior to posterior fashion so that the pupil and the optic nerve were in one section (PO section). The slides were stained with hematoxylin-eosin (HE) and Periodic Acid Schiff (PAS) and reviewed by light microscopy in a masked fashion by a single pathologist. Each histologic abnormality was assigned a severity grade between 0 and 5, representing normal, slight, mild, moderate, marked and severe respectively based on the estimated extent of corneal involvement (slight: ≤5%, mild: 6–30%, moderate: 31–60%, marked: 61–90%, and severe: 91–100%). The score (sum of grades) was calculated for each eye. Mean severity score was assessed (sum of scores of individual eyes divided by total number in group).

4.13. Serum drug flux

1 mL of blood was drawn from the rabbit's central ear artery at predetermined time points after administration of the Dex-DS or dexamethasone drops in order to determine the serum dexamethasone levels that resulted from the respective treatments in normal rabbit eyes. For 0.1% dexamethasone ophthalmic solution, one drop was placed every hour for 8 consecutive hours (n = 4, 2 male and 2 female). Blood was drawn from anesthetized animals approximately 1 h following administration of the 8th drop. For the Dex-DS, blood was collected from anesthetized animals during the biocompatibility study after 4, 8, and 24 h, 3 days and 7 days of wear. The blood collected was centrifuged at 4 °C at 2500 rpm for 15 min and the serum separated was pipetted off from the top and stored at −20 °C. Dexamethasone concentration in the serum was measured by LC/MS/MS as described earlier.

4.14. Statistical analysis

Ordinal data is expressed as median and interquartile range (IQR) and compared by Mann-Whitney *U* test. This was also used in studies of continuous data where the results did not follow a normal distribution, namely the vitreous humor protein concentrations. All other continuous data is expressed as mean and standard deviation, and statistical analysis was done by either Student t-test or ANOVA.

Author contributions

AER developed Dex-DS formulation. AER and RT fabricated Dex-DS

AER performed all OCT and sterilization. AER, LBC, RT and LK performed *in vitro* release studies. AER quantified *in vitro* release. LK quantified drug loading. AER, LBC, DEM, BSC, PEK, DGV and JBC designed animal studies. AER, LBC, DEM, BSC, HK, HZ and JBC performed animal studies. RT and KT quantified *in vivo* drug release. MM performed histological analysis. DEM, HK, MM, DGV and JBC performed masked image grading. AER performed light transmission studies. AER, LBC, DEM, DGV, DSK and JBC performed data analysis and interpretation. AER, LBC, LK, MM and JBC created figures. AER, LBC, DSK and JBC drafted the article and/or edited it critically for important intellectual content.

Funding

This research was funded by DOD (JBC) (DOD Vision Research Program - Translational Research Award Grant #W81XWH-15-1-0034), NEI 1K08EY019686-01 (JBC), Boston Area Diabetes Endocrinology Research Center (JBC) Grant, National Eye Institute P30EY003790 (Schepens Eye Research Institute Core), NIGMS GM 073263 (DSK). This study was supported in part by an unrestricted grant from Research to Prevent Blindness Inc., New York, NY (JBC).

Conflicts of interest

JBC and DSK are listed as inventors on a patent.

Acknowledgments

The authors wish to thank Chun Yu, Ph.D., for assistance with light transmission studies, David Zurakowski, Ph.D., for statistical advice, Bianai Fan and Philip Seifert for advice and preparation of tissue sections, Brian McAlvin, M.D., for study suggestions, Jessica Hoadley and Oscar Morales for assisting with the animal studies and Dr. Joseph Bonanno for advice on dexamethasone effect on corneal edema.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biomaterials.2019.119285.

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