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TITLE: Supersaturated Oxygen Emulsion as a Novel Topical Treatment for Ocular Chemical Injury

PRINCIPAL INVESTIGATOR: Jia Yin, MD, PhD, MPH

CONTRACTING ORGANIZATION: Schepens Eye Research, Boston, MA

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14. ABSTRACT The purpose of the project is to develop a novel supersaturated oxygen emulsion (SSOE) for ophthalmic use to treat acute chemical injury to the eye. The major goals are: 1) Manufacture, optimize, and characterize SSOE for ocular application; 2) Determine the safety and efficacy of immediate SSOE application in mitigating acute ocular burn in vivo; and 3) Determine the efficacy of delayed SSOE application in preventing tissue damage and promoting tissue repair after ocular burn in vivo. In the first year of the project, we have accomplished the following tasks: 1) SSOE containing three concentrations of the active ingredient perfluorodecalin (PFD, 15, 25, and 35%) were successfully manufactured for ophthalmic use; 2) All three formulations of SSOE release oxygen immediately and potently with 35% SSOE having the longest release duration; 3) SSOE was found to be safe to culture human corneal cells (epithelial, stromal, and endothelial cells) in vitro; and 4) SSOE, either applied one-time only or daily for 2 weeks, was found to be safe to mouse eyes in vivo.						
15. SUBJECT TERMS Ocular chemical injury, supersaturated oxygen emulsion (SSOE), oxygenated emulsion						
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1. INTRODUCTION:

The purpose of the project is to develop a novel supersaturated oxygen emulsion (SSOE) for ophthalmic use to treat acute chemical injury to the eye. The scope of the research is: 1) Manufacture, optimize, and characterize SSOE for ocular application; 2) Determine the safety and efficacy of immediate SSOE application in mitigating acute ocular burn *in vivo*; and 3) Determine the efficacy of delayed SSOE application in preventing tissue damage and promoting tissue repair after ocular burn *in vivo*.

2. KEYWORDS:

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

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Major Tasks	Timeline (months)	Projected completion date	Actual completion date
Major Task 1: Engineering and characterization of SSOE for tunable oxygen delivery	1-6	3/14/2021	6/14/2021
Major Task 2: Determine the safety, biocompatibility and toxicity of SSOE <i>in vitro</i> and <i>in vivo</i>	7-9	6/14/2021	9/14/2021
Major Task 3. Determine the efficacy of SSOE in reducing ocular tissue damage after acute burn	10-18	3/14/2022	6/14/2022
Major Task 4. Determine the mechanisms by which SSOE preserves tissue integrity after acute burn	19-24	9/14/2022	Ongoing
Major Task 5. Determine therapeutic window of effective SSOE treatment	25-30	ongoing	ongoing
Major Task 6. Determine whether delayed application of SSOE at high dosage and high frequency can promote tissue regeneration.	31-36	ongoing	ongoing

What was accomplished under these goals?

1) Major activities:

In the previous reporting year (Year 1), we achieved the following: 1) Local IACUC and ACURO approvals for animal work, 2) contract and IP agreement between our institute the Schepens Eye Research Institute of Mass Eye and Ear and our collaborator Coruna Medical LLC, 3) manufacture of the supersaturated oxygen emulsion (SSOE) (Major Task 1), and 4) demonstration of great biocompatibility and minimal ocular toxicity of SSOE (Major Task 2).

SSOE manufacture was scheduled to start 1/20/2021 but was delayed due to COVID-19 pandemic-related supply shortage. The emulsion was eventually made on 3/31/2021 and delivered to SERI for testing in early April in 2021. Therefore, there is a 3-month delay to the overall project.

In the current reporting year (Year 2), we tested the efficacy of SSOE in reducing ocular tissue damage after acute burn (Major Task 3) and began determining the mechanisms by which SSOE preserves tissue integrity after acute burn (Major Task 4).

2) Specific objectives:

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

- Corneal opacification, neovascularization (NV), 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:

- Acute alkali burn (1 mol/L sodium hydroxide) resulted in corneal epithelial defect and persistent fluorescein staining, SSOE treatment, compared with PBS and vehicle control, led to faster epithelial wound closure at 24 hours and less epitheliopathy at 48 hours.
- Within 2 days after burn, mice started developing corneal opacification, NV, ocular inflammation, and cataract formation that intensified over the course of 1 month. SSOE treatment, but not the un-oxygenated vehicle control, led to significant reduction in optical opacity, inflammation, and cataract formation.
- The anterior chamber oxygen concentration was reduced by 60% immediately after burn. SSOE treatment led to a significant increase in the intraocular oxygen concentration. Using pimonidazole hydrochloride, a hypoxia marker, we observed reduced cellular hypoxia with SSOE treatment.
- SSOE led to preservation of normal ocular microanatomy (Using AS-OCT) of the eye, particularly a reduction in anterior chamber exudation and maintenance of anterior chamber depth, after burn.
- SSOE had limited effects on corneal edema after burn.
- Histology showed massive leukocyte infiltration, tissue fibrosis, and distortion of the cornea, as well as significant exudation within the anterior chamber and adhesion of the iris/lens to the cornea after burn. In SSOE-treated eyes, alkali-induced infiltration of inflammatory cells into the cornea was reduced, the corneal stroma was less disorganized, and the anterior chamber was maintained.
- Corneal fibrosis, assessed with the immunostaining of α -smooth muscle actin, was reduced by SSOE treatment.

4) Other achievements:

None.

What opportunities for training and professional development has the project provided?

Nothing to Report

How were the results disseminated to communities of interest?

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?

We have completed Major Task 3 and most of Major Task 4 in the current reporting period. We plan to complete Major Task 4 and proceed to start Major Tasks 5&6 in the next reporting period. In these tasks, we will seek to determine the therapeutic window of delayed SSOE treatment.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

We demonstrated that a single topical application of SSOE to the eye after alkali injury significantly promotes the recovery of normal tissue integrity during the wound healing process and reduces the negative impact of chemical eye injury. SSOE is a novel topical therapeutic in the acute treatment of ocular chemical injuries.

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS:

Due to the COVID-19 pandemic, there was a 3-month delay in the manufacture of SSOE. Since we received the emulsion in April 2021, we have been able to keep our proposed schedule and proceed with the Major Tasks outlined in our SOW with an approximate 3-month delay.

Changes in approach and reasons for change

Actual or anticipated problems or delays and actions or plans to resolve them

Except for the aforementioned manufacture delay, we have been keeping our schedule and do not foresee other major delays.

Changes that had a significant impact on expenditures

Nothing to report

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

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:

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

 - **Journal publications.**

 - Nothing to report

 - **Books or other non-periodical, one-time publications.**

 - Nothing to report

 - **Other publications, conference papers and presentations.**

 - Nothing to report

- **Website(s) or other Internet site(s)**

- Nothing to report

- **Technologies or techniques**

- Nothing to report

- **Inventions, patent applications, and/or licenses**

 - Patient application: OXYGENATED EMULSION FOR TREATMENT OF OCULAR INJURY. This application claims the benefit of United States (US) Patent Application No. 62/989,354, filed Mar. 13, 2020. This application claims the benefit of U.S. Patent Application No. 63/133,865, filed Jan. 5, 2021. This application is a continuation in part of U.S. patent application Ser. No. 17/172,403, filed Feb. 10, 2021.

- **Other Products**

- Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

<i>Name:</i>	<i>Jia Yin</i>
<i>Project Role:</i>	<i>PI</i>
<i>Researcher Identifier (e.g. ORCID ID):</i>	<i>0000-0003-1340-6758</i>
<i>Nearest person month worked:</i>	<i>1</i>
<i>Contribution to Project:</i>	<i>Dr. Yin designed and supervised SERI studies</i>
<i>Name:</i>	<i>Asmaa Zidan</i>
<i>Project Role:</i>	<i>Postdoctoral Fellow</i>
<i>Research Identifier:</i>	<i>n/a</i>
<i>Nearest person month worked:</i>	<i>12</i>
<i>Contribution to project:</i>	<i>Dr. Zidan performed experiments</i>
<i>Name:</i>	<i>Kate Pate</i>
<i>Project Role:</i>	<i>PI Coruna Medical LLC</i>
<i>Research Identifier:</i>	<i>n/a</i>
<i>Nearest person month worked:</i>	<i>0</i>
<i>Contribution to project:</i>	<i>Dr. Pate is the sub-PI of the Coruna Subcontract and responsible for SSOE manufacture</i>
<i>Name:</i>	<i>Sharon Lake</i>
<i>Project Role:</i>	<i>Engineer Coruna Medical LLC</i>
<i>Research Identifier:</i>	<i>n/a</i>
<i>Nearest person month worked:</i>	<i>0</i>
<i>Contribution to project:</i>	<i>Ms. Lake is an engineering consultant at Coruna for SSOE manufacture</i>

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

What other organizations were involved as partners?

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES: