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TITLE: Noninvasive Optical Monitoring of Spinal Cord Hemodynamics and Oxygenation after Acute Spinal Cord Injury

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Our objectives in Year 2 are fully addressed. Following a series of technical refinements and pilot studies. OXT5 (V1) NIRS sensor prototype and our Multi-Wavelength NIRS system were developed. This setup was required for monitoring spinal cord oxygenation, hemodynamics and cytochrome c oxidase (CCO) activity in our animal model of acute SCI (Experiment 1). We successfully completed Experiment 1 as scheduled in year 2. Nine animals were studied and comparative statistical analysis was completed. Outcomes of Experiment 1 are prepared for publication in the Journal of Neurotrauma. We will also present our results at 2018 Neuroscience conference in San Diego and at 2019 SPIE Photonics West conference in San Francisco. Following completion of Experiment 1, we proceed by conducting the 3rd round of NIRS technology development to design and prototype miniaturized V2-NIRS sensor and modified NIRS system enabled for continuous long-term data collection. V2-NIRS sensor is being tested in a series of pilot animal studies. In year three we conduct Experiment 2 and will focus on refining the technology to engineer a clinical spinal cord NIRS sensor.

15. SUBJECT TERMS

Spinal Cord Injury, Hemodynamic Support, Spinal Cord hemodynamics, Spinal Cord Blood Flow, Near Infrared Spectroscopy, Intraparenchymal Pressure

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1 INTRODUCTION

The hemodynamic management of acute spinal cord injury (SCI) represents an under-appreciated opportunity to improve neurologic recovery in human patients. A major limitation in our ability to optimize hemodynamic management in acute SCI is the lack of a real-time method for measuring blood flow, oxygenation, metabolic responses, and hydrostatic pressure within the injured spinal cord. Near-infrared spectroscopy (NIRS) offers the potential to provide a relatively non-invasive measure of these important parameters within the injured spinal cord. NIRS works by transmitting near infrared light through tissue, and based on the absorption of this light by chromophores such as oxygenated and deoxygenated hemoglobin (O_2Hb and HHb), microcirculatory oxygen and perfusion can be derived. Additionally, alterations in the O_2Hb waveform that are caused by tissue pressure can be potentially utilized to monitor changes in hydrostatic pressure within the cord. Finally, NIRS measures of the redox state of cytochrome-c-oxidase (CCO) can provide information not just about tissue O_2 but also about downstream cellular O_2 metabolism. The overall objective of this initiative is to develop an implantable NIRS sensor and system that can be used to provide non-invasive real-time measurements of spinal cord oxygenation, blood flow, pressure, and oxidative metabolism in acute human SCI. We will test the hypothesis that a NIRS sensor positioned extra-durally can provide real-time measurements of tissue oxygenation, perfusion, oxidative metabolism and hydrostatic pressure within the underlying spinal cord adjacent to the site of traumatic injury over the course of seven-day post-injury. To test this hypothesis, we will conduct a series of preclinical studies using our pig model of thoracic SCI, alongside efforts to refine the NIRS technology into a clinically applicable device. Our animal studies aim to establish the relationship between non-invasive NIRS measurements of oxygenation, perfusion, metabolism and pressure with invasive IP monitoring that is made possible by the large calibre of the pig spinal cord. First, we will conduct a non-survival study in 8 animals to verify the relationship between NIRS and IP spinal cord monitoring after various intra-operative stimuli, including systemic hypoxia, contusive SCI, sustained spinal cord compression, and alterations in blood pressure. After refining the technology to engineer a sensor that can potentially be used in humans, we will test the NIRS system in another pig study with a seven-day post-injury survival period to determine how well the NIRS system monitors tissue changes in comparison to Intraparenchymal (IP) monitoring in awake, mobile animals (a more clinically relevant scenario).

2 KEYWORDS

- Spinal Cord Injury
- Hemodynamic Support
- Spinal Cord hemodynamics
- Spinal Cord Oxygenation
- Spinal Cord Blood Flow
- Near Infrared Spectroscopy
- Intraparenchymal Pressure

3 ACCOMPLISHEMENTS

3.1 Protocol and Activity Status

- **Human Use Regulatory Protocols**
  No human subject research will be performed to complete the Statement of Work

- **Use of Human Cadavers for RDT&E, Education or Training**
  No RDT&E, education or training activities involving human cadavers will be performed to complete the Statement of Work

- **Animal Use Regulatory Protocols**

  Total Protocols: Two animal use research protocol will be required to complete the Statement of Work

- **Protocol: 1 of 1**
- **Protocol [ACURO Assigned Number]:** Conveyed from SC130007 and SC130008
- **Title:** Optical Monitoring of Spinal Cord Hemodynamics
- **Target required for statistical significance**: n=8 / each of two experiments
- **Target approved for statistical significance**: n=8 / each of two experiments
- **Submitted to and Approved by:** Bryan K. Ketzenberger, DVM, DACLAM
- **Status:** Approved - August 15, 2016

3.2 Approved Statement of Work

The approved statement of work is described below. A current Gantt chart is provided in Table 1 for reference.

Table 1: Approved Statement of Work (Gantt chart).

Specific Aim 1: *Refine our current NIRS sensor (V1) configuration, and software algorithms to optimize the measurement of spinal cord oxygenation (O_2Hb, HHb, TOI%), perfusion*	**YEAR 1**	**YEAR 2**	**YEAR 3**	**Extended YEAR 4**

(THb), metabolism (CCO) and hydrostatic pressure.																
Activity	**Q 1**	**Q 2**	**Q 3**	**Q 4**	**Q 1**	**Q 2**	**Q 3**	**Q 4**	**Q 1**	**Q 2**	**Q 3**	**Q 4**	**Q 1**	**Q 2**	**Q 3**	**Q 4**
1a) UBC & ACURO approvals																
1b) Technology assessment																
1c) IP assessment																
1d) V1 Sensor development																
Specific Aim 2: *NIRS system evaluation and verification of the relationship between the NIRS and IP assessments of oxygenation, perfusion, pressure, and metabolic responses in our pig SCI model in anesthetized (n=8 immobile) animals (Experiment 1)*	**YEAR 1**				**YEAR 2**				**YEAR 3**				**Extended YEAR 4**			
Activity	**Q 1**	**Q 2**	**Q 3**	**Q 4**	**Q 1**	**Q 2**	**Q 3**	**Q 4**	**Q 1**	**Q 2**	**Q 3**	**Q 4**	**Q 1**	**Q 2**	**Q 3**	**Q 4**
2a) NIRS system evaluation and collecting NIRS and IP data in Experiment 1																
2b) Data analysis: NIRS vs. IP measurements (SC oxygenation & perfusion)																

2c) Data analysis: NIRS vs. IP measurements (SC pressure)																
2d) Data analysis: NIRS vs. IP measurements (SC metabolic responses)																
2e) Final data analysis and dissemination																
Specific Aim 3: *Refine and miniaturize the NIRS sensor to develop an implantable (but also removable), NIRS sensor (V2) that can be used to monitor the spinal cord for a clinically relevant time frame post-injury in the "non-ideal" setting of an awake (and moving) individual.*	**YEAR 1**				**YEAR 2**				**YEAR 3**				**Extended YEAR 4**			
Activity	**Q 1**	**Q 2**	**Q 3**	**Q 4**	**Q 1**	**Q 2**	**Q 3**	**Q 4**	**Q 1**	**Q 2**	**Q 3**	**Q 4**	**Q 1**	**Q 2**	**Q 3**	**Q 4**
3a) V2 sensor development																
3b) V2 sensor calibration																
3c) NIRS system refinement for long term (7-day) monitoring																

Specific Aim 4: *Establish the ability of V2 sensor to assess oxygenation, perfusion, pressure, and metabolic responses for 7-day post-injury by comparing the NIRS and IP measurements in pigs that are awake and mobile for 7 days post-injury. (targeted n=8)*	**YEAR 1**				**YEAR 2**				**YEAR 3**				**Extended YEAR 4**			
Activity	**Q 1**	**Q 2**	**Q 3**	**Q 4**	**Q 1**	**Q 2**	**Q 3**	**Q 4**	**Q 1**	**Q 2**	**Q 3**	**Q 4**	**Q 1**	**Q 2**	**Q 3**	**Q 4**
4a) NIRS vs. IP measurements (SC oxygenation & perfusion)																
4b) NIRS vs. IP measurements (SC pressure)																
4c) NIRS vs. IP measurements (SC metabolic responses)																
4d) Data analysis, dissemination																
Specific Aim 5: *Obtain local ethics approval to conduct a clinical trial of our finalized NIRS system in human acute SCI patients.*	**YEAR 1**				**YEAR 2**				**YEAR 3**				**Extended YEAR 4**			

Activity	Q 1	Q 2	Q 3	Q 4	Q 1	Q 2	Q 3	Q 4	Q 1	Q 2	Q 3	Q 4	Q 1	Q 2	Q 3	Q 4
5a) Final refinements of V3 sensor																
5b) Obtaining Canada Health approval for the V3 sensor																
5c) Obtaining UBC CREB approval																

Aim 1. Refine our current NIRS sensor (V1) configuration, and software algorithms to optimize the measurement of spinal cord (SC) oxygenation (O_2Hb, HHb, TOI%), perfusion (THb), metabolism (CCO) and hydrostatic pressure.

Task 1: Submit documents for Institutional (UBC) and ACURO approval. [Months 1-3]
Task 2: NIRS technology assessment. [Months 1-6]
Task 3: Intellectual Property (IP) protection assessment, through UBC University Industrial Liaison office (UILO). [Months 1-6]
Task 4: Develop SC NIRS sensor V1 to be used in experiment 1. [Months 5-9]

Milestone(s) Achieved:

- ***(a)*** *UBC and ACURO approvals are obtained.*
- ***(b)*** *NIRS technology assessment is completed.*
- ***(c)*** *The first round of the Invention Disclosure assessment was completed by the UBC University Industry Liaison Office (UILO); file number: 17-037. Filing a provisional application with the United States Patent and Trademark Office is under process.*
- ***(d)*** *The first SC-NIRS sensor (V1) was developed, modified and examined in a pilot study on five pig models of SCI. The final version of the V1 NIRS sensor was prototyped and being used in Experiment 1.*
- ***(e)*** *After extensive consultations with various NIRS manufacturers, such as Hamamatsu during technology assessment stage (Task 2), we came to the conclusion that currently available NIRS technology and equipment do not have the capacity to reliably and accurately monitor changes of spinal cord cytochrome c oxidase (COO), at least in a manner that would be applicable to eventual clinical application. Almost all researchers conducting CCO measurements are using experimental lab-based systems that are not applicable in clinical settings. To approach this challenge, we worked with a biophotonics consulting company in January 2017 to design and prototype a unique NIRS system that may enable us to detect spinal cord CCO changes. Pathonix Innovation Inc. is a Canadian biophotonics technology consulting company that has been actively in the field of design and customizing NIRS systems applied by the clinical researcher in different universities*

and institutions including US National Institute of Health (NIH). After consultations and reviewing the task with Pathonix engineers, a novel multi-wavelength NIRS prototype for monitoring changes of tissue CCO as well as O_2Hb and HHb was developed. Following a series of sensor and software refinements, the new MW-NIRS sensor (V1) could successfully collect CCO traces in three pilot animal models of acute SCI. The final waterproof and advanced version of V1 prototype, was used to collect all NIRS traces in Experiment 1.

(f) *The protocol of Experiment 1 was tested and refined.*

Aim 2. Verify the relationship between the NIRS and intraparenchymal (IP) assessments of oxygenation, perfusion, pressure, and metabolic responses in our pig SCI model in anesthetized (immobile) animals.

Task 1: Evaluating the NIRS system function in a pig model of SCI in conjunction with IP measurements of oxygen, perfusion, pressure, and metabolism. (Non-survival experiment with NIRS & IP monitoring under anesthesia, n=8)

Task 2: Comparing tissue oxygenation & perfusion as assessed by 1) NIRS measures of O_2Hb, HHb, THb, TOI% and Hbdiff, and 2) an IP oxygen/blood flow sensor. [Months 10-14]

Task 3: Comparing hydrostatic pressure as assessed by 1) a novel analysis of the NIRS O_2Hb waveform that accounts for how cardiac-induced oscillations in the waveform are influenced by tissue pressure, and 2) an IP pressure sensor. [Months 10-14]

Task 4: Comparing metabolic responses as assessed by 1) NIRS measures of cytochrome-c-oxidase (CCO), and 2) an IP microdialysis catheter to monitor lactate, pyruvate, and the lactate/pyruvate ratio. [Months 10-14]

Task 5: Data analysis. [Month 15]

Milestone(s) Achieved:

(a) *Experiment 1 is completed. Spinal cord NIRS data were successfully collected by OXT5 NIRS system, in conjunction with IP measurements of spinal cord tissue oxygenation, perfusion, pressure, and metabolism. NIRS sensor placement and on-going monitoring were completed in all animals and were not associated with any complications.*

(b) *Comparisons of NIRS measures of spinal cord oxygenation and hemodynamics with IP measures of spinal cord oxygenation and blood flow were completed.*

(c) *Comparison of NIRS measure of spinal cord CCO activity with IP measure of spinal cord metabolism, L/P ratio, was completed.*

(d) *Data analysis of Experiment 1 is completed.*

Aim 3. Refine and miniaturize the NIRS sensor, cables, and fixation devices to develop an implantable (but also removable), NIRS sensor (V2) that can be used to monitor the spinal cord for a clinically relevant time frame post-injury in the "non-ideal" setting of an awake (and moving) individual.

Task 1: NIRS monitoring system adjustment/refinement for long term (7-day) continuous monitoring, data storage and management. [Months 16-20]
Task 2: Sensor prototyping and development. [Months 16-19]
Task 3: Sensor calibration and testing. [Months 19-20]

Milestone(s) Achieved:

- ***a)*** *Development of the V2-NIRS sensor is completed and sensor is tested in a series of pilot trials.*
- ***b)*** *To provide an stable and consistent contact between the NIRS sensor optode and the spinal cord tissue during data collection, we have designed an adjustable magnetic fixator, with three degrees of freedom. This fixator system is successfully tested during recent pilot studies. The system provided an stable fixation for the V2 NIRS sensor over the spinal cord of our animal models of SCI for a week. We could pull the sensors out at the end of the week with no damage or difficulty.*
- ***c)*** *To support a long-term continuous data collection, the NIRS hardware and software were upgraded.*
- ***d)*** *Upgradet sensor were calibrated and tested.*

Aim 4. Establish the ability of this refined NIRS system to assess oxygenation, perfusion, pressure, and metabolic responses for 7-day post-injury by comparing the NIRS and IP measurements in pigs that are awake and mobile in their cages for 7 days post-injury.

Task 1: Comparing changes in tissue oxygenation & perfusion as assessed by 1) NIRS measures of O_2Hb, HHb, THb, and TOI%, and 2) an IP oxygen/blood flow sensor. [Months 21-28]
Task 2: Comparing changes in hydrostatic pressure as assessed by 1) a novel analysis of the NIRS O_2Hb waveform that accounts for how cardiac-induced oscillations in the waveform are influenced by tissue pressure, and 2) an IP pressure sensor. [Months 21-28]
Task 3: Comparing changes in metabolic responses as assessed by 1) NIRS measures of cytochrome-c-oxidase (CCO), and 2) an IP microdialysis catheter to monitor lactate, pyruvate, and the lactate/pyruvate ratio. [Months 21-28]
Task 4: Data analysis and dissemination. [Month 29]

Milestone(s) Achieved:

- ***a)*** *Experiment 2 is completed. We have collected one-week post-SCI continues spinal cord NIRS data in six animals by V2 sensor and the upgraded NIRS system. IP measurements of spinal cord tissue oxygenation, perfusion and pressure were collected in conjunction with NIRS measures of spinal cord tissue hemodynamics and oxygenation. This 7-days survival animal experiment includes physiological manipulation before and after induction of acute spinal cord injury under anesthesia on day 1, MAP alterations in the conscious animal on day 3, and physiological manipulations under anesthesia followed by euthanasia on Day 7. Our data analysis is indicative of good correlations between NIRS oxygenations (O_2Hb, TOI, Hbdiff) and hemodynamics (THb) parameters, and IP PO_2 and SCBF measures, respectively.*

- ***b)*** *Developing TPSA method (Tissue Pressure Signal Analysis) to monitor changes of spinal cord intraparenchymal pressure (SCP), using O_2Hb waveform analysis of spinal cord*

tissue pulsation, is under process. The objective of this sub-project is to derive a quantitative measure of tissue perfusion and interstitial pressure (either MAP or IP) from photoplethysmography (PPG) signals collected directly from the spinal cord tissue in our pig models of acute SCI during Experiment 1. The focus of our study so far has been on modelling the MAP from PPG. The PPG data from the spinal cord is affected by both MAP and tissue IP variations. Therefore, it is essential for the TPSA technique to be able to differentiate between the two pressure sources. To address this challenge, we first focused on E2 data, which contains PPG collected from animal limbs that are expected to be only impacted by MAP. Having confirmed the MAP estimation performance, we are processing E1 data collected directly from the spinal cord. It should be noted that the technical details of the method are mostly the same, but this approach allows us to assess the two variables separately.

c) *Further validation of NIRS measures of cytochrome-c-oxidase (CCO) requires an in vivo experiment that involves Cyanide administration. Since applications of this extremely toxic agent was not initially included in our ethics approvals and our access to a research fascility in which Cyanide administration is possible is limited, we decided to cancel this task.*

d) *Results of Experiment 2 are included in "Overall project Summary" – Page 17.*

Aim 5. Obtain local ethics approval to conduct a clinical trial of our finalized NIRS system in human acute SCI patients.

Task 1: Final refinements and development of a clinical sensor (V3) for a future pilot trial in human. [Months 30-34]

Task 2: Obtaining Canada Health Approval for the V3 sensor. [Months 32-36]

Task 3: Obtaining UBC CREB approval for conducting a clinical trial in acute SCI patient to test the V3 feasibility and safety. [Months 32-36]

Milestone(s) Achieved:

a) *We have started working on the design of the V3 Clinical sensor based on information and feedback obtained from the animal experiments. Further miniaturization and reshaping the sensor is the first step in our sensor re-design. For this purpose, we have established a collaboration with Dr. Peyman Servati and his team at the Department of electronics and computer science of the University of British Columbia. Dr. Servati is a professor of electronics with a particular focus on flexible nanoelectronics and smart textiles applied in the medical device.*

b) *To apply for regulatory health approvals for the V3 sensor, we need to provide the complete technical specifications, design and modelling of the V3 clinical sensor initially.*

c) *While being confident of our ability to design and prototype the V3 sensor, we decided to request a one-year no-cost extension, "Extension Without Funding (EWOF)", on our DoD grant. Our request was accepted, and our program is extended to 31 August 2020 (#P00001, 06 June 2019). This extension enables us to spend more time and efforts on designing the V3 clinical sensor, as well as obtaining the Canada Health approval and the UBC CREB approval for conducting the first clinical pilot study in 2020.*

3.3 Current Progress on Statement of Work

A Gantt chart indicating actual completed works is provided in Table 2 for reference.

Aim 1. Refine our current NIRS sensor (V1) configuration, and software algorithms to optimize the measurement of spinal cord (SC) oxygenation (O_2Hb, HHb, TOI%), perfusion (THb), metabolism (CCO) and hydrostatic pressure.

- **Task 1:** Submit documents for Institutional (UBC) and ACURO approval.

  Completed. *We have obtained UBC and ACURO approval. This protocol was approved by the University of British Columbia, Vancouver IACUC on January 31, 2016. Dated August 15, 2016, ACURO approval was conveyed to project protocol from USAMRMC protocols SC130007 and SC130008 which were previously approved for the use of swine.*

- **Task 2:** NIRS technology assessment.

  Completed. *An updated literature review on the technology applied in this project is completed. The NIRS technologies and engineering components required for the development of the first (V1) sensor prototype is defined. The first series of equipment related to V1 sensor development and Experiment 1 are provided.*

- **Task 3:** Intellectual Property (IP) protection assessment, through UBC University Industrial Liaison office (UILO).

  In Progress. *Our intellectual property application was reviewed and confirmed by UBC UILO and filing a provisional application with the United States Patent and Trademark Office is under process.*

- **Task 4:** Develop SC-NIRS sensor V1 to be used in Experiment 1.

  Completed.

Aim 2. Verify the relationship between the NIRS and intraparenchymal (IP) assessments of oxygenation, perfusion, pressure, and metabolic responses in our pig SCI model in anesthetized (immobile) animals.

- **Task 1:** Evaluating the NIRS system function in a pig model of SCI in conjunction with IP measurements of oxygen, perfusion, pressure, and metabolism.

  Completed.

- **Task 2:** Comparing tissue oxygenation & perfusion as assessed by 1) NIRS measures of O_2Hb, HHb, THb, and TOI%, and 2) an IP oxygen/blood flow sensor.

Completed.

- **Task 3:** Comparing hydrostatic pressure as assessed by 1) a novel analysis of the NIRS O_2Hb waveform that accounts for how cardiac-induced oscillations in the waveform are influenced by tissue pressure, and 2) an IP pressure sensor.

  Completed.

- **Task 4:** Comparing metabolic responses as assessed by 1) NIRS measures of cytochrome-c-oxidase (CCO), and 2) an IP microdialysis catheter to monitor lactate, pyruvate, and the lactate/pyruvate ratio.

  Completed.

- **Task 5:** Final data analysis and dissemination.

  Completed. *A paper including results of the study is published by the Journal of Neurotrauma in May 2019 (doi: 10.1089/neu.2018.6208)[3]. A copy of the paper is attached at Appendices. Results of Experiment 1 were presented at 2018 SFN conference in San Diego and at 2019 SPIE Photonics West in San Francisco.*

Aim 3. Refine and miniaturize the NIRS sensor, cables, and fixation devices to develop an implantable (but also removable), NIRS sensor (V2) that can be used to monitor the spinal cord for a clinically relevant time frame post-injury in the "non-ideal" setting of an awake (and moving) individual.

- **Task 1:** NIRS system adjustment/refinement for long term (7-day) continuous monitoring of the spinal cord, data storage and management.

  Completed.

- **Task 2:** Sensor prototyping and development.

  Completed.

- **Task 3:** Sensor calibration and testing.

  Completed.

Aim 4. Establish the ability of this refined NIRS system to assess oxygenation, perfusion, pressure, and metabolic responses for 7-day post-injury by comparing the

NIRS and IP measurements in pigs that are awake and mobile in their cages for 7 days post-injury.

- **Task 1:** Comparing changes in tissue oxygenation & perfusion as assessed by 1) NIRS measures of O_2Hb, HHb, THb, and TOI%, and 2) an IP oxygen/blood flow sensor.

  Completed.

- **Task 2:** Comparing changes in hydrostatic pressure as assessed by 1) a novel analysis of the NIRS O_2Hb waveform that accounts for how cardiac-induced oscillations in the waveform are influenced by tissue pressure, and 2) an IP pressure sensor.

  In Progress.

- **Task 3:** Comparing changes in metabolic responses as assessed by 1) NIRS measures of cytochrome-c-oxidase (CCO), and 2) an IP microdialysis catheter to monitor lactate, pyruvate, and the lactate/pyruvate ratio.

  Nothing to report

- ***Task 4:*** *Data analysis and dissemination.*

  Completed.

Aim 5. Obtain local ethics approval to conduct a clinical trial of our finalized NIRS system in human acute SCI patients.

- **Task 1:** Final refinements to develop a clinical sensor (V3) for a future pilot trial in human.

  In Progress.

- **Task 2:** Obtaining Canada Health Approval for the V3 sensor.

  Nothing to report.

- **Task 3:** Obtaining UBC CREB approval for conducting a clinical trial in acute SCI patient to test the V3 feasibility and safety.

  Nothing to report.

Table 2: Gantt chart of current work. Blue sections reflect actual work completed. Green sections indicate in progress tasks. This Gantt chart of current work matches the Gantt chart of approved statement of work (Table 1).

Specific Aim 1: *Refine our current NIRS sensor (V1) configuration, and software algorithms to optimize the measurement of spinal cord oxygenation (O_2Hb, HHb, TOI%), perfusion (THb), metabolism (CCO) and hydrostatic pressure.*	**YEAR 1**				**YEAR 2**				**YEAR 3**				**Extended YEAR 4**			
Activity	**Q 1**	**Q 2**	**Q 3**	**Q 4**	**Q 1**	**Q 2**	**Q 3**	**Q 4**	**Q 1**	**Q 2**	**Q 3**	**Q 4**	**Q 1**	**Q 2**	**Q 3**	**Q 4**
1a) UBC & ACURO approvals																
1b) Technology assessment																
1c) IP assessment																
1d) V1 Sensor development																
Specific Aim 2: *NIRS system evaluation and verification of the relationship between the NIRS and IP assessments of oxygenation, perfusion, pressure, and metabolic responses in our pig SCI model in*	**YEAR 1**				**YEAR 2**				**YEAR 3**				**Extended YEAR 4**			

anesthetized (n=8 immobile) animals (Experiment 1)																
Activity	**Q 1**	**Q 2**	**Q 3**	**Q 4**	**Q 1**	**Q 2**	**Q 3**	**Q 4**	**Q 1**	**Q 2**	**Q 3**	**Q 4**	**Q 1**	**Q 2**	**Q 3**	**Q 4**
2a) NIRS system evaluation and collecting NIRS and IP data in Experiment 1																
2b) Data analysis: NIRS vs. IP measurements (SC oxygenation & perfusion)																
2c) Data analysis: NIRS vs. IP measurements (SC pressure)																
2d) Data analysis: NIRS vs. IP measurements (SC metabolic responses)																
2e) Final data analysis and dissemination																
Specific Aim 3: *Refine and miniaturize the NIRS sensor to develop an implantable (but also removable), NIRS sensor (V2) that can be used to monitor the spinal cord for a clinically relevant time frame post-injury in the "non-ideal"*	**YEAR 1**				**YEAR 2**				**YEAR 3**				**Extended YEAR 4**			

setting of an awake (and moving) individual.																
Activity	**Q 1**	**Q 2**	**Q 3**	**Q 4**	**Q 1**	**Q 2**	**Q 3**	**Q 4**	**Q 1**	**Q 2**	**Q 3**	**Q 4**	**Q 1**	**Q 2**	**Q 3**	**Q 4**
3a) V2 sensor development																
3b) V2 sensor calibration																
3c) NIRS system refinement for long term (7-day) monitoring																
Specific Aim 4: *Establish the ability of V2 sensor to assess oxygenation, perfusion, pressure, and metabolic responses for 7-day post-injury by comparing the NIRS and IP measurements in pigs that are awake and mobile for 7 days post-injury. (targeted n=8)*	**YEAR 1**				**YEAR 2**				**YEAR 3**				**Extended YEAR 4**			
Activity	**Q 1**	**Q 2**	**Q 3**	**Q 4**	**Q 1**	**Q 2**	**Q 3**	**Q 4**	**Q 1**	**Q 2**	**Q 3**	**Q 4**	**Q 1**	**Q 2**	**Q 3**	**Q 4**
4a) NIRS vs. IP measurements (SC oxygenation & perfusion)																
4b) NIRS vs. IP measurements (SC pressure)																

4c) NIRS vs. IP measurements (SC metabolic responses)																
4d) Data analysis, dissemination																
Specific Aim 5: *Obtain local ethics approval to conduct a clinical trial of our finalized NIRS system in human acute SCI patients.*	**YEAR 1**				**YEAR 2**				**YEAR 3**				**Extended YEAR 4**			
Activity	**Q 1**	**Q 2**	**Q 3**	**Q 4**	**Q 1**	**Q 2**	**Q 3**	**Q 4**	**Q 1**	**Q 2**	**Q 3**	**Q 4**	**Q 1**	**Q 2**	**Q 3**	**Q 4**
5a) Final refinements of V3 sensor																
5b) Obtaining Canada Health approval for the V3 sensor																
5c) Obtaining UBC CREB approval																

4 OVERALL PROJECT SUMMARY

The overall objective of this project is to develop a NIRS sensor, system and method that can provide real-time monitoring of spinal cord oxygenation, blood flow, pressure, and metabolic responses after acute SCI. The ability to monitor these parameters within the injured spinal cord will provide clinicians with potentially critical information to optimize the hemodynamic management of the acutely injured patient. In this grant, we propose a sequence of preclinical studies aimed to translate this approach to human SCI patients. Following initial NIRS technology assessmsnt, prototype design and development, and a series of pilot animal studies and technology refinements in year one, Experiment 1 was completed in year two.

EXPEWRIMENT 2

4.1 METHODS

4.1.a V2 NIRS System and Sensor Prototype Development

The “V1” Experiment demonstrated that our NIRS system was able to monitor spinal cord oxygenation and hemodynamics in a fully anesthetized animal in an experiment that lasted about 10 hours. However, because the period of aggressive hemodynamic monitoring in humans is approximately 7 days post-injury, we sought to achieve a longer-term continuous data collection. This required the NIRS hardware and software to be upgraded. Figure 1 shows a screenshot of the monitor during data collection using the new software.

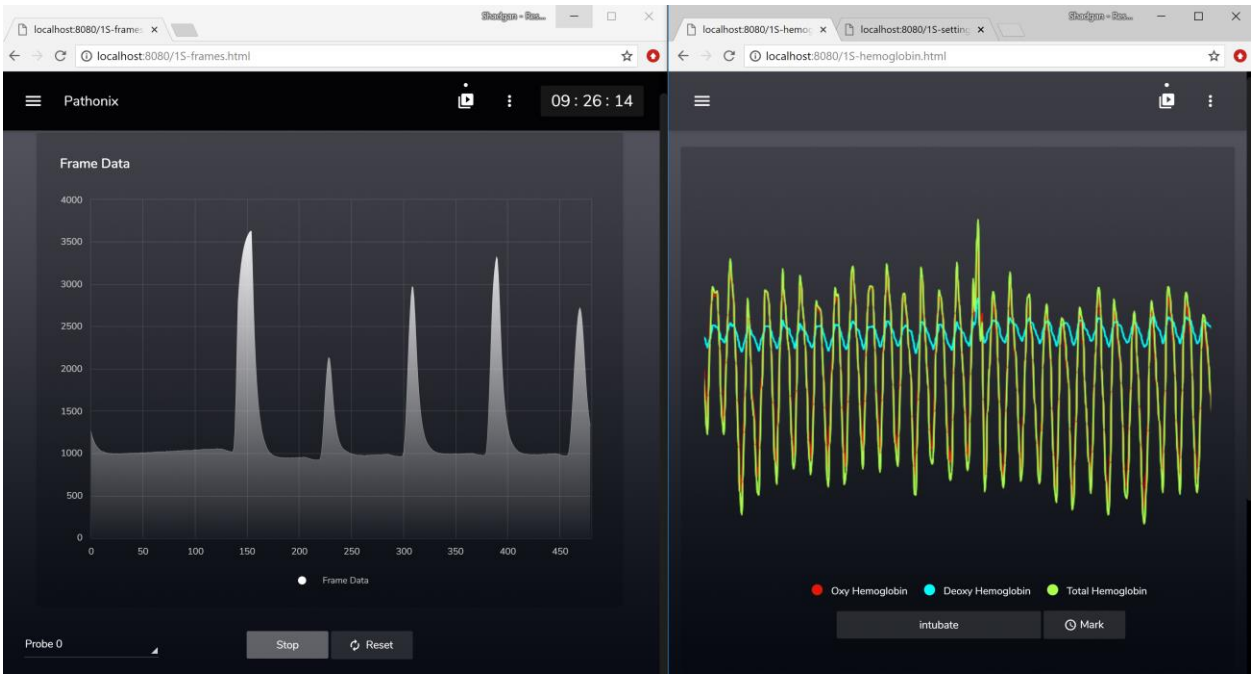


Figure 1. This screenshot shows NIRS data collected from the spinal cord in an animal model of SCI of Experiemnt 2 using the new upgraded NIRS software. Five signal picks (left panel), indicating healthy function of V2 NIRS sensor, can be observed during data collection. Clear effect of cardiac pulsations on the spinal cord tissue hemodynamics (right panel) indicate high definition of data collected by the V2 NIRS sensor.

The custom V2 NIRS sensor is a modification of V1 SC-NIRS sensor (Figures 2-4). Essential specifications of this sensor include:

1. Implantable,
2. Multi wavelength light source – Current LED supports 5 NIR wavelengths,
3. Smaller size – Sensor diameter: 6 mm,

4. With a long (250cm length) shielded wire, to better protect against potential electromagnetic interference (EMI),
5. Water/fluid resistant,
6. With an option for adjusted fixation over the dura,
7. Safely removable.

Figure 2. V2 SC-NIRS sensor prototype model - optode components and structure.

A notable feature of the V2 sensor is that it is tubular and comparable in size to a ¼ inch Hemovac surgical drain (actually it is slightly smaller). This would allow for the sensor to be implanted onto the exposed dura at the time of surgery, externalized like a Hemovac drain, and then pulled out after the week of monitoring.

Figure 3. V2 vs. V1 SC-NIRS prototype

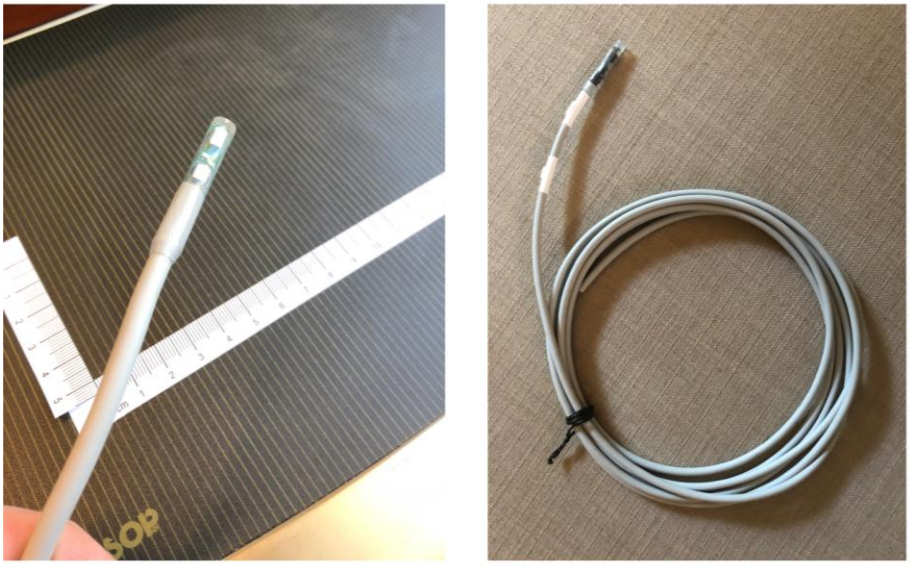


Figure 4. V2 SC-NIRS Sensor Prototype

Sensor Fixation System - To place and fix the V2 SC-NIRS sensor optode over the dura for a long-term (one-week) data collection, in a 7-day survival animal model of SCI, a system for fixing the NIRS sensor over the dura was designed (Figure 5). This fixator provides 3 degrees of freedom for a fine adjustment of the sensor's optode. A small component of N52 magnet, mounted at the fixator's arm, absorbs and stabilizes the V2 sensor's optode over the dura. This magnetic fixation system allows for the sensor to be held on the dura in stable but non-rigid manner so that the sensor can be removed by pulling it out (i.e. without requiring a second surgery to remove it).

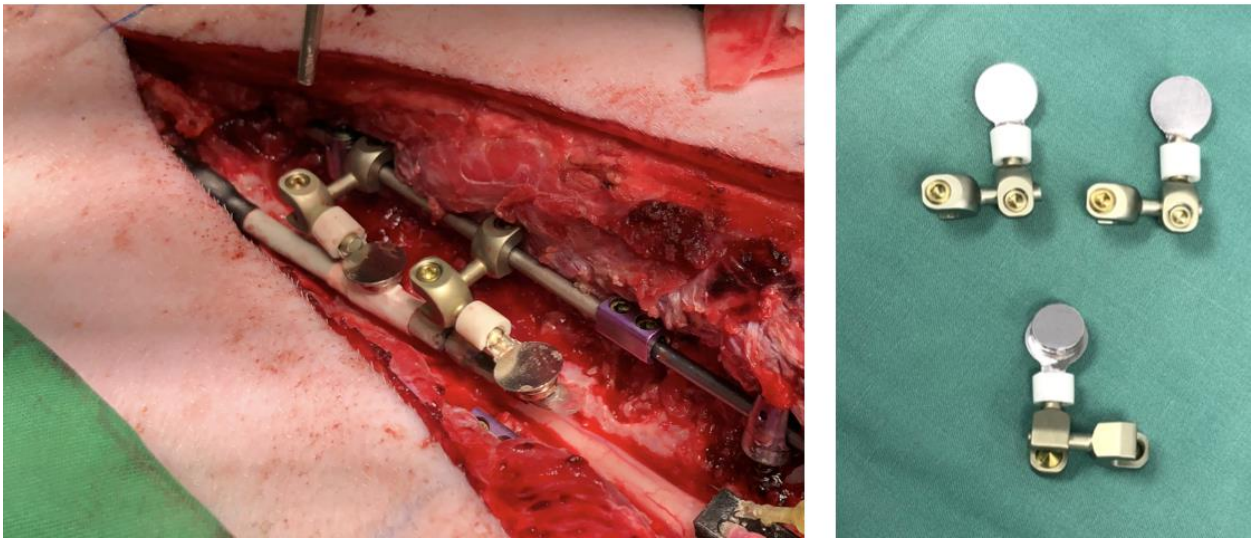


Figure 5. Magnet-based sensor fixation system

4.1.b Animal and Surgical Preparation

In this experiment, we used a porcine model of thoracic contusion-compression SCI to investigate the feasibility and sensitivity of our V2 NIRS sensor for continuous, real-time monitoring for 7 days post-injury. All procedures were approved by the UBC Committee on Animal Care and follow the regulations of the Canadian Council on Animal Care. The anesthesia/analgesia protocols were established by the UBC Centre for Comparative Medicine.

Six 25-31 kg female Yucatan miniature pigs were used in this study. On the first day, a pig was anesthetized and intubated with a combination of 1.4 L (70%) nitrogen and 0.6 L (30%) oxygen. A pulse oximeter was attached to the pig's ear to monitor arterial oxygen saturation and heart rate. The right jugular vein and carotid artery were exposed by blunt dissection, and catheters were inserted to monitor central venous pressure and MAP respectively. Then, the pig was carefully repositioned for the spinal cord surgery. A dorsal laminectomy was performed between T5 and T15 in order to expose the spinal cord. In anticipation of a weight drop injury, the T10 area was identified as the impact site.

IP probes and V2-NIRS sensor were positioned on the cord, as shown in the schematic diagram (Figure 6). Two sets of two IP probes were inserted into the spinal cord approximately 1.5 cm and 3.5 cm caudal from the impact site to a depth of approximately 3-4 mm under the dura. To keep the probe tips in place, they were inserted into catheters and a custom 3-D printed housing unit. In each set of probes, one is used for dual monitoring of oxygenation and blood flow (0.45 mm diameter - OxyLite and OxyFlo combined oxygen/laser Doppler sensors, Oxford Optronix, Abingdon, UK), while the other is used for monitoring hydrostatic pressure (0.31 mm diameter - Fiso FOP-LS-NS-1006A,

FISO, Quebec, Canada). Ultrasounds were performed to verify proper probe placement inside the cord and determine the actual probe tip position.

V2-NIRS sensor was inserted transcutaneously from 10 cm proximal to the surgical exposure, using a Hemovac surgical trocar. The sensor was positioned on top of the dura approximately 3.5 cm rostral to the impact site and held in place using rods and magnet-based fixators (Figure 7). The sensor was covered with fibrin bio-adhesive sealant (Tisseel®, Baxter Healthcare, Deerfield, IL, USA) to further maintain gentle contact with the cord and prevent blood from interfering with photon transmission (Figure 8). The wires from the IP probes and NIRS sensor were brought out of the surgical field and tunneled through the skin.

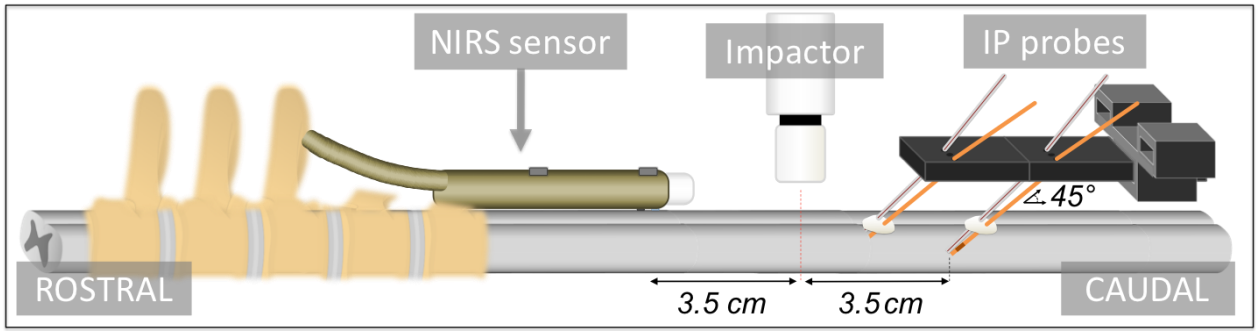


Figure 6. Schematic of probe placement. The NIRS sensor was placed approximately 3.5 cm rostral from the impact site (T10), while the IP probes measuring oxygenation, blood flow, and tissue pressure were placed 1.5 and 3.5 cm caudal to the site. The IP probes were inserted into catheters and a custom 3-D printed housing unit for probe tip stabilization and protection.

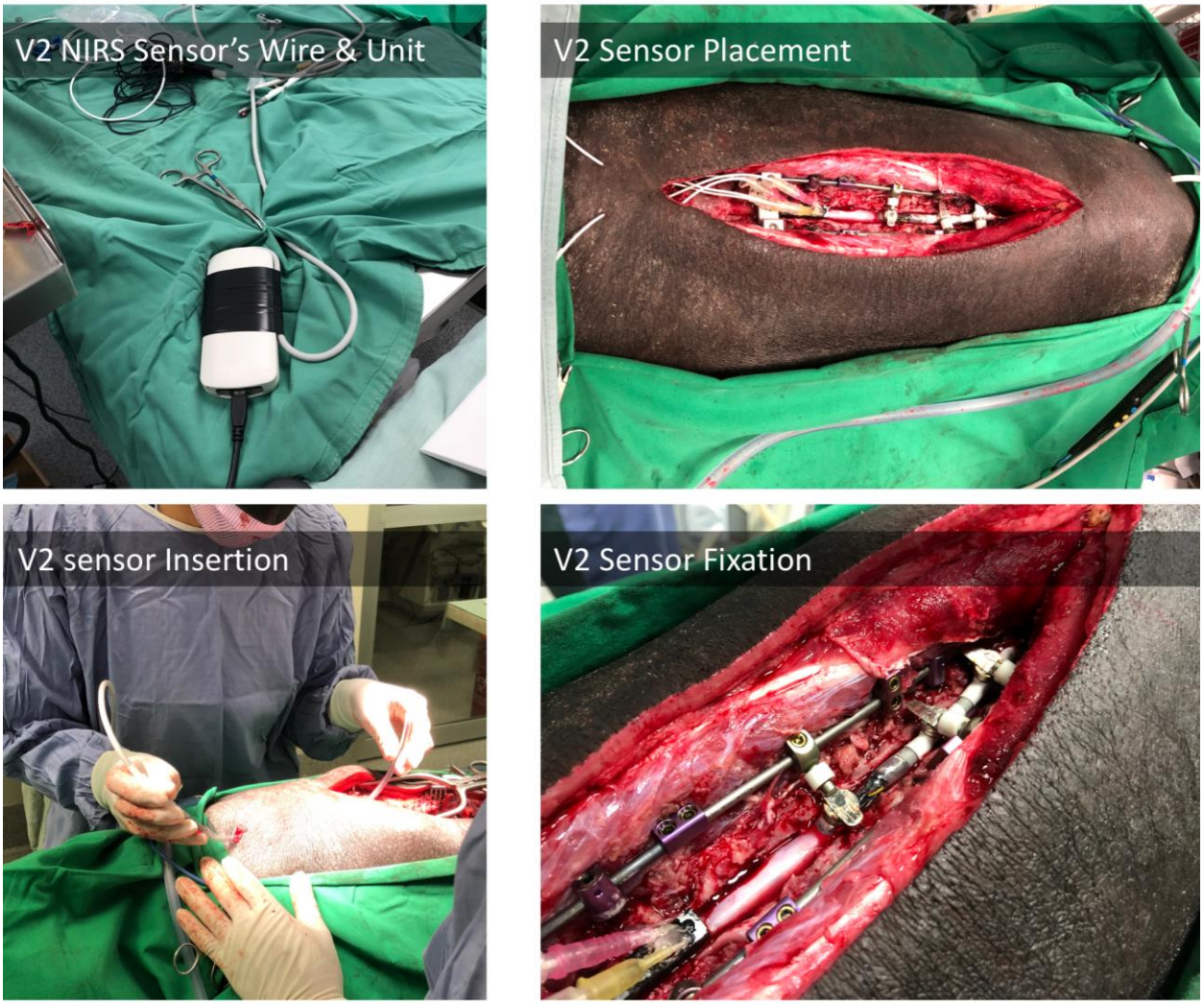


Figure 7. Demonstrates the process of V2 sensor insertion and placement on the spinal cord at T10 in a pilot animal model of SCI that was continuously monitored for a week.

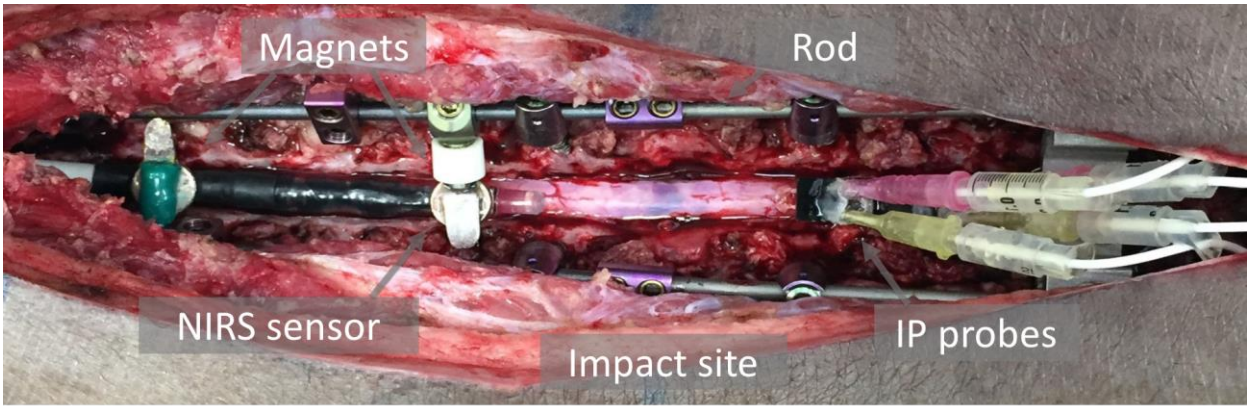
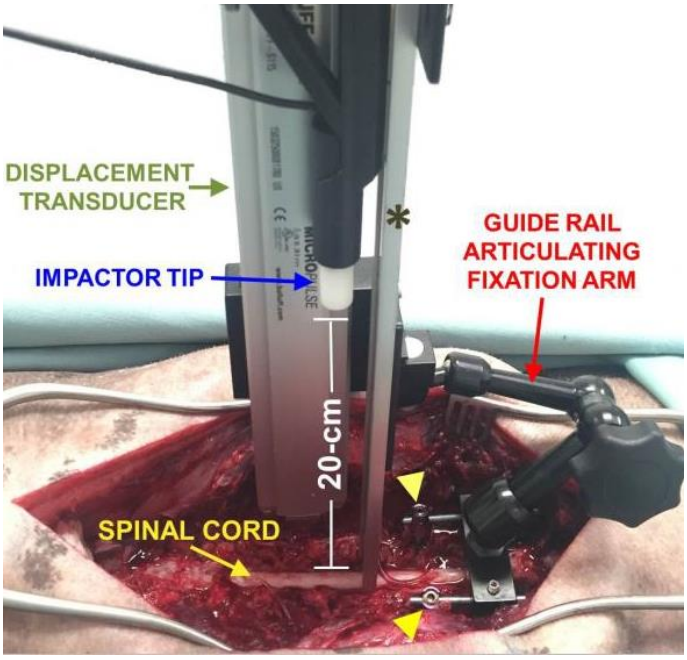


Figure 8. Surgical image of the NIRS sensor, IP probes, and impact site at T10. The NIRS sensor was kept in place on top of the dura using rods and magnets.

4.1.c Spinal cord injury procedure

As shown in Figure 9, An articulating arm (660, Starrett, Athol, Massachusetts, USA) was fixed to the T9, T10, and T11 positioned pedicle screws with titanium rods. This arm positions in place the impact device, which consists of an impactor (diameter, 0.953 cm) fitted with a load cell (LLB215; Futek Advanced Sensor Technology, Irvine, CA) that slides down a guide rail equipped with a Balluff Micropulse® linear position sensor (BTL6-G500-M0102-PF-S115, Balluff Canada Inc., Mississauga ON, Canada) to record the force and impactor position from which displacement and velocity were determined. A fentanyl bolus was administered five minutes prior to the contusion-compression SCI. The contusion was induced by dropping a 50 g weight along the guide rail onto the cord at T10 from a height of 20 cm. Then, a further 100 g weight was gently added, for a total compression weight of 150 g. After 30 minutes of sustained compression, the cord was decompressed by removing the weights. Following 30 minutes of decompression, MAP was increased by 20 mmHg by infusing norepinephrine via the jugular vein catheter. The target MAP was maintained for 30 minutes. The incision was then closed. The recording wires were attached to a suspended beam to prevent tangling, and to allow the pig to recover and move freely inside its clamshell. Figure 10 shows a schematic figure of the experimental setup including the position of the impactor.

Figure 9. The guide-rail impactor apparatus used to induce a weight-drop SCI at T10. The rail was vertically aligned using the articulating fixation arm. The 50 g impactor was dropped along the guide-rail from 20 cm above the spinal cord to induce a SCI.



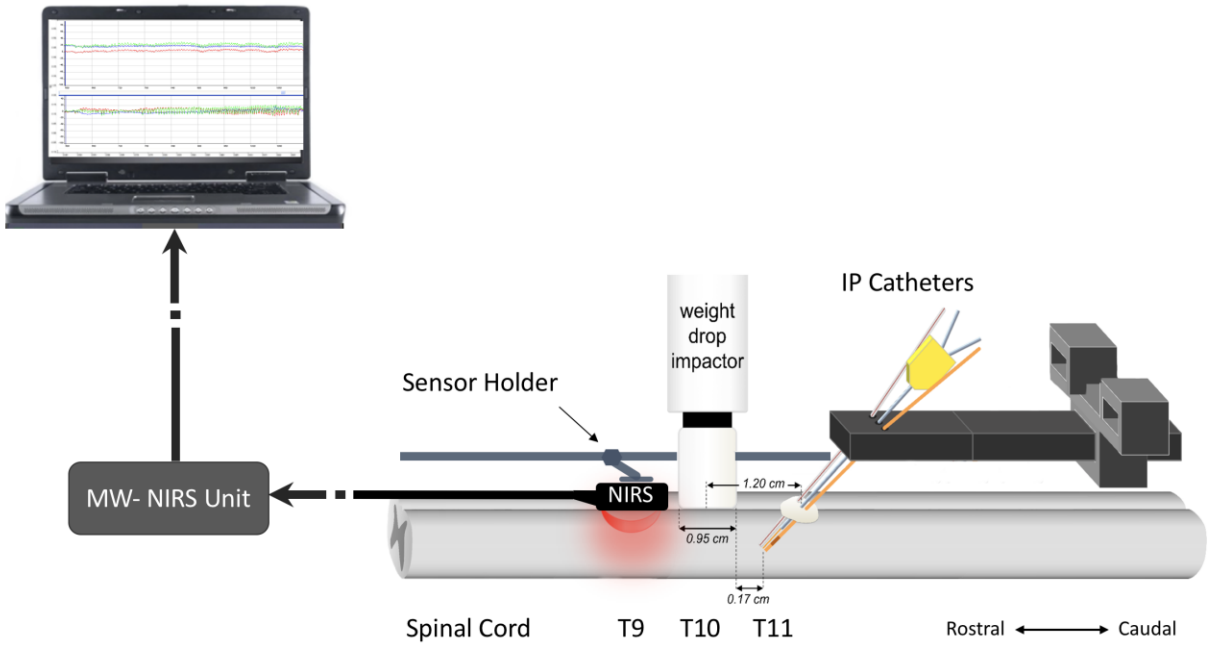


Figure 10. A schematic figure of the experimental setup that shows position of the impactor.

4.1.d Experimental Protocol

On the first day, after the probes and sensor were positioned in the pig, they began to continuously record physiological parameters of interest. Baseline monitoring occurred for approximately 60 minutes, followed by a series of events as shown in the experimental timeline (Figure 11). Two mild hypoxic episodes were induced in the pig by detaching the ventilator. It was re-attached when oxygen saturation reached 80%. Blood was drawn before, during, and after hypoxia for use in i-Stat tests to compare oxygen saturation between the different measuring methods. Each hypoxic episode lasted for approximately 30 seconds. The pig was then allowed to recover for 15 minutes.

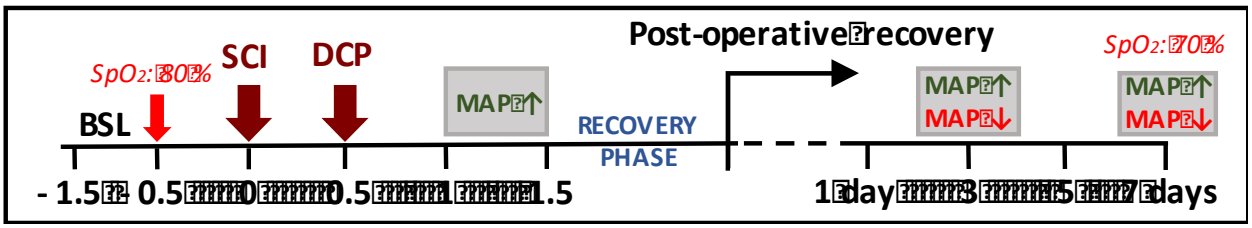


Figure 11. 7-day continuous monitoring timeline. After baseline monitoring (BSL) and hypoxia (SpO_2: 80%), a SCI and compression was induced at T10, followed by a 30-min decompression (DCP) and a 20 mmHg MAP increase. MAP alterations (increase and decrease) were performed on day 3 and 7, with hypoxic episodes (SpO_2: 70%) on day 7.

On the third day, MAP alterations were performed. Norepinephrine was infused via the jugular vein catheter to increase MAP by 20 mmHg. The target MAP was maintained for 30 minutes, followed by a 30-minute recovery period. The alteration was repeated using nitroprusside to decrease MAP. On the seventh day, the pig was sedated and intubated. MAP alterations were performed in a similar manner as previous days, except the target MAP was maintained for 60 minutes instead of 30 minutes. Each alteration was followed by a 30-minute recovery period. After the MAP alteration portion, two severe hypoxic episodes were induced until the oxygen saturation reached 70%. Each episode lasted for about 45 seconds, followed by 15 minutes of recovery time. The pig was then euthanized. After 5 minutes, we stopped recording IP probe and NIRS sensor data. The probes and sensor were then removed and the cord was harvested and preserved in formalin for subsequent histologic analysis.

4.1.e Data analysis and statistics.

All data, including NIRS measures of spinal cord chromophore concentrations (O_2Hb, HHb), spinal cord IP measures ($PaPO_2$, SCBF) and vital signs (SaO_2, heart rate, respiratory rate) were recorded continuously during the 7-day experiment. The raw optical data were converted into changes in NIRS parameters (O_2Hb, HHb), and THb, Hbdiff and TOI% were calculated via the NIRS software. The changes for each variable during each event, including spinal cord NIRS-derived O_2Hb, HHb, THb, Hbdiff, TOI%, and spinal cord IP $PaPO_2$ and SCBF were compared using Wilcoxon signed-rank test to determine statistical significance. Pearson correlation coefficients were calculated to establish the pairwise relationships between the IP and NIRS measurements during episodes of induced hypoxia, and MAP changes. Combined-intervals sensitivity and specificity of NIRS parameters for predicating positive and negative changes of $PaPO_2$ and SCBF were performed. Data are presented as means ± standard error (SEM) and the level of significance set at $p<0.05$ for all statistical analysis and comparisons. Data were analyzed using SAS v9.4 (SAS Institute, Cary, North Carolina).

4.2. Results

4.2.a Subject parameters

Six 25-31 kg female Yucatan miniature pigs were studied (Table 3).

Table 3. Measures of weight and injury parameters. SEM, standard error of the mean.

Animal Subject	Weight (kg)	Injury Force (kdynes)	Survival
E2E1	31	4548	7 days
E2E2	29	3595	7 days
E2E3	26	3759	7 days
E2E4	25	4271	7 days
E2E5	27.5	3860	1 day*
E2E6	26.5	4107	7 days

*E2E5 was euthanized 1 day post-injury due to cardiac and respiratory issues.

4.2.b. IP vs. NIRS parameters.

The ability for the V2-NIRS sensor to monitor changes in NIRS measures during hypoxia events and MAP alterations on day 1 post-SCI were maintained on day 7 post-SCI (Figure 12 and 13). These examples demonstrate that the V2-NIRS sensor can maintain physiologic measurements over the 7 day post-SCI period.

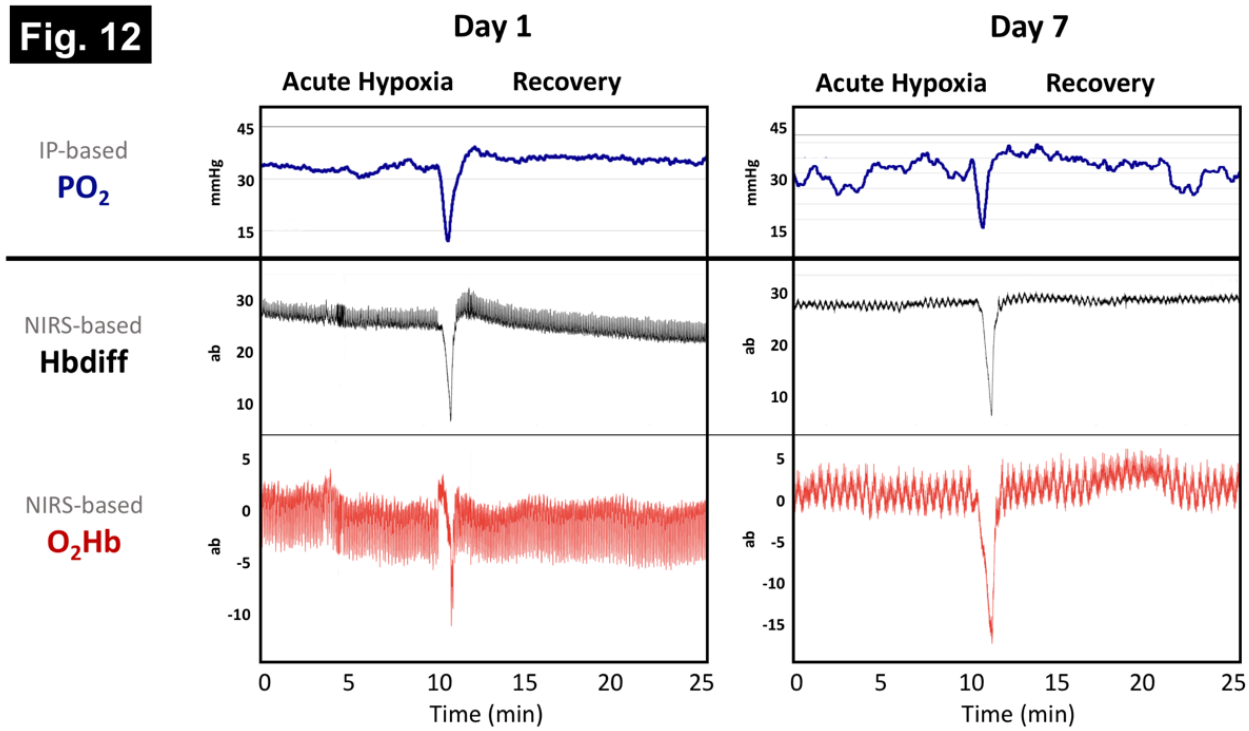


Figure 12. Hypoxic challenge on day 1 results in a significant drop in PO_2 measured with intraparenchymal (IP) sensors, which is also visualized in the NIRS O_2Hb waveform. This is also seen on day 7 in this same animal of Experiment 2.

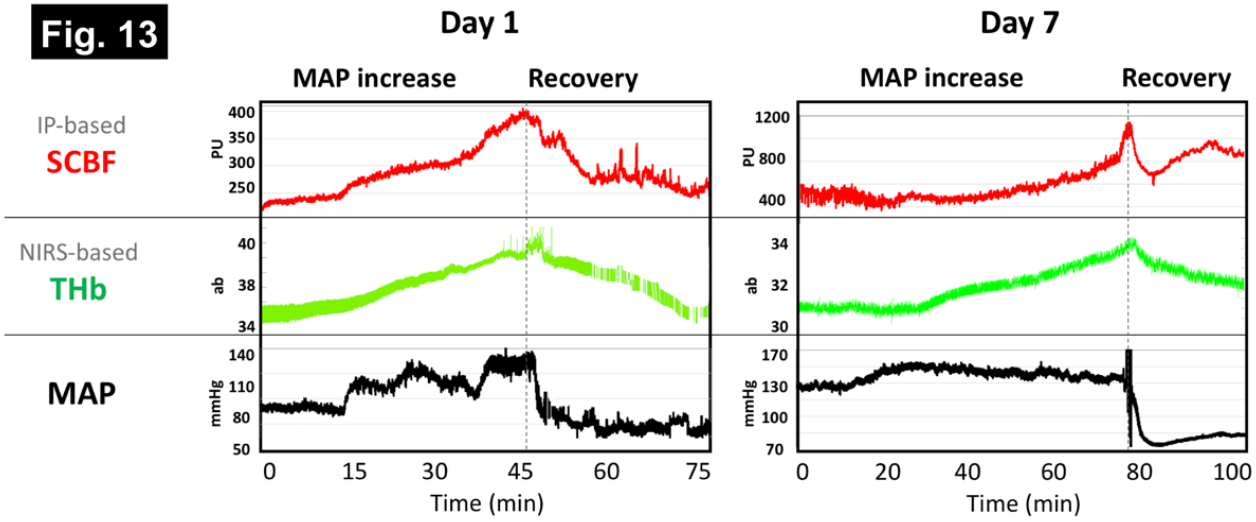
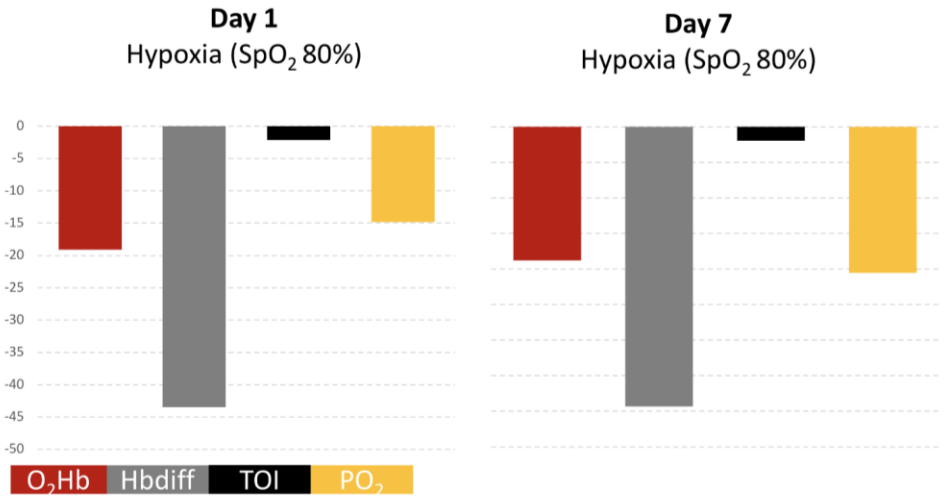


Figure 13. Increasing the MAP on day 1 results in an increase in spinal cord blood flow (SCBF) measured with the IP sensor. This is mirrored by an increase in the NIRS THb. Both responses are also maintained on day 7 in this same animal of Experiment 2.

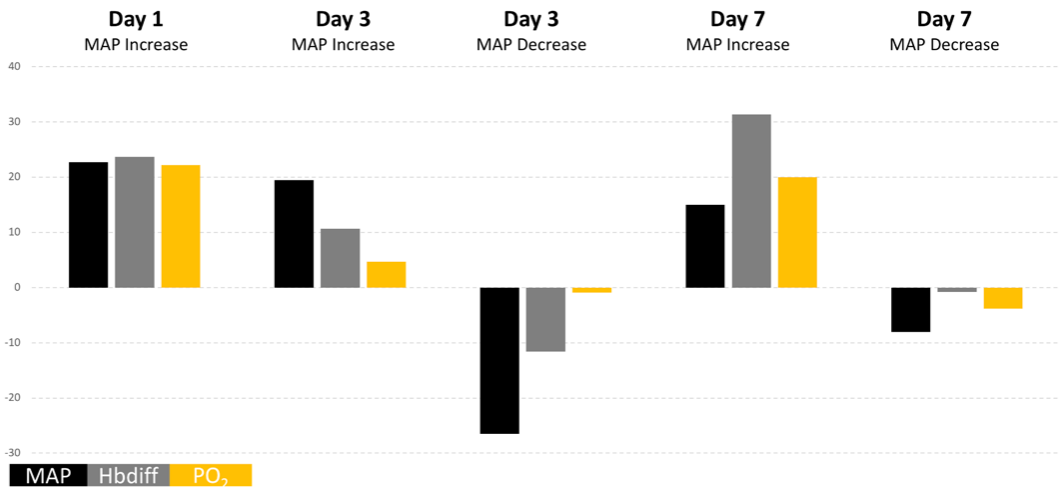
Strong correlations between IP-based PO_2 and NIRS-based oxygenation parameters were observed during hypoxia events on Day 1 and on Day 7, suggesting that the fixation system was effective at securing the NIRS sensor on the dura (Figure 14). Strong correlations between IP-based PO_2 and NIRS-based Hbdiff were observed during MAP manipulations on Day 1, Day 3 and Day 7 (Figure 15). Strong correlations were also observed between IP-based SCBF and NIRS-based THb (spinal cord tissue hemodynamics parameter) during MAP manipulations on Day 1, Day 3 and Day 7 (Figure 16). Importantly, at the end of 7 days, we were able to easily pull the entire NIRS sensor and cable out through the skin puncture site, demonstrating that while the fixation system was effective at holding the NIRS sensor on the dura, it did so in a manner that still allowed for the sensor to be pulled out.



	Day 1	Day 7
	Hypoxia	Hypoxia
O_2Hb (ab)	22.72 (10.90)	19.46 (5.35)
Hbdiff (ab)	23.68 (14.31)	10.07 (16.27)
TOI (%)	22.20 (7.84)	4.68 (3.90)
PO2 (mmHg)	23.40 (17.44)	17.55 (12.06)

All values are mean – standard error of the mean.

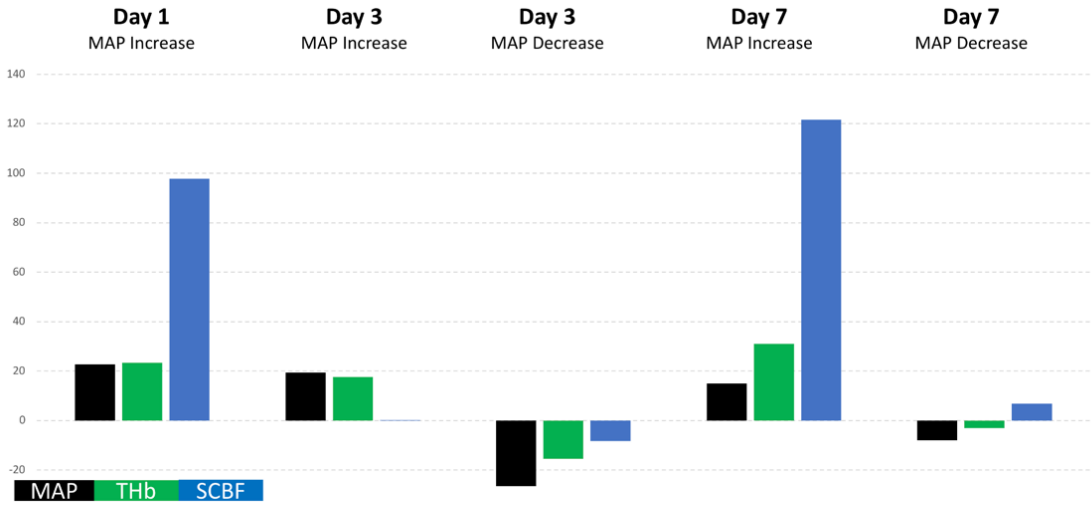
Figure 14. Relationship between non-invasive NIRS O_2Hb, Hbdiff and TOI% and invasive IP PO_2 during induced Hypoxia changes in Day 1 and Day 7.



	Day 1 MAP Increase	Day 3 MAP Increase	Day 3 MAP Decrease	Day 7 MAP Increase	Day 7 MAP Decrease
MAP (mmHg)	22.72 (10.90)	19.46 (5.35)	-26.54 (15.61)	15.01 (9.23)	-8.04 (18.75)
Hbdiff (ab)	23.68 (14.31)	10.07 (16.27)	-11.63 (13.60)	31.34 19.71)	-0.81 (4.52)
PO_2 (mmHg)	22.20 (7.84)	4.68 (3.90)	-0.9 (1.74)	20.04 (23.16)	-3.8 (7.67)

All values are mean – standard error of the mean.

Figure 15. Relationship between non-invasive NIRS Hbdiff and invasive IP PO_2 during induced MAP changes in Day 1, Day 3 and Day 7.



	Day 1 MAP Increase	Day 3 MAP Increase	Day 3 MAP Decrease	Day 7 MAP Increase	Day 7 MAP Decrease
MAP (mmHg)	22.72 (10.90)	19.46 (5.35)	-26.54 (15.61)	15.01 (9.23)	-8.04 (18.75)
THb (ab)	23.40 (17.44)	17.55 (12.06)	-15.44 (9.92)	30.98 (20.68)	-3 (9.43)
SCBF (APU)	97.73 (83.57)	0.06 (36.40)	-8.2 (57.02)	121.69 (112.1)	6.8 (59.55)

All values are mean – standard error of the mean.

Figure 16. Relationship between non-invasive NIRS THb and invasive IP SCBF during induced MAP changes in Day 1, Day 3 and Day 7.

The statistical analysis of the continuous 7-day data collected non-invasively via NIRS and invasively via the IP sensors demonstrates statistically significant correlations between the NIRS and IP measures of oxygenation and hemodynamics. Importantly, the NIRS measures (O_2Hb, TOI, Hbdiff, and THb) were significantly changed with MAP alterations and strongly correlated to MAP (Table 4). **This is a key result, because it indicates that the NIRS sensor is able to detect local tissue changes within the injured spinal cord that are reflective of systemic hemodynamic changes (MAP) over the first 7 days post-injury.**

Table 4. Relationship between NIRS vs IP measures and NIRS vs MAP.

MEASURE	MEASURE	PEARSON CORRELATION COEFFICIENT	P VALUE
NIRS O_2Hb	**IP PO_2**	0.736	p=0.000
NIRS TOI	**IP PO_2**	0.613	p=0.000
NIRS HbDiff	**IP PO_2**	0.717	p=0.000
NIRS THb	**IP PO_2**	0.717	p=0.000
NIRS THb	**IP SCBF**	0.492	p=0.000
NIRS O_2Hb	**MAP**	0.715	p=0.000
NIRS TOI	**MAP**	0.434	p<0.001
NIRS HbDiff	**MAP**	0.653	p=0.000
NIRS T Hb	**MAP**	0.718	p=0.000

One NIRS parameter that was particularly interesting was the Hbdiff (the calculated difference between the oxygenated and deoxygenated hemoglobin). The Hbdiff is reflective of the oxygenation status of the tissue, as when the oxygenated hemoglobin increases, the deoxygenated hemoglobin decreases, and the "difference" between the two increases. Conversely, as the oxygenated hemoglobin decreases, the deoxygenated hemoglobin will increase, and the difference between the two decreases. In this 7-day experiment, we observed that the Hbdiff increased and decreased with the induced MAP increases and decreases on Day 1, Day 3, and Day 7, and was typically more sensitive to changes in MAP than the intraparenchymal PO_2 (Figure 15).

Combined-intervals sensitivity and specificity of NIRS parameters for predicting positive and negative changes of intraparenchymal PO_2 and SCBF are shown in Table 5. This demonstrates high sensitivity, specificity, and positive predictive value (PPV) for NIRS-derived O_2Hb, Hbdiff, TOI, and THb for predicting changes of PO_2 and SCBF.

Table 5. Sensitivity/Specificity and Positive Predictive Values for NIRS and IP Measures.

NIRS MEASURES PREDICTING POSITIVE IP MEASURES

	Sensitivity	Specificity	PPV	NPV
ΔPAPO$_2$ *VS.* ΔO$_2$Hb	89.47	85.71	94.44	75
ΔPAPO$_2$ *VS.* Δ HbDiff	84.21	71.43	88.89	62.5
ΔPAPO$_2$ *VS.* ΔTOI	78.95	57.14	83.33	50
ΔSCBF *VS.* ΔTHb	87.5	60	77.78	75

NIRS MEASURES PREDICTING NEGATIVE IP MEASURES

	Sensitivity	Specificity	PPV	NPV
ΔPAPO$_2$ *VS.* ΔO$_2$Hb	85.71	89.47	75	94.44
ΔPAPO$_2$ *VS.* Δ HbDiff	71.43	84.21	62.5	88.89
ΔPAPO$_2$ *VS.* TOI	57.14	78.95	50	83.33
ΔSCBF *VS.* ΔTHb	60	87.5	75	77.78

Furthermore, the combined-intervals sensitivity and specificity analysis indicates high sensitivity, specificity, and PPV of TOI and THb to predict changes of MAP over 7-day of spinal cord monitoring (Table 6). **This important data confirms the predictive values of NIRS-derived measures of spinal cord oxygenation and hemodynamics when MAP is altered.** It is particularly important to show this, because in a clinical setting, we would not have invasive monitoring of oxygenation and flow – we would only have the NIRS sensor and would need some assurance that the measured changes with NIRS do in fact reflect “real” intraparenchymal measure when the MAP is altered.

Table 6. Sensitivity/Specificity and Positive Predictive Values for NIRS Measures and MAP.

NIRS MEASURES PREDICTING POSITIVE MAP MEASURES

	Sensitivity	Specificity	PPV	NPV
ΔTOI *VS.* ΔMAP	94.44	87.5	94.44	87.5
ΔTHb *VS.* ΔMAP	94.44	87.5	94.44	87.5

NIRS MEASURES PREDICTING NEGATIVE MAP MEASURES

	Sensitivity	Specificity	PPV	NPV
ΔTOI *VS.* ΔMAP	87.5	94.44	87.5	94.44
ΔTHb *VS.* ΔMAP	87.5	94.44	87.5	94.44

5 KEY RESEARCH ACCOMPLISHEMENTS

- V2-NIRS sensor and system were successfully developed and tested over 7-day monitoring of spinal cord oxygenation and hemodynamics.
- Experiment 2 is completed as scheduled.
- Data clearly demonstrate that the V2-NIRS system can indeed monitor spinal cord tissue oxygenation and hemodynamics in this more clinically relevant paradigm where the animal is recovered from the experimental SCI and then monitored over seven days post-SCI.

6 CONCLUSIONS

This research focuses on establishing a novel NIRS-based system and sensor that can be used to monitor changes of spinal cord oxygenation and hemodynamics from the early post-injury period to a week post-injury. Such an intervention will enable clinicians to optimize patient care by providing them real-time information about the physiology of the injured spinal cord. We have been successful to achieve our third year goals; Experiment 2 was successfully completed. The outcomes of Experiment 2 suggest that the proposed transdural MW-NIRS technique has the potential to monitor spinal cord oxygenation and hemodynamics noninvasively and continuously for a week post-injury. Designing an uptimized implantable clinical SC-NIRS sensor for monitoring spinal cord oxygenation and hemodynamics in first patient with acute spinal cord injury will finalize this research project.

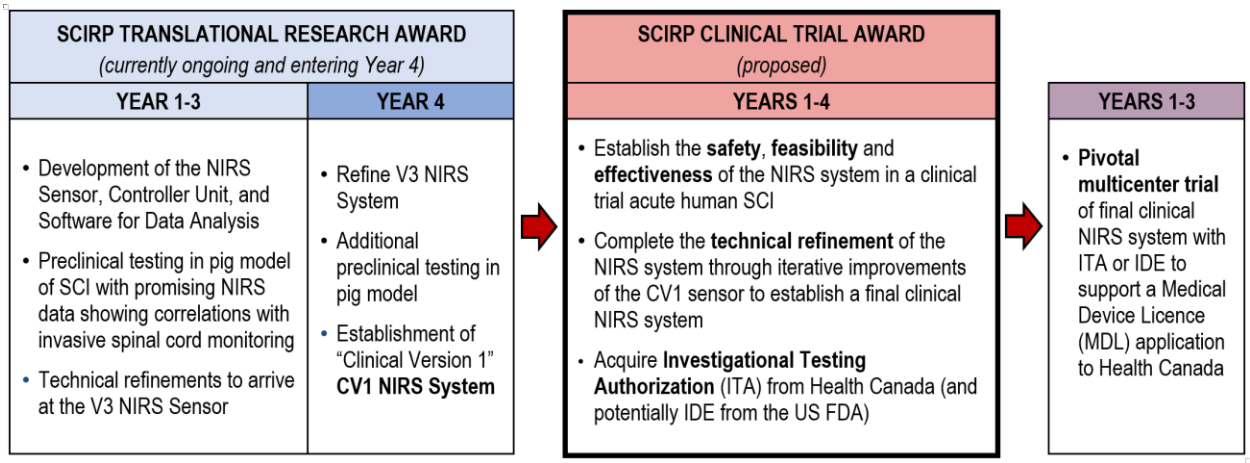
WHERE DOES THIS LEAVE US AND WHAT WILL WE DO IN YEAR 4?

Now that we have concluded Year 3 of the grant, we are very excited by the V2 experiment results and are currently writing this up for publication. The V2 experiment shows that in our pig model of SCI, we can non-invasively monitor the injured spinal cord for 7 days post-injury, with good correlation between the NIRS measures of oxygenation and hemodynamics and the invasive measures.

Our long-term goal at the outset, however, was to develop a NIRS sensor for human application. While the scope of work for this Translational Research Award did not include the conduct of a human clinical trial, our stated intention was to move towards being able to test the NIRS sensor in human patients. And so, the purpose of Year 4 of this grant (for which we have received an Extension Without Funds, "EWOF"), is to move towards human application of the NIRS sensor. *What activities then, will this entail?*

1. Positioning the NIRS sensor concept to be funded for future clinical testing.

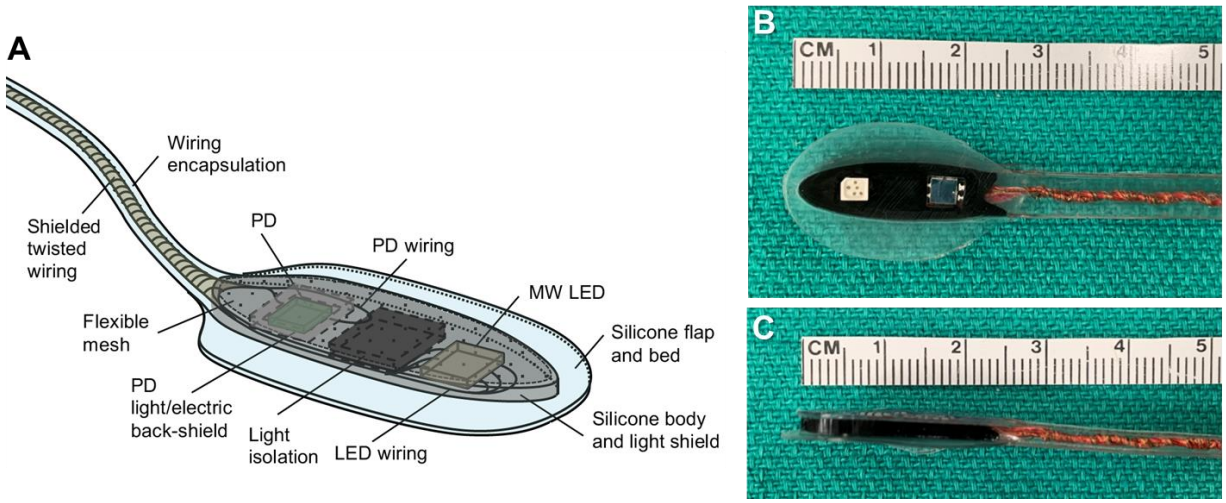
Obviously, if we are thinking about a future clinical trial for the NIRS sensor, it is imperative to begin considering about how such a trial would be funded and what would need to occur in between now and then. We decided that if we were seriously aiming to translate this approach into humans, we should not wait until the final human sensor design was established before looking for funding. So, to this end, we submitted a Clinical Trial Award to the FY19 SCIRP program to conduct a pilot study for the NIRS system. This was submitted in mid-September, 2019, (SC190035 - *"Monitoring Spinal Cord Hemodynamics with Near-Infrared Spectroscopy After Acute Spinal Cord Injury"*). We envision the following pathway:



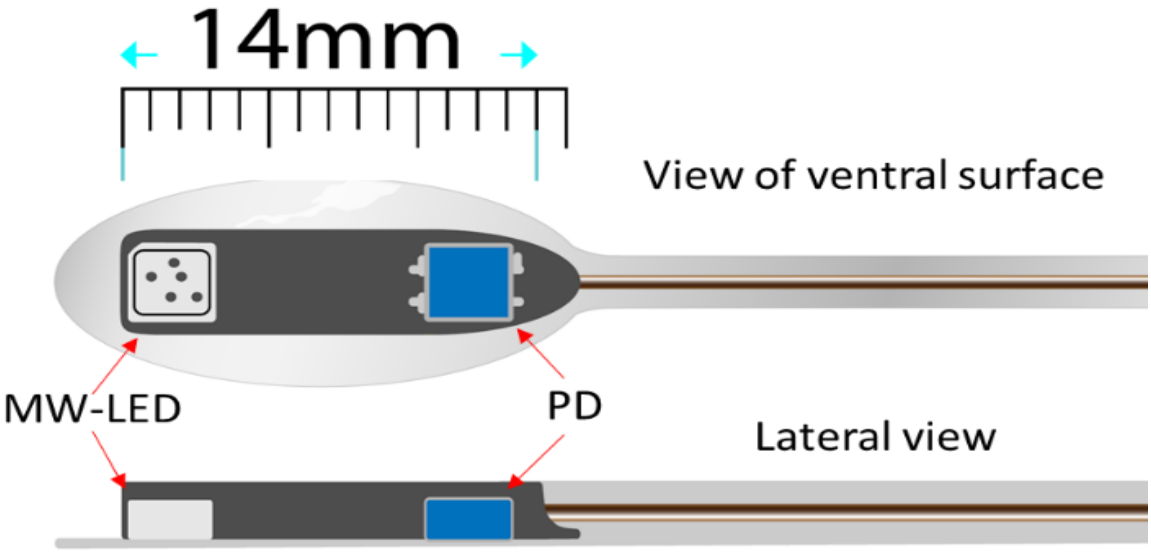
2. Refining the V2 sensor into a V3 sensor for 7d implantation without magnets

While we confirmed that the V2 sensor can non-invasively monitor the spinal cord over 7days, we feel that it would be preferable to establish a fixation system that does not include magnets (in case, for example, the patient needs an MRI post-operatively, and the magnets create severe distortion of the imaging). So, we think that we should try to

refine the V3 system with a design that allows for “non-rigid” fixation and extraction, but without the magnets. We envision developing a sensor with soft silicone flaps and have engaged **Dr. Peyman Servati** at UBC (Electrical Engineering) to help us develop such a sensor. Our first prototypes of the sensor are shown below:



We do feel that there are still improvements that we could make in the design of the sensor to minimize its footprint. For example, we will re-configure the placement of the MW-LED and PD in order to provide an even “slimmer” sensor. The envisioned sensor is shown below. While maintaining the core components, these refinements will help with the application to the human spinal cord in the clinical setting.



3. Testing the revised sensor for 7d monitoring with new fixation system.

We will confirm the function of the refined sensor in 7d survival experiments in the pig, using the TISSEEL for sealing the sensor to the dura. We expect that these pig experiments will be conducted with the smaller NIRS sensor in the next 6 months.

4. Manufacturing the sensor to prepare it for human clinical testing.

We recognize that there are a number of tasks that still need to be addressed in order to be able to implant this device in humans. Here, we have enlisted the help of our **Brendan Gribbons** who works in the Biomedical Engineering Department at Vancouver General Hospital (VGH). He will support the technical development of the NIRS sensor to fulfill the manufacturing and safety standards required to implant the clinical NIRS system. Some of these include:

a. Receiving an electrical certification mark, with assessment against the Canadian Standards Association (CSA) SPE-3000:19 which pertains to hazards such as electric shock, fire, and mechanical hazard.

b. Assessment of electromagnetic transmission with an electromagnetic spectrum analyzer according to standard IEC 60601-1-2.

c. Electrical leakage test and mechanical integrity and robustness evaluation.

d. Inspection with an optical microscope to ensure the catheter's enclosure is sealed, providing immunity to fluid/particular ingress.

In addition to the requirements of Biomedical Engineerins, in discussion with our Infection Control Department we have clarified the steps that need to be taken to ensure that the sensor can be sterilized adequately before implantation in humans. Sterility testing will be performed by an independent lab certified by IOS, FDA, or Health Canada, to establish that the sensor reprocessing steps consistently yield a sterile outcome. Independent laboratories identified by Infection Control to conduct such sterility validation include Nelson Labs (Salt Lake City, UT; www.nelsonlabs.com) and High-Power Validation Testing and Lab Services (Rochester, NY; https://higpowervtls.com) Validation will include:

a. Bioburden testing - to ensure the device is in fact sterile after cleaning and sterilization.

b. Mechanical testing – to ensure that the device is not negatively affected by the sterilization technique.

c. Dose mapping – confirmation of the sterilization technique at the parameters determined by bioburden testing (applicable to items being reprocessed using radiation).

We anticipate sterile packaging of individual NIRS sensors for single-use, which can be potentially provided locally by Iotron Industries Canada, Inc. (Port Coquitlam, BC; https://iotron.com), after e-beam sterilization.

Importantly, we have clarified with Health Canada that for building such a device locally and testing it within a small pilot study in human patients at our site, we will NOT require an Investigational Testing Authorization (ITA) (the equivalent of an IDE from the US FDA).

5. Establishing the human protocol.

Over the next 12 months, we will begin to assemble the protocol for a human clinical trial. Obviously, we hope that we will be funded by the CDMRP SCIRP program to begin in the fall of 2020, but either way, we will start to prepare the documents for conducting a human clinical trial of the NIRS sensor.

7 PUBLICATIONS, ABSTRACTS, PRESENTATIONS

PODIUM / POSTER PRESENTATIONS:

Presented:

1. *"Studying the effects of mean arterial pressure changes on spinal cord hemodynamics in a large animal model of acute spinal cord injury, using a novel optical sensor." Presentation#: 568.22.*
   Neuroscinece 2018, San Diego, November 6th, 2018

2. *"Changes of mean arterial pressure affect spinal cord oxygenation as monitored by an implantable near-infrared spectroscopy sensor in an animal model of acute spinal cord injury."*
   SPIE Photonics West Congress, San Francisco, USA, February 2nd, 2019.

3. *"Optical Monitoring of Spinal Cord Oxygenation in Acute SCI."*
   2019 ICORD Research Day, Vancouver, Canada, Mar 2019.

4. *"Optical Monitoring of Spinal Cord Oxygenation in Acute SCI."*
   Canadian Spinal Cord Injury Research Biannual Meeting; Mar 2019, Vancouver, (Winner of the Best Poster Award).

5. *"Optical Monitoring of Spinal Cord Hemodynamics."*
   Peter Wall Institute for Advanced Studies Theme Development Workshop: Biomedical Imaging and Artificial Intelligence, Apr 2019.

6. *"Optical Monitoring of Spinal Cord Hemodynamics."*
   9th Annual ICORD Trainee Symposium; May 2019, Vancouver, BC.

7. *"Optical Monitoring of Spinal Cord Hemodynamics."*
   10th Annual GF Strong Rehabilitation Research Day; May 2019, Vancouver, BC. (Winner of the Best Poster Award).

8. *"Optical Monitoring of Spinal Cord Hemodynamics."*
   2nd Annual Symposium of UBC School of Biomedical Engineering; June 2019, Vancouver, Canada.

9. *"Continuous optical monitoring of spinal cord hemodynamics during the first 7 days post-injury in a porcine model of acute spinal cord injury."*
   Annual Meeting of the Society for Neuroscience, Chicago, USA, October 2019.

PUBLICATIONS:

Published:

1. Tahereh Rashnavadi, Andrew Macnab, Amanda Cheung, Brian K. Kwon, Babak Shadgan. Monitoring Spinal Cord Hemodynamics and Tissue Oxygenation: A Review of the Literature with Special Focus on the Near-Infrared Spectroscopy Technique. Journal of Spinal Cord (Nature), 2019.
   https://www.nature.com/articles/s41393-019-0304-2.

2. Shadgan B, Macnab A, Fong A, Manouchehri N, So K, Shortt K, Streijger F, Cripton P, Sayre E, Guy D, Pagano R, Kim K, Kwon B.K., Optical Assessment of Spinal Cord Tissue Oxygenation Using a Miniaturized Near Infrared Spectroscopy Sensor. Journal of Neurotrauma, 2019 May 2. doi: 10.1089/neu.2018.6208.
   https://www.ncbi.nlm.nih.gov/pubmed/31044642

In progress:

- *"Continuous monitoring of spinal cord tissue oxygenation and hemodynamics using a miniaturized near infrared spectroscopy sensor".* Babak Shadgan, Amanda Cheung, Lorna Tu, Andrew Macnab, Neda Manouchehri, Kitty So, Femke Streijger, Peter A. Cripton, Eric C. Sayre, Brian K. Kwon.
  Prepared for submission to J Neurotrauma in December 2019.

8 INVENTIONS, PATENTS AND LICENSES

To explore the potential intellectual property that might result from this project we approached the UBC University-Industry Liaison Office (UILO) and have filed an Invention Disclosure & Assignment application (file number of 17-037) in the Q1 period. Our invention disclosure was assessed by the UILO. The assessment included prior art searchers using the Thomson Innovation database as well as PubMed and Google Scholar for relevant publications. The prior art search identified a number of NIRS-based methods for monitoring spinal cord. However, none of them were similar to our design and capability of integrated real-time monitoring of spinal cord hemodynamics, oxygenation, and Intraparenchymal pressure. The UILO has determined that our system design is patentable. A provisional patent entitled "METHODS AND APPARATUS FOR NEAR INFRARED SPECTROSCOPY" was filed on September 6, 2019.

9 REPORTABLE OUTCOMES

Nothing to report

10 OTHER ACHIEVEMENTS

Nothing to report

11 REFERENCES

1. Streijger, F., So, K., Manouchehri, N., Tigchelaar, S., Lee, J.H.T., Okon, E.B., Shortt, K., Kim, S.E., McInnes, K., Cripton, P., Kwon, B.K. (2017). Changes in Pressure, Hemodynamics, and Metabolism within the Spinal Cord during the First 7 Days after Injury Using a Porcine Model. J Neurotrauma 34, 3336-3350.
2. Pollard, V., Prough, D.S., DeMelo, A.E., Deyo, D.J., Uchida, T., Stoddart, H.F. (1996). Validation in volunteers of a near-infrared spectroscope for monitoring brain oxygenation in vivo. Anesth. Analg. 82, 269-277.
3. Shadgan, B., Macnab, A., Fong, A., Manouchehri, N., So, K., Shortt, K., Streijger, F., Cripton, P., Sayre, E., Guy, D., Pagano, R., Kim, K., Kwon, B.K., (2019) Optical Assessment of Spinal

Cord Tissue Oxygenation Using a Miniaturized Near Infrared Spectroscopy Sensor. Journal of Neurotrauma. doi: 10.1089/neu.2018.6208.

12 APPENDICES

Shadgan B, Macnab A, Fong A, Manouchehri N, So K, Shortt K, Streijger F, Cripton P, Sayre E, Guy D, Pagano R, Kim K, Kwon B.K., Optical Assessment of Spinal Cord Tissue Oxygenation Using a Miniaturized Near Infrared Spectroscopy Sensor. Journal of Neurotrauma, 2019 May 2. doi: 10.1089/neu.2018.6208.

FINANCIAL HEALTH

Date Range: From 1-SEP-2018 to 31-AUG-2019			
	Actual Expenditures	Commitments	Projected Actuals
	YTD	YTD	
Funding/Revenues			
Carry Forward Allocation	609,551.63	0.00	609,551.63
Expense Funding Allocation	851,300.68	0.00	851,300.68
Total Funding/Revenues	1,460,852.31	0.00	1,460,852.31
Expenses			
Salaries			
Student Salaries	34,843.46	4,110.00	38,953.46
Staff Salaries	152,021.81	21,747.00	173,768.81
Sessional Salaries	27,400.94	0.00	27,400.94
Total Salaries	214,266.21	25,857.00	240,123.21
Benefits	31,957.90	4,120.00	36,077.90
Supplies & Sundries	570,971.92	24,660.76	595,632.68
Capital Expenditures			
Equipment	57,919.35	41,694.02	99,613.37
Total Capital Expenditures	57,919.35	41,694.02	99,613.37
Travel	3,840.54		
Total Expenses	878,955.92	96,331.78	975,287.70
Revenue Less Expenses	581,896.39	-96,331.78	485,564.61