**AWARD NUMBER: W81XWH-20-1-0869**

**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 Institute of Mass Eye and Ear**
**20 Staniford St, Boston, MA, 02114**

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

**DISTRIBUTION STATEMENT:** Approved for Public Release; Distribution Unlimited

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**4. TITLE AND SUBTITLE**

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

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**5b. GRANT NUMBER**
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**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**
Jia Yin, MD, PhD, MPH

E-Mail: Jia_Yin@meei.harvard.edu

**5d. PROJECT NUMBER**
VR190039

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Schepens Eye Research Institute of Mass Eye and Ear

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

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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 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

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
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| Unclassified | Unclassified | Unclassified | Unclassified | 15 | 19b. TELEPHONE NUMBER *(include area code)* |

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**Page**

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

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:** *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: 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 *in vitro* and *in vivo* | 7-9 | 6/14/2021 | 9/14/2021 |
| Major Task 3. Determine the efficacy of SSOE in reducing ocular tissue damage after acute burn | 10-17 | ongoing | ongoing |
| Major Task 4. Determine the mechanisms by which SSOE preserves tissue integrity after acute burn | 19-24 | ongoing | 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?**
*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:**

We worked on and submitted our animal protocol related to this project to our local IACUC, then submitted it to the Animal Care and Use Review Office (ACURO). We also worked on and submitted Cadaver tissue protocol to the Office of Research Protections. PI's institution (SERI, Schepens Eye Research Institute) worked with sub-contract Coruna Medical LLC (sub-PI Dr. Pate) on contract and IP agreement negotiation.

We began the production of the supersaturated oxygen emulsion (SSOE). The manufacture was scheduled to start 1/20/2021 but was delayed due to COVID-19-related supply shortage. The emulsion was finally made on 3/31/2021 and delivered to SERI for testing in early April. We then tested the oxygen releasing capacity, safety, biocompatibility, and toxicity of SSOE in vitro and in vivo.

**2) Specific objectives:**

- Obtain approvals of animal and human cadaver tissue protocols and sign sub-contract and IP agreements
- Design and optimize SSOE with a range of PFD concentrations for tunable delivery of oxygen
- Determine the oxygen release amplitude, duration, and storage stability of different SSOE formulations
- Determine the toxicity of SSOE on cultivated human corneal cells using a live/dead cell viability assay
- Determine the biocompatibility of SSOE using a modified Draize test in live mice

**3) Significant results or key outcomes:**

We obtained ACURO approval of our animal protocol on 9/22/2020 and Cadaver Activity Approval from the Office of Research Protections on 11/08/2020. SERI and Coruna signed subcontract and IP agreements in October 2020.

Three concentrations of SSOE, containing 15, 25, and 35% of the active ingredient perfluorodecalin (PFD), were manufactured and tested for oxygen release:

- 35% PFD SSOE released oxygen above 600 umol/L at 1 min and reached peak level of 1000 at 2 hours. It maintained levels above 600 for more than 24 hours and levels above atmospheric levels for more than 1 week.
- 25% PFD SSOE released oxygen above 600 umol/L at 1 min and reached peak level of >700. It maintained levels above 600 for more than 14 hours and levels above atmospheric levels for more than 1 week.
- 15% PFD SSOE released oxygen above 600 umol/L at 2 min and reached peak level of >700 at 1 hr. It maintained levels above 600 for 1 hr and levels above atmospheric levels for 16 hours.

We tested the toxicity of SSOE containing 25% PFD in cultured corneal cells. We applied PBS control, unoxygenated emulsion (vehicle) control, or SSOE to cultured human corneal epithelial cells (hCECs), stromal fibroblast cells (hCFCs), and endothelial cells (hCEnCs) for 1 hour and determined cell death using Live/Dead Assay. In all 3 cell types, cell death rates in SSOE groups were comparable or lower than those of PBS and vehicle controls. Similarly, SSOE 35% showed no significant toxicity in cultured human corneal cells in vitro. The potential toxicity of SSOE originates from the active ingredient perfluorodecalin (PFD). Given that SSOE containing higher concentrations of PFD (25 and 35%) showed no toxicity, SSOE 15% was not tested.

We then performed a modified Draize test, a standard test to evaluate ocular toxicity of medications in mice. 25% and 35% SSOE or vehicle control were applied topically on mouse eyes in two regimens: 1) a single-application of 1 hour; and 2) a daily application of 10-minute for 2 weeks. SSOE showed no eyelid irritation or redness, no conjunctival injection or discharge, no corneal opacification or edema, no signs of intraocular inflammation, no anterior chamber exudation, or loss of lens transparency. Corneal thickness, corneal fluorescein staining score, intraocular pressure, and retinal thickness remained comparable between control vehicle-treated and SSOE-treated mice.

**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 have completed Major Tasks 1&2 in this reporting period and will proceed to Major Task 3 - Determine the efficacy of SSOE in reducing ocular tissue damage after acute burn.

*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 have successfully manufactured supersaturated oxygen emulsion (SSOE) for ophthalmic use. We demonstrated its immediate, robust, and long-lasting oxygen releasing capacity. We also demonstrated that SSOE is safe to cultured corneal cells and the mouse eyes when applied topically. Collectively, these results prove that SSOE is safe to the eye and effective in releasing oxygen, paving ways for our subsequent tasks to test its efficacy in treating acute ocular chemical injury.

**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:*

| |
|---|
| 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. |

**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.*

| |
|---|
| Except for the aforementioned manufacture delay, we have been keeping our schedule in terms of completing Major Tasks 1&2. We do not foresee other major delays and will proceed to Major Task 3 as outlined. |

**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).*

| |
|---|
| Kunpeng Pang, Sanming Li, shuyan zhu, Kathryn Pate, Jia Yin; Perfluorodecalin-based oxygenated emulsion mitigates ocular hypoxia and inflammation after alkali burn. *Invest. Ophthalmol. Vis. Sci.* 2021;62(8):536. (Published), acknowledgement of federal support (yes). |

**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.*

| |
|---|
| Perfluorodecalin-based oxygenated emulsion mitigates ocular hypoxia and inflammation after alkali burn. *ARVO 2021 Annual meeting (Virtual)** |

- **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".*

*<u>Example:</u>*

| | |
|---|---|
| *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:* *Jia Yin*
*Project Role:* *PI*
*Researcher Identifier (e.g. ORCID ID):* 0000-0003-1340-6758
*Nearest person month worked:* *1*
*Contribution to Project:* *Dr. Yin designed and supervised SERI studies*

*Name:* *Kunpeng Pang*
*Project Role:* *Postdoctoral Fellow*
*Research Identifier:* *n/a*
*Nearest person month worked:* *9*
*Contribution to project:* *Dr. Pang performed experiments*

*Name:* *Kate Pate*
*Project Role:* *PI Coruna Medical LLC*
*Research Identifier:* *n/a*
*Nearest person month worked:* *0*
*Contribution to project:* *Dr. Pate is the sub-PI of the Coruna Subcontract and responsible for SSOE manufacture*

*Name:* *Sharon Lake*
*Project Role:* *Engineer Coruna Medical LLC*
*Research Identifier:* *n/a*
*Nearest person month worked:* *0*
*Contribution to project:* *Ms. Lake is an engineering consultant at Coruna for SSOE manufacture*

**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.*