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TITLE: Mobile, Multi-modal, Label-Free Imaging Probe Analysis of Choroidal Oximetry and Retinal Hypoxia

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14. ABSTRACT

Coherent anti-stokes Raman spectroscopy (CARS) can be used to detect differences in the oxygen content in aqueous hemoglobin solutions. Our current setup is not ideal for accurately calibrating these measurements, but changes in the microscope setup and in the addition of gaseous oxygen to the physical sample are promising next steps.

15. SUBJECT TERMS
Primary blast injury, PBI, hypoxia, ischemia, oxygen, eye, retina, photoreceptor, neuron, TRPM7, neurodegeneration, neurotoxicity, coherent anti-Stokes Raman spectroscopy, CARS, mouse

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Section 1: Introduction

The study is based on the premise that Coherent Anti-Stokes Raman scattering (CARS) imaging provides a cellular resolution, label-free method to evaluate the effect of blast injury on the retina and could provide a diagnostic tool for clinical use. We have proposed to develop and test a novel imaging approach, based on successful CARS imaging techniques currently in place in our lab. After some minor delays at the onset of the project, the work is currently on track for completion in 2017. We have developed the blast injury model proposed in the original research plan and used a transgenic mouse model with fluorescently-labeled neurons to examine neuronal-specific effects of our blast injury under fluorescence microscopy (not label-free). Work has begun to re-calibrate our label-free CARS imaging system to the needs of this project and we anticipate applying it to blast-injured animals in the next two months. The specific aims have not been significantly changed from the original approved statement of work.

Section 2: Keywords

CARS, imaging, microscopy, blast-injury, retina, neuron

Section 3: Accomplishments

The major research objectives during the year 2 are listed below:

1) Create PBI animal model
2) Calibrate label-free probe for O_2 measurement
3) Detect and map hypoxic regions in injured eyes
4) Measure TRPM7 and cellular/apoptosis biomarkers in retinas
5) Measure neuronal death and cell-specific biomarker in retinas
6) Whole-cell electrophysiology for TRPM7 function
7) Modulate TRPM7 activity in *ex vivo* retinas

During the past one year, we have been working on objectives 1-5. Although we dealt with many technical issues on the CARS and animal parts, we still strived to implement the proposed objectives. Our major activities and achievements are listed below.

1) Create PBI animal model

In Quarter 1, Dr. Gilliam designed a pressure device and a holding platform for creating PBI-induced eye injury mouse model; optimized the procedures suitable for our IACUC animal protocol with the HMRI veterinary staff; revised the IACUC protocol and received approval from the HMRI IACUC committee; and submitted documents for ACURO. In Quarter 2, Dr. Li joined the research team. Following Dr. Gilliam's design and the protocol in literature, Dr. Li added the pressure sensor, transductor, and amplifier to calibrate the blast pressure for this blast ocular injury system. We introduced a Thy1-YFP transgenic mouse model into this study in order to easily examine the retinal neuronal injury in objective 3/4/5. IACUC protocol was amended, and documents were sent for ACURO approval. In Quarter 3, we set up the blast part of our PBI device, but the transductor and amplifier for calibrating have been delayed. With approval of ACURO, we ordered and expanded Thy1-YFP transgenic mice. Our use of this animal was delayed by a 3.5 months quarantine period due to an unexpected murine norovirus contamination in June. In Quarter 4, after the quarantine was lifted, we set up our PBI device (Figure 1) and optimized the blast-relevant parameter for producing ocular injury model (Figure 2). We generated YFP Tg mice and wild type mice for the animal work; obtained ophthalmic drugs for animal work and tested the selected parameters according to our IACUC protocol. In addition, we checked the injury on eyeball following PBI-treatment via stereomicroscopy with Ful-Glo fluorescence dye.

2) Calibrate label-free probe for O_2 measurement.

In Quarter 1, Dr. Gilliam was working on setting up multi-modal label-free imaging system and calibrating our system to measure blood oxygen levels in the eye. The reported problems with our coherent anti-Stokes Raman spectroscopy (CARS) laser system alignment were repaired to working order. Human hemoglobin protein solution was used to optimize the system by altering the oxy:deoxy hemoglobin ratio in situ, but some technical issues remain to be overcome. In Quarter 2: Our CARS group realigned the picosecond optical parametric oscillator (OPO) in order to enhance the parametric amplification and the output power. We worked on the instability issue of the Nd:YVO4 laser in order to generate sufficient power to pump the OPO. The manufacturer of the laser was requested to inspect this issue and repairs are still in progress. In Quarter 3: We continued working on the instability issue of our Nd:YVO4 laser, we contacted the manufacturer and sent our Bio-optics researchers to our collaborators at Purdue University to optimize this bio-optic experimental system.

Human hemoglobin arrived. In Quarter 4: The laser is now fixed by working with the vendor. OPO are now under tuning and getting closed to a functional status, which convert an input laser wave with frequency ωp into two output waves of lower frequency (ωsωi) by means of second-order nonlinear optical interaction. We are now ready to test human hemoglobin with our CARS system.

3) Detect and map hypoxic regions in injured eyes

In Quarter 1-2: We optimized our protocol to isolate the whole retina for protein analysis and pathological assays, introduced three different triple whole-mount staining to examine the morphology of neurite, cell body, and blood vessel network to determine the vulnerable retinal regions for future biochemical and pathological analysis. In Quarter 3-4: The next steps in this work await the end of the animal room quarantine period.

4) Measure TRPM7 and cellular/apoptosis biomarkers in retinas

In Quarter 1-2: We tested TRPM7 and other retinal layer-marker in the isolated retina from dissected mouse eyeball with western blot assay. We determined the distribution of TRPM7 in YFP-labeled retinal cells by whole mount staining with the retina isolated from discarded mouse eyeball (YFP Tg). TRPM7 can be seen in some YFP-labeled neuronal cells. In Quarter 3-4: We selected, purchased, and received an apoptosis detection kit; further experiments await the end of the quarantine period.

5) Measure neuronal death and cell-specific biomarker in retinas

In Quarter 1-2: In isolated retina from dissected mouse eyeballs (YFP Tg, Figure 3a-e), we examined the morphology of the retinal cells in YFP Tg mouse by imaging the genetically-encoded fluorescence protein label. In the retina from each eyeball, the soma, dendrites, and axon can be visualized in 40-50 retinal cells. In Quarter 3-4: Our amendment to use YFP mice in our IACUC protocol has been approved by ACURO.

6) Whole-cell electrophysiology for TRPM7 function – Not Started

7) Modulate TRPM7 activity in *ex vivo* retinas – Not Started

Dissemination of Results

Nothing to report.

Goals in the Next Reporting Period

In the next year, we intend to apply our novel CARS imaging approach to the mouse blast injury model implemented in the previous year. By the end of this year we plan to:

(1) survey the neuronal-specific retinal damage that occurs in response to blast injury by label-free CARS and

(2) develop and validate criteria to assess severity of retinal damage based on label-free imaging.

Section 4: Impact

Impact on Development of the Principal Discipline of the Project

Nothing to report.

Impact on Other Disciplines

Nothing to report.

Impact on Technology Transfer

Nothing to report.

Impact on Society Beyond Science and Technology

Nothing to report.

Section 5: Changes/Problems

In Quarter 2: Dr. Gilliam was no longer working at HMRI; Dr. Li was added. In Quarter 2-4: We have worked with the manufacturer on some technical issue of our laser, and sent our Bio-optics researchers to Purdue University to learn how to optimize our bio-optic experimental system. In Quarter 3-4: our animal room was contaminated by murine norovirus (MNV) and our mice were under quarantine for 3.5 months. We worked with CMP staff for the procedures and expanded our Tg mice during this period.

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

Our most significant problems over the first two years of the funding period have been with lack of power in our imaging setup. A great deal of time and effort has been spent over the last two years, in conjunction with the vendor, optimizing the system for use in this work. We do not anticipate further delays. Use of the fluorescent-labeled animal model also adds some redundancy into the work which ensures that the biological questions will be answered even if technical problems remain.

Significant changes in use or care of vertebrate animals

We have added a new transgenic animal line that allows us to use fluorescence microscopy to survey neuronal subtype-specific damage in conjunction with our label-free approach. ACURO approval has been obtained.

Section 6: Products

Nothing to report.

Section 7: Participants and Other Collaborating Organizations

Name: Stephen T.C. Wong, Ph.D., P.E
Project Role: Principle Investigator
Contribution to Project: Overall research direction, systems design, scientific management, and project execution.

Name: Jared C. Gilliam, Ph.D. **(Q1)**
Project Role: Research Associate
Contribution to Project: Developing tools and procedures for animal handling and testing using label-free optical imaging.

Name: Xuping Li, Ph.D. **(Q2-4)**
Project Role: Postdoctoral Fellow
Contribution to Project: Developing tools and procedures for animal handling and testing using label-free optical imaging.

Additional Preliminary data

Figure 1. PBI-device build up and optimization. (**1A-F**)The component of our PBI-devices, output pressure detection sensor, amplifier, input pressure panel. (**1G)** Correlation between input-output pressures. (**1H)** Measurement of the pressure change at different distance from the barrel.

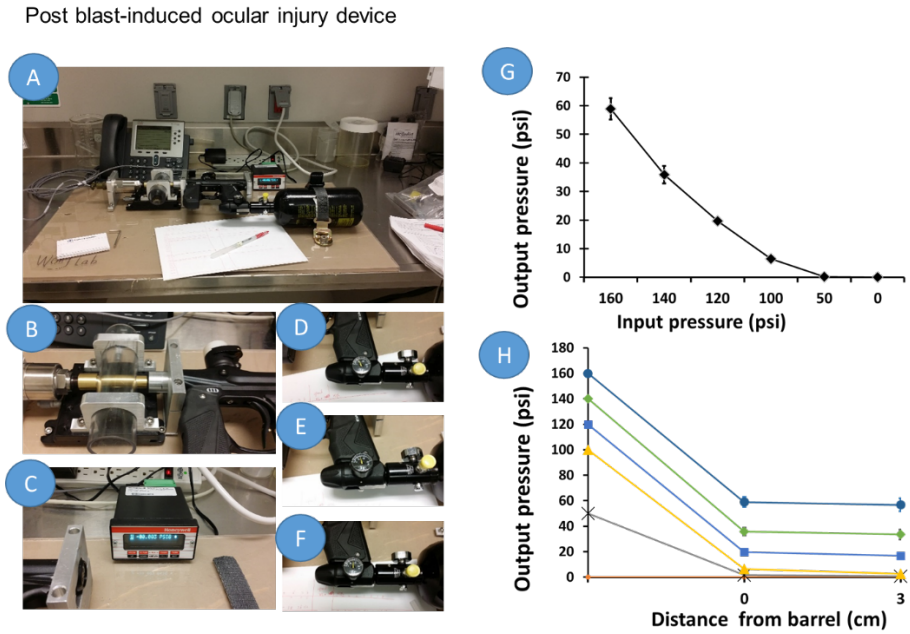

Figure 2. Optimization of output pressure by changing the setting of blast generator. (2A-B) Correlation between out-put pressure and blast time duration. **(2C)** After PBI-treatment, the eyes of dead animal were labeled by Ful-Glo fluorescence, observed under the close-global injury and morphology of eye ball with a stereomicroscope with blue light. **(2D-G)** In the control and all three blasted groups, the overall globe and the cornea are intact while the dye appear smooth across the entire surface of the eye with treatment. Blast pressure at these levels did not cause open-globe injury.