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TITLE: Advancing Supersaturated Oxygen Emulsion as a Topical Treatment for Ocular Chemical Injury in Rabbits

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Fort Detrick, Maryland 21702-5012

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5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Schepens Eye Research Institute of Mass Eye and Ear

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

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12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The purpose of the project is to examine the safety and efficacy of a novel supersaturated oxygen emulsion (SSOE) for ophthalmic use to treat acute chemical injury to the rabbit eyes. The scope of the research is: 1 Determine the ocular safety of SSOE in rabbits; 2 Determine the efficacy of SSOE in mitigating acute ocular alkali burn in rabbits.

In the current reporting year (Year 1), we have accomplished the following tasks: 1) Local IACUC and ACURO approvals for animal work; 2) manufacture of the SSOE by our collaborator Coruna Medical LLC, and 3) Safety of single topical application of SSOE and vehicle in rabbits; and 4) The efficacy of SSOE in reducing ocular tissue damage in rabbits after acute burn. Single application of SSOE reduced the infiltration of inflammatory cells and stromal fibrosis in the cornea after acute alkali burn.

15. SUBJECT TERMS

Ocular chemical injury, supersaturated oxygen emulsion (SSOE), oxygenated emulsion

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
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TABLE OF CONTENTS

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

The purpose of the project is to examine the safety and efficacy of supersaturated oxygen emulsion (SSOE) for ophthalmic use to treat acute chemical injury to rabbit eyes. The scope of the research is: 1 Determine the ocular safety of SSOE in rabbits; 2 Determine the efficacy of SSOE in mitigating acute ocular alkali burn in rabbits.

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

Ocular chemical injury, supersaturated oxygen emulsion (SSOE), oxygenated emulsion

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.

Major Tasks	**Timeline (months)**	**Projected completion date**	**Actual completion date**
Major Task 1: Determine the ocular safety of SSOE in rabbits	1-9	04/30/2023	ongoing
Subtask 1.1: Local IACUC and ACURO Approval	1	09/30/2022	12/20/2022
Subtask 1.2: Ocular toxicity of single-dose SSOE application	2-4	1/31/2023	ongoing
Subtask 1.3: Ocular toxicity of repeated SSOE application	5-8	05/30/2023	ongoing
Major Task 2: Determine the efficacy of SSOE in mitigating acute ocular alkali burn in rabbits	9-24	08/30/2024	ongoing
Subtask 2.1: Immediate single application of SSOE	9-16	12/31/2023	ongoing
Subtask 2.2: Delayed and daily application of SSOE	17-24	08/30/2024	ongoing

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.

1) Major activities:

In the current reporting year (Year 1), we achieved the following: 1) Local IACUC and ACURO approvals for animal work (Major Task 1, Subtask 1.1); 2) manufacture of the supersaturated oxygen emulsion (SSOE) by our collaborator Coruna Medical LLC, and 3) **testing ocular safety of SSOE in normal rabbits (Major Task 1, Subtask 1.2); and 4) testing efficacy of SSOE in reducing ocular tissue damage in rabbits after acute burn (Major Task 2, Subtask 2.1)** and began determining the mechanisms by which SSOE preserves tissue integrity after acute burn.

2) Specific objectives:

(A) A single application of SSOE (or vehicle) will be applied to intact rabbit eyes, the objectives are to determine the following parameters:

- Corneal transparency, corneal fluorescein staining (CFS), intraocular inflammation (IOP)
- Intraocular oxygen concentration
- Microanatomy of the eye will be determined using OCT and fundus photography
- Histology of the eye using H&E staining

(B) Acute corneal alkali burn will be induced in rabbits and SSOE (or vehicle) will be applied topically immediately after burn, the objectives are to determine the following parameters:

- Corneal opacification, neovascularization (NV), CFS, intraocular inflammation, and cataract formation,
- Intraocular oxygen concentration and tissue hypoxia
- Microanatomy of the eye will be determined using anterior segment-OCT (AS-OCT)
- Histology of the eye using H&E staining
- Corneal fibrosis

3) Significant results or key outcomes:

We tested the ocular safety of SSOE and vehicle control in 2 rabbits (1 in each group). In addition, we tested the efficacy of immediate application of SSOE or vehicle control in mitigating acute ocular alkali burn in 2 rabbits (1 in each group). The low animal number is due to SERI's rabbit housing and handling capacity. In these 4 rabbits, we found that:

- A single SSOE application (or vehicle) was safe to the rabbit eye and did not result in corneal epithelial damage, corneal edema, opacity, NV, or impairment to normal ocular microanatomy (using AS-OCT and infrared fundoscopy) of the eye. Histological analysis showed good tissue integrity of the cornea and retina after a single SSOE application (or vehicle).
- Acute alkali burn (sodium hydroxide) resulted in corneal epithelial defect and persistent fluorescein staining. Compared to the vehicle control (one eye), the SSOE-treated eye (one eye) showed similar epithelial wound healing rate.
- After alkali burn, rabbits started developing corneal opacification and edema immediately. One week after alkali burn, corneal NV developed and intensified over the following 3 weeks. SSOE treatment, but not the un-oxygenated vehicle control, led to a reduction in corneal NV.
- Histology showed massive leukocyte infiltration, tissue, and fibrosis of the cornea. In SSOE-treated eye, alkali-induced infiltration of inflammatory cells into the cornea was reduced.
- Corneal fibrosis, assessed by the immunostaining of α-smooth muscle actin, was reduced by SSOE treatment.

These results are attached at the end of the report as an appendix.

4) Other achievements:

None.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to Report

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.

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

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

We plan to complete Major Task 1 and proceed to complete Major Task 2 in the next reporting period. In these tasks, we will test the safety of SSOE (and vehicle control) in more rabbits and determine the efficacy of SSOE in treating alkali burn in rabbits when given in both immediate and delayed fashion.

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 that a single topical application of SSOE to the rabbit eyes is safe without obvious impairment of tissue integrity. We also showed that a single application of SSOE after alkali injury reduced the inflammatory infiltration and the stromal fibrosis in the cornea during wound healing. Although the in vivo experiment was conducted in a small number of animals and more data in the ongoing study is needed, topical application SSOE appears safe and at least partially effective in the acute treatment of ocular chemical injuries.

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.*

Nothing to Report

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:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

There are no significant changes in the project, its direction, objectives or scope. There is change in the animal facility where the rabbit experiments are performed. In our original grant application, we proposed to contract the Pine Acres Research Facility (PARF) to conduct in vivo rabbit experiments. The reason for contracting an outside animal facility is because our home institute the Schepens Eye Research Institute (SERI) of Mass Eye and Ear has limited animal research capacity for larger animals such as rabbits. The intention to contract PARF was approved by SERI animal research leadership at time of grant application. After receiving the award, we negotiated contract with PARF and submitted animal protocol to their IACUC in July 2022. Unfortunately, PARF lost its AAALAC accreditation, and SERI does not allow research collaboration with non-accredited facility, we had to seek alternative animal facility. We subsequently negotiated with the DaVinci Biomedical Research Facility and the Tufts Medical Center Animal Research Facility in good faith, under the guidance of SERI Director Dr. Patricia D'Amore and SERI Animal Facility Director Marie Ortega. These negotiations took months but eventually failed. The DaVinci facility's budget was much higher than what the current award could afford, and the Tufts facility did not have the necessary ophthalmic equipment to carry out these experiments. We then made the decision to complete the proposed experiments in house at SERI. These challenges were communicated with the DOD VRP, and we were approved to carry out these experiments at SERI. We submitted rabbit animal protocol to SERI IACUC and received its approval and subsequent ACURO approval in 12/2022.

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.

There is an approximate 6-month delay to the project. The aforementioned animal facility issue delayed the initiation of the project by approximately 4 months. Working with our collaborator Coruna Medical LLC, we began manufacturing SSOE in January 2023. However, we did not receive the emulsion (and vehicle control) until April 2023 due to equipment issue at the manufacturer. In addition to these challenges, the SERI rabbit capacity remains limited due to both space restrictions and personnel shortage. Our animal facility experienced flooding in the spring of 2023 and several animal technicians left our facility this year. Despite these challenges, we were able to successfully carry out initial rabbit experiments. We anticipate the SERI rabbit space issue to be ongoing, but will work with our Animal Facility Director Marie Ortega closely to ensure the project proceeds at normal pace. We may have to ask for no-cost extension of the current project, but do not expect any budgetary changes.

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.

Nothing to Report

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

Nothing to Report

Significant changes in use or care of vertebrate animals

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

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).*

Nothing to Report

  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).*

Nothing to Report

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.*

Nothing to Report

- **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.

Nothing to Report

- **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".

Name: *Jia Yin*
Project Role: *PI*
Researcher Identifier (e.g. ORCID ID): 0000-0003-1340-6758
Nearest person month worked: *0.6*
Contribution to Project: *Dr. Yin supervised the project*

Name: *Zhirong Lin*
Project Role: *Postdoctoral Fellow*
Research Identifier: *n/a*
Nearest person month worked: *4.8*
Contribution to project: *Dr. Lin performed experiments*

Name: *Kate Pate*
Project Role: *Consultant*
Research Identifier: *n/a*
Nearest person month worked: *0*
Contribution to project: *Dr. Pate is the CEO of Coruna Medical, LLC, our collaborator and manufacturer of the emulsion*

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.*

Nothing to Report

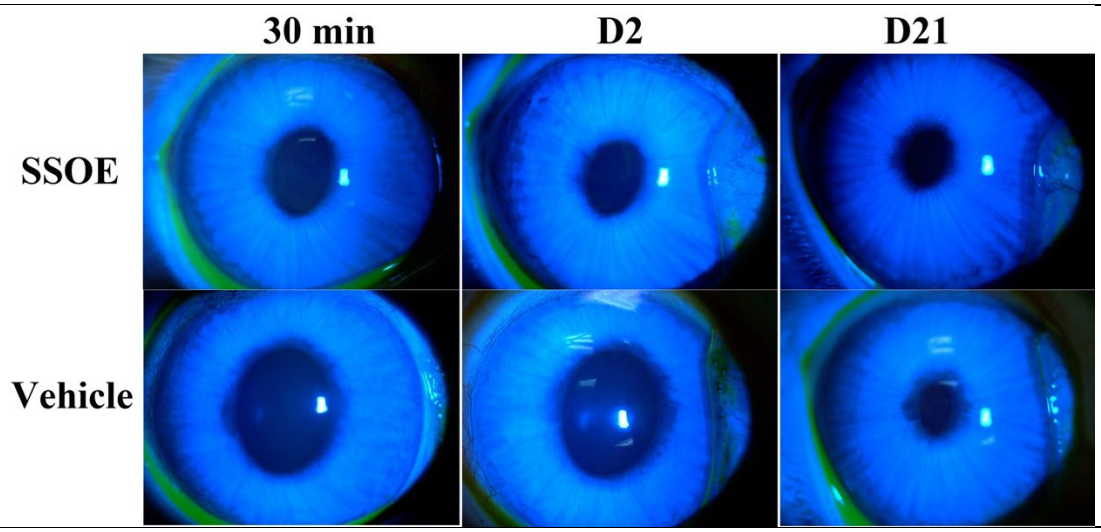
8. SPECIAL REPORTING REQUIREMENTS

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://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

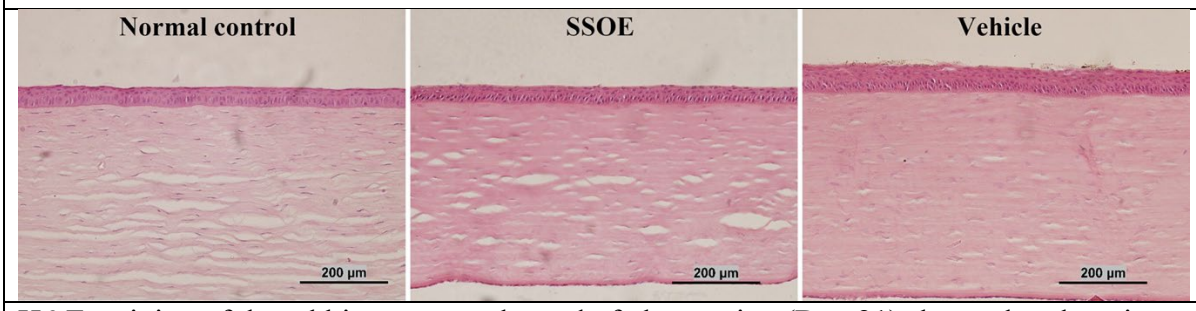
QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) should be updated and submitted with attachments.*

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.*

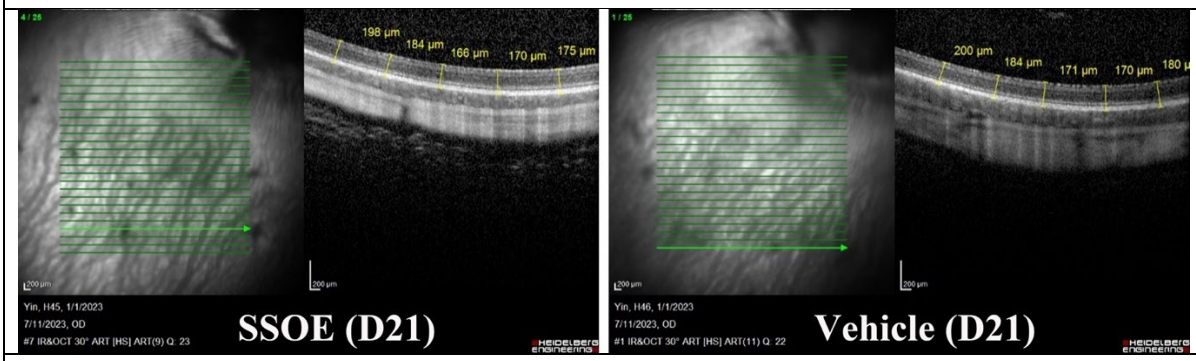
Safety of SSOE and vehicle in a single application to rabbit eyes.



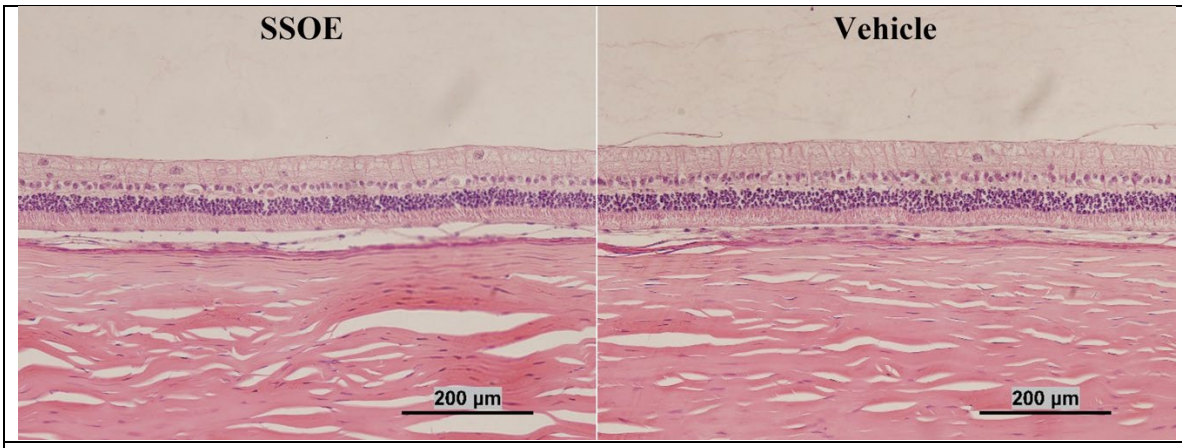
Corneal fluorescein staining demonstrates that SSOE (or vehicle) did not issue corneal epithelial defect or staining.



H&E staining of the rabbit cornea at the end of observation (Day 21) shows that there is no changes in corneal micro-anatomy.

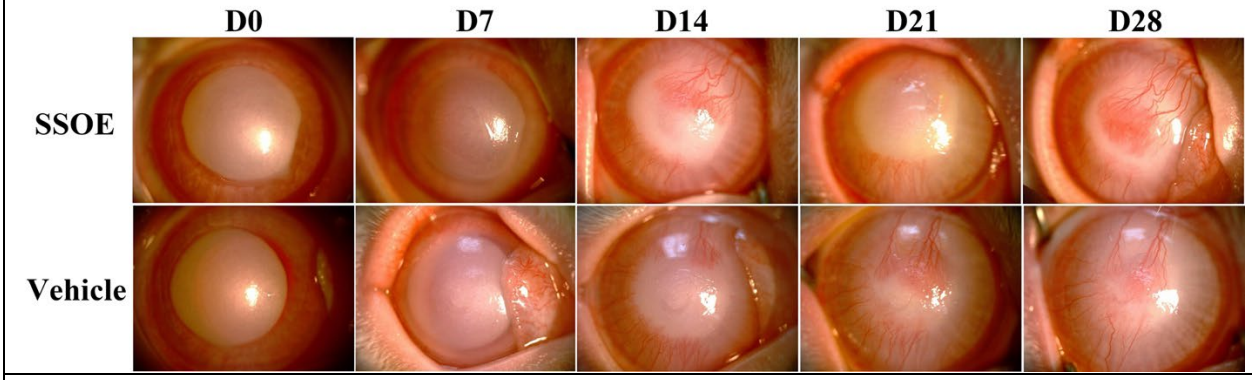


OCT images of the retina shows that there is no retinal structure changes after SSOE or vehicle application after 21-day observation.

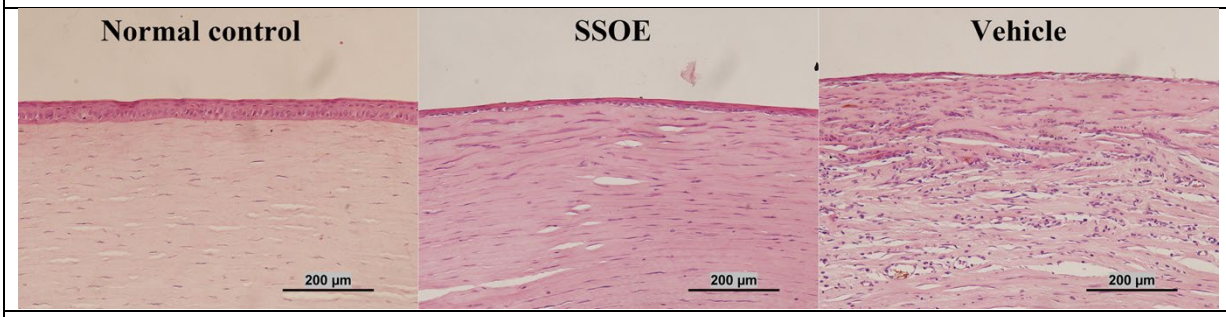


H&E staining of the rabbit retina at the end of observation (Day 21) shows that there is no changes in retinal structure.

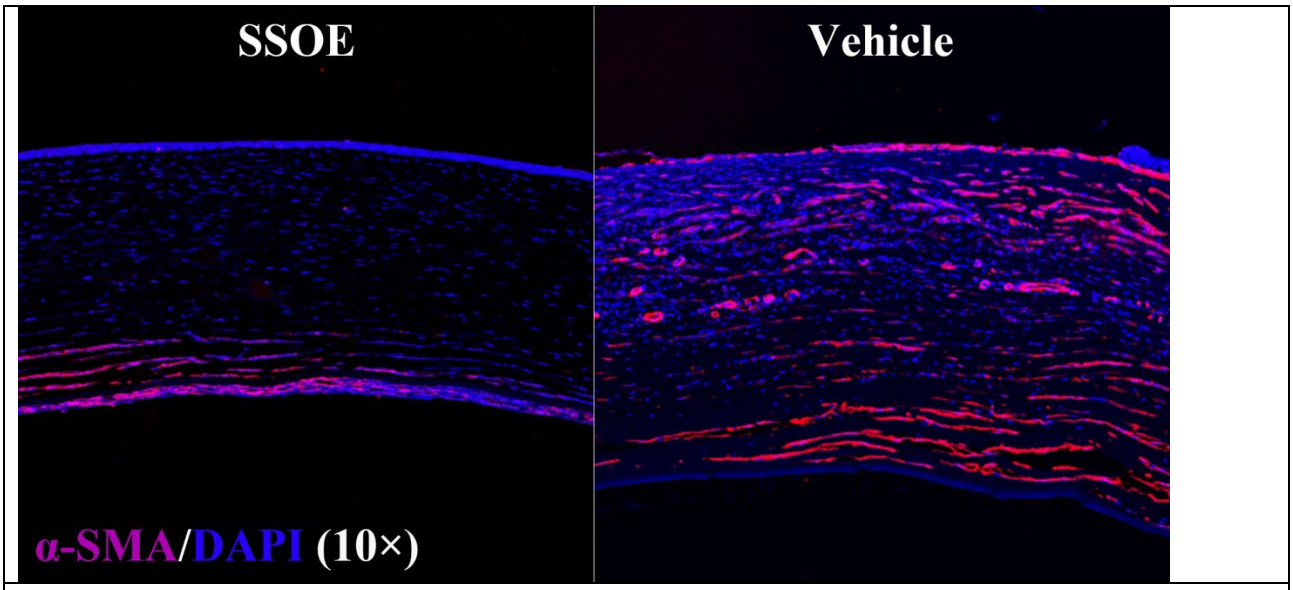
Efficacy of SSEO in reducing corneal neovascularization (NV), inflammation, and fibrosis (alpha-smooth muscle actin, SMA staining) after alkali burn.



Slit lamp photography demonstrates corneal opacity after alkali burn. SSOE, compared to the vehicle control, reduced corneal NV.



H&E staining of the rabbit cornea shows that SSOE application reduced inflammatory cell infiltration into the corneal stroma.



Alpha-SMA staining demonstrates that SSOE reduced corneal stromal fibrosis after alkali burn.