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RESEARCH ARTICLE

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Percutaneously introduced wireless intramuscular near-infrared spectroscopy device detects muscle oxygenation changes in porcine model of lower extremity compartment syndrome

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Abstract

Serial examination and direct measurement of intracompartmental pressure (ICP) are suboptimal strategies for the detection of acute compartment syndrome (CS) because they are operator-dependent and yield information that only indirectly reflects intracompartmental muscle perfusion. As a result, instances of unnecessary fasciotomy and unrecognized CS are relatively common. Recently, near-infrared spectroscopy (NIRS)-based systems for compartment monitoring have generated interest as an adjunct tool. Under ideal conditions, NIRS directly measures the oxygenation of intracompartmental muscle (StO_2), thereby obviating the challenges of interpreting equivocal clinical examination or ICP data. Despite these potential advantages, existing NIRS sensors are plagued by technical difficulties that limit clinical utility. Most of these limitations relate to

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their transcutaneous design that makes them susceptible to both interference from intervening skin/subcutaneous tissue, underlying hematoma, and instability of the skin-sensor interface. Here, we present a flexible, wireless, Bluetooth-enabled, percutaneously introducible intramuscular NIRS device that directly and continuously measures the StO_2 of intracompartmental muscle. Proof of concept for this device is demonstrated in a swine lower extremity balloon compression model of acute CS, wherein we simultaneously track muscle oxygenation, ICP, and compartment perfusion pressure (PP). The observed StO_2 decreased with increasing ICP and decreasing PP and then recovered following pressure reduction. The mean change in StO_2 as the PP was decreased from baseline to 30 mmHg was −7.6%. The mean difference between baseline and nadir StO_2 was −17.4%. Cross-correlations (absolute value) describing the correspondence between StO_2 and ICP were >0.73. This novel intramuscular NIRS device identifies decreased muscle perfusion in the setting of evolving CS.

KEYWORDS

compartment syndrome, intramuscular, near-infrared spectroscopy, perfusion monitoring

1 | INTRODUCTION

Acute compartment syndrome (CS) is caused by swelling and increased pressure within a closed muscle compartment. This increased intracompartmental pressure (ICP) results in a decreased perfusion pressure (PP) that can impair circulation. In the absence of prompt diagnosis and treatment, this process can cause muscle necrosis, contracture, or even necessitate amputation.[1–3] CS most commonly results from skeletal trauma, but it can also be seen with ischemia/reperfusion or burn injuries. The treatment for CS is surgical decompressive fasciotomy, wherein the fascia enclosing the muscle compartment is incised to allow expansion of the muscle and reduce ICP. Failure to release the fascia within a few hours of CS development results in permanent damage; however, it is challenging to recognize and diagnose CS in a timely fashion.

A diagnosis of CS is made primarily based upon a physical examination which may demonstrate pain out of proportion to the injury, pain upon passive stretch of muscles in the affected compartment, swelling, sensory deficit, and motor deficit.[4] However, accurate and timely clinical evaluation of the patient may be unobtainable if the patient is anesthetized, sedated, distracted, or intoxicated.[5] Direct ICP measurement is an adjunctive tool which has been used to assist in making the diagnosis of CS. This is accomplished by obtaining continuous or intermittent compartment access with a pressure transducer catheter or needle.[6–8]

As CS is defined by the presence or absence of intercompartmental issue ischemia, pressure is only an indirect indicator of CS pathophysiology. The ICP threshold indicating the need for fasciotomy is a matter of debate but is commonly approximated by a PP (diastolic pressure [DBP] – ICP) ≤ 30 mmHg.[9] However, using ICP to diagnose CS may not accurately reflect the presence of CS and recent studies have reported a high false-positive rate of diagnosis of CS based on ICP measurements.[10–12] This results not only in unnecessary fasciotomy but also necessitates secondary wound closure/reconstruction and results in extensive cutaneous scarring. For this reason, direct and more specific measures of intracompartmental tissue perfusion have attracted recent interest.

Near-infrared spectroscopy (NIRS) offers the continuous real-time capability to monitor tissue oxygenation and has been shown to aid in the diagnosis of CS.[8,13] In a swine model of lower extremity acute CS, transcutaneous NIRS devices detected significant changes in tissue oxygenation corresponding with CS and NIRS was found to be a superior predictor of neuromuscular dysfunction than compartment PP.[14–16] However when deployed clinically, the utility of transcutaneous NIRS is substantially limited by the effect of skin pigmentation, ambient light, fracture hematoma, and variable subcutaneous tissue thickness.[13,17–19] Additionally, there have been a number of technical difficulties during attempts to use NIRS for CS diagnosis in the clinical environment.[13,18]

To circumvent the limitations of current transcutaneous NIRS devices, it was our aim to bypass the skin and percutaneously deploy a NIRS device directly within the intracompartmental muscle tissue. Here, we introduce a flexible, lightweight, wireless, percutaneously introducible intramuscular NIRS device that measures the oxygen saturation (StO_2) of intracompartmental muscle continuously and in real time. Proof of concept for this device is demonstrated in a balloon compression model of acute CS in the lower extremities of swine. Our hypothesis was that the intramuscular NIRS device would detect changes in muscle StO_2 that correspond with the decreasing PP associated with progressive ICP elevation.

2 | METHODS

2.1 | Intramuscular wireless NIRS device

The intramuscular NIRS device introduced here is flexible, lightweight, and allows wireless measurement of deep tissue StO_2 (Figure 1). An mm-scale flexible substrate (polyimide; thickness, 75 μm; width, 4 mm) supports a collection of thin conductive metal traces (copper; thickness, 18 μm; width, 70 μm) that interconnect two light-emitting diodes (LED) at different wavelengths (Red: 640 nm, size: length, 550 μm; width, 350 μm; thickness, 200 μm, and IR: 940 nm, size length, 1 mm; width, 500 μm; thickness, 500 μm), and two silicon-based PIN photodiodes (PD) (length, 2 mm; width, 1.25 mm; thickness, 850 μm). A thin encapsulation layer (parylene-C, thickness, 14 μm) together with a soft layer of biocompatible silicone elastomer (Silbione RTV4420) conformally coated onto the probe prevents penetration of biofluids into the electronic components. The probe connects to a flexible Bluetooth-based electronic module via the Cu/PI/Cu traces. This system offers a high degree of bendability (minimum bending curvature, 2 mm) without affecting the optoelectronic performance. The distance between the LEDs and the nearest PD is 4 mm and the distance between the two PDs is 3 mm. This arrangement optimizes the signal-to-noise ratio and ensures sufficient light-tissue interactions. Figure 1C shows the layout and overall size and thin, flexible form factor of the intramuscular probe encapsulated with silicone elastomer.

The intramuscular NIRS device exploits Bluetooth Low Energy (BLE) technology for wireless control and monitoring. The microcontroller module (NRF52832; Nordic Semiconductor, Inc.) controls LEDs, processes and transmits the digital signals in the form of analog to digital convertor values converted from a transimpedance amplifier with photocurrent generated by the two PDs. During operation, the microcontroller drives the red and IR LEDs alternatively at a frequency of 50 Hz and 5% duty cycle, then acquires and transmits signals from two PDs. Data is wirelessly transmitted to a bedside smart device where it is displayed continuously and in real time. Complete information on the materials, design, development characterization, calibration, validation, and mathematics associated with this device will be reported separately, but two critical benchtop calibration and in-vivo validation experiments are described in Appendices A and B, respectively.

2.2 | Porcine anesthesia

Animal research was performed with the approval of the Institutional Animal Care and Use Committee at Washington University School of Medicine. This was performed as per U.S. Department of Agriculture Animal Welfare Regulations at an accredited facility. In this study, four swine were utilized in separate experiments. Anesthesia was induced with Telazol, ketamine, and xylazine followed by maintenance with isoflurane. At the end of the experiment, each animal was euthanized with pentobarbital.

2.2.1 | Compartment syndrome porcine model

The swine balloon model of acute CS has been shown to be a reproducible model of ischemic-reperfusion and consistently results in experimental acute CS.[14,20] CS was induced by inserting a balloon catheter (2.5 mm diameter, 40 mm length, #PDZ339; B-Braun Interventional Systems) between the anterior muscle compartment of the hind limb and the anterior face of the tibia, as previously described by Budsberg et al (Figure 2).[14] We found it easiest to guide this balloon into place by first accessing the pretibial space using a 19-f round surgical drain on a rigid metal trocar. The rigid trocar was

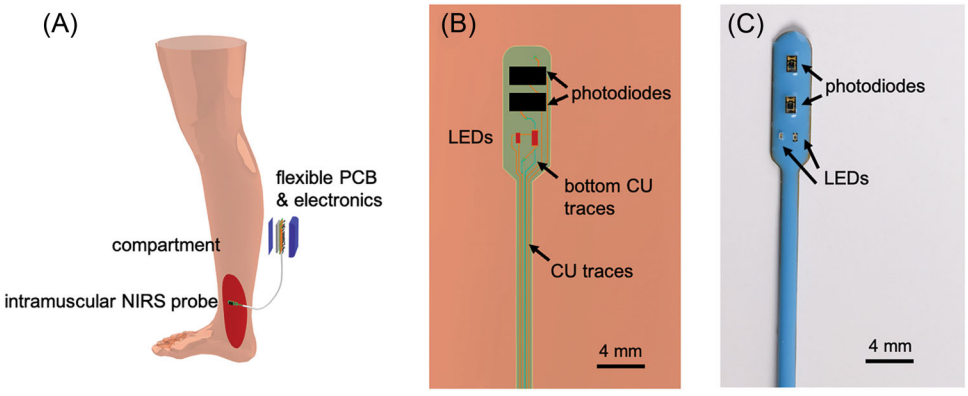

FIGURE 1 An implantable intramuscular probe for providing real-time analysis of near-infrared spectroscopy (NIRS) on compartment. (A) Schematic illustration of the use of an implanted intramuscular NIRS probe for monitoring tissue oxygenation inside the compartment. The system consists of an intramuscular NIRS probe, interfaced to a wireless Bluetooth-based data acquisition system for signal collection and transmission to a computer for real-time analysis and control. (B) Top-down view schematic illustration of the design of an intramuscular NIRS probe, which consists of a flexible printed circuit board, two light-emitting diodes (LEDs), and sensing components. (C) Image of an intramuscular NIRS probe, which highlights the sensing components and LEDs. The probe is fully encapsulated with transparent, biocompatible parylene and has a diameter of 4 mm.

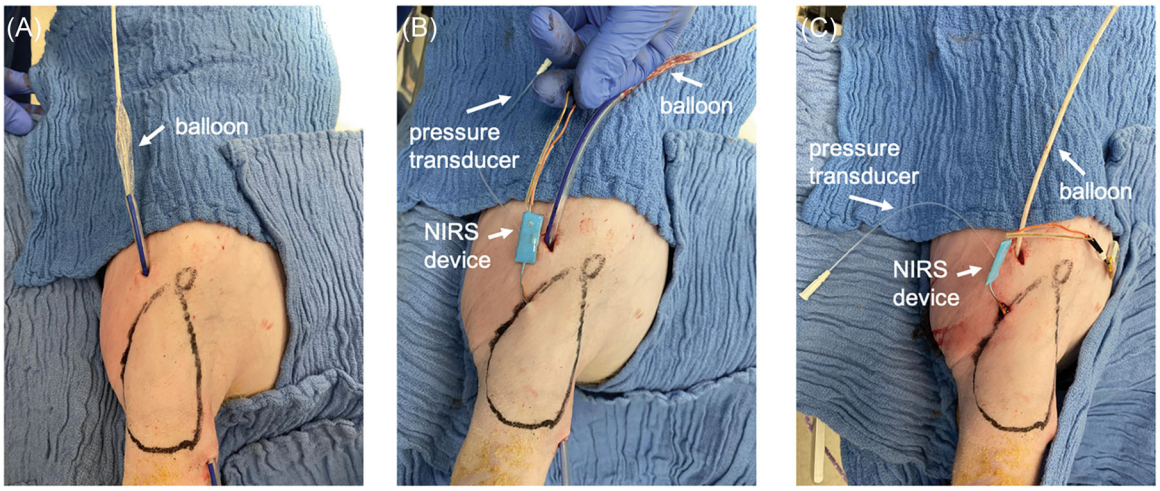

FIGURE 2 (A) Image of a balloon catheter inserted into a swine leg to create the acute compartment syndrome model. (B) The pressure transducer and intramuscular near-infrared spectroscopy (NIRS) device are then inserted into the compartment. (C) The final experimental set-up in the swine leg.

introduced through a stab incision at the proximal–lateral aspect of the compartment and then navigated underneath the compartment musculature, just anterior to the tibia, using tactile feedback. After the trocar exited just distal and medial to the limits of the compartment, the balloon catheter was intussuscepted into the trailing end of the drain and the trocar was pulled through, bringing the balloon catheter into the established pretibial position. The drain was then separated from the catheter and the balloon was pulled back into intracompartmental position.

2.3 | Experimental design

Following placement of the balloon catheter, a slit catheter was inserted into the anterior compartment musculature and attached to a pressure transducer.[6] The slit catheter was placed approximately 1 cm lateral to the tibia and the intramuscular NIRS probe was placed 5–10 cm distal to the slit catheter. To deploy the intramuscular NIRS probe, a 15 blade was used to make a stab incision (approximately 3 mm) and the probe was inserted into the anterior compartment musculature. The entire deployment procedure requires less than 30 s (Video S1). The wireless probe is connected to an external recording software via Bluetooth. To monitor blood pressure, a carotid arterial line was placed via cutdown and connected to a pressure transducer. During all experiments, the systolic and diastolic blood pressure (mmHg), mean arterial pressure (MAP; mmHg), balloon volume (ml), and ICP (mmHg) from the pressure transducer were recorded every minute. The intramuscular NIRS device continuously recorded muscle oxygenation. After device deployment, 10 min were allowed before the beginning of any experimentation to allow stabilization of a pressure and oxygenation baseline.

Experiment #1: Seven milliliters of saline were placed in the balloon to establish a baseline level of moderate intracompartmental hypertension. At 1-min intervals, 1 ml of saline was added, then 1 ml of saline was removed in a repeating pattern for three cycles. This pattern was repeated by adding 2 ml of saline and then removing 2 ml of saline every minute for three cycles. This experiment was conducted on one animal.

Experiment #2: One milliliter of saline was added to the balloon every minute until ICP approximated 30 mmHg above MAP.[14,20] The volume of the balloon was maintained for 10 min and then decreased by 1 ml every minute until all the saline was removed. The entire cycle was then repeated using the same limb. This experiment was conducted on four swine on separate days using different probes to demonstrate reproducibility.

2.4 | StO_2 calculations

Muscle oxygenation was calculated utilizing methods previously described by Bai et al.[21] Briefly, blood saturation was evaluated in the swine model using optical densities corresponding to red light (640 nm) and NIR light (940 nm). Optical density was calculated by converting averages of the respective signals collected by the data acquisition system and then using Lambert–Beer law to determine changes in hemoglobin and deoxyhemoglobin concentrations.[22] The oxygen saturation of the muscle was then calculated using:

$$StO_2 = \frac{HbO_{20} + \Delta HbO_2(i)}{HbO_{20} + \Delta HbO_2(i) + Hb_O + \Delta Hb(i)}.$$

2.5 | Statistical methods

Cross-correlation at zero lag was used to describe the correspondence between the wireless intramuscular NIRS device and pressure transducer. Central tendency is reported as mean ± standard deviation.

3 | RESULTS

3.1 | Experiment #1

The alternating addition, then removal of water from the balloon, resulted in incremental and proportional increases then decreases of ICP (Figure 3). The ICP changes corresponded with decreases and then increases in oxygen saturation. As the volume of the balloon increased/decreased, the magnitude of pressure and oxygen saturation changes also increased/decreased. This testing demonstrated that the intramuscular NIRS device was capable of assessing muscular perfusion changes resulting from ICP changes. The cross-correlation at zero lag describing the correspondence between the intramuscular NIRS device (StO_2) and pressure transducer (ICP) was −0.86.

3.2 | Experiment #2

ICP elevation/normalization was successfully achieved with balloon volume modulation, as confirmed by the pressure transducer data. Continuous StO_2 monitoring was accomplished in all cases without technical complications. Figure 4 shows two cycles of ICP elevation for each swine (four swine total). Muscle StO_2 measured by the intramuscular NIRS device decreased with increasing compartment pressure and then recovered following pressure release. Figure 5 highlights that intramuscular NIRS values decreased substantially with decreasing PP. Across the eight experimental trials (four swine, two cycles each), the mean difference between baseline and StO_2 when CS began during infusion (defined by PP = 30 mmHg) was −7.6 ± 4.6%. The mean difference between baseline and nadir StO_2 was −17.4 ± 6.5%. The cross-correlations at zero lag describing the correspondence between the intramuscular NIRS device (StO_2) and pressure transducer (ICP) were −0.73, −0.78, −0.89, and −0.84, respectively, for the four swine. The cross-correlations at zero lag describing the correspondence between the intramuscular NIRS device (StO_2) and PP were 0.75, 0.78, 0.89, and 0.73, respectively, for the four swine.

4 | DISCUSSION

The diagnosis of CS based on ICP has to date been unreliable with a false positive rate of 35% and specificity of 0.53.[10–12] Tissue damage ultimately occurs in CS when there is a decrease in local tissue perfusion and pressure within the compartment is only one of several variables which determine local blood flow and oxygen delivery.[1] Direct measurement of local tissue oxygen saturation with the use of NIRS has demonstrated promising diagnostic potential for CS in both animal and human studies.[8,13–16] However, current NIRS devices are limited to transcutaneous measurements which are limited by the interference of the skin and subcutaneous tissues and instability of the probe-skin interface and have to date been ineffective for CS monitoring in clinical settings.[13,18] In this study, we offer proof of concept for a novel, wireless, percutaneously deployed intramuscular NIRS probe which effectively measures local muscle oxygenation, and circumvents the challenges associated with transcutaneous spectroscopy.

Our hypothesis was that the intramuscular NIRS device would generate muscle oxygenation measurements that correspond with the decreased tissue perfusion associated with CS. This hypothesis was tested using a previously established porcine balloon compression model of acute CS. This model operates by percutaneous introduction of an empty balloon catheter just anterior to the tibia, underneath the muscles of the anterior compartment. Because the balloon is introduced percutaneously, the fascial which defined the compartment remains effectively intact. As the volume of the balloon is increased during the experiment, the pressure within the compartment (which is minimally distensible) increases. As this increased ICP climbs toward and above the PP, circulation within the intercompartmental muscle is compromised and muscle tissue hypoxia ensues.

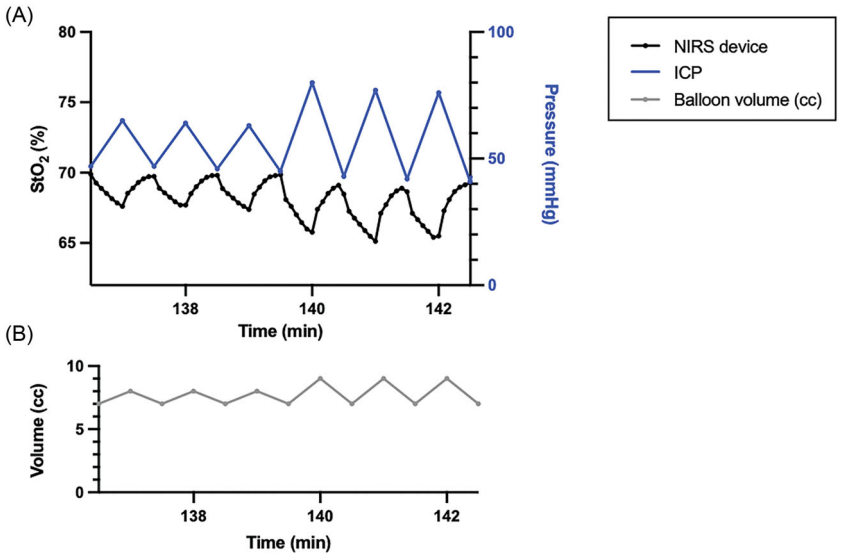

FIGURE 3 Simultaneous StO_2 oxygen saturation) traces from the intramuscular near-infrared spectroscopy (NIRS) and intracompartmental pressure (ICP) from the pressure transducer (A) during cycles of infusion and extraction of water from the balloon catheter (B; three cycles of 1 cc; three cycles of 2 cc; Animal 3).

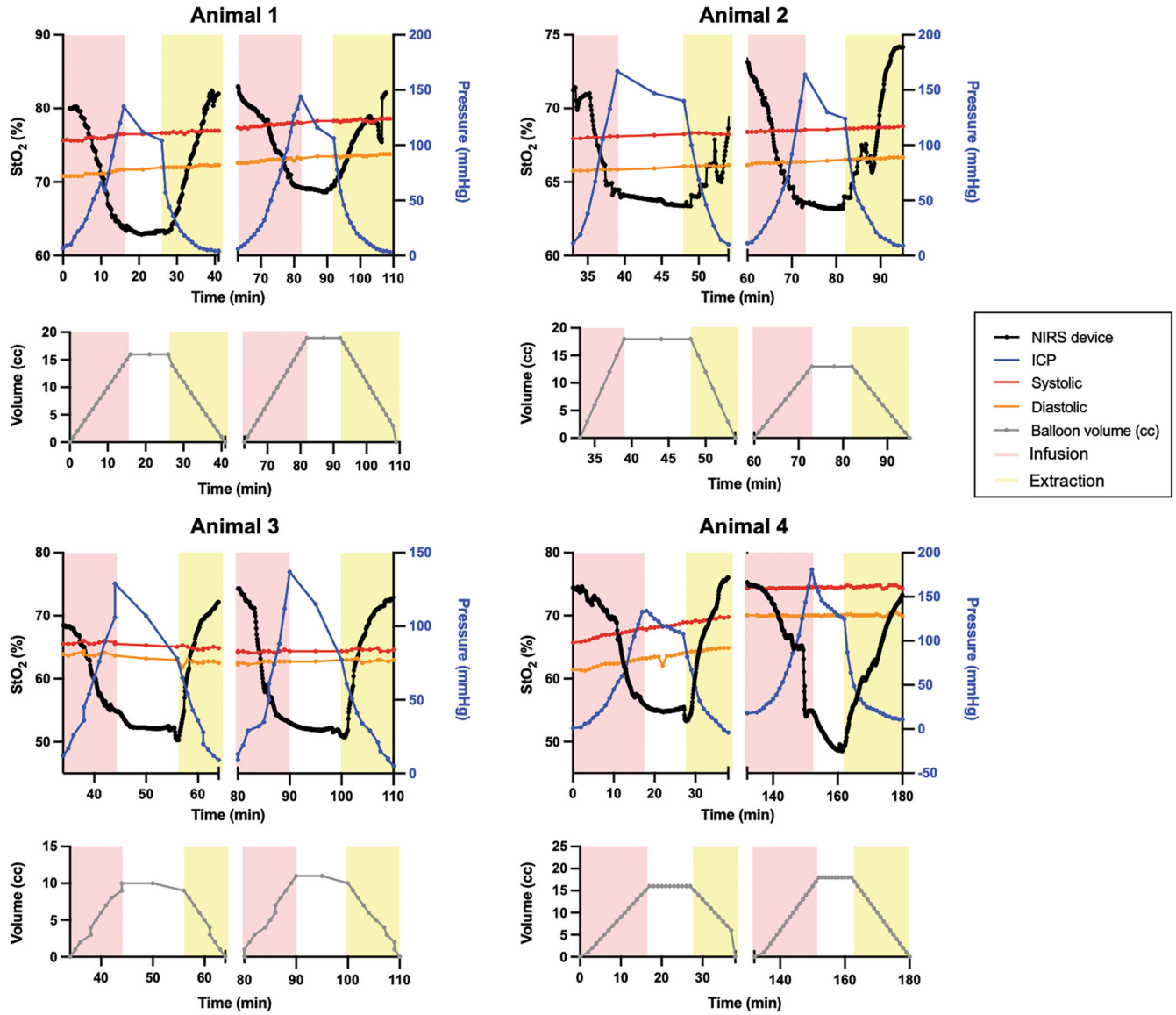

FIGURE 4 For four animals, simultaneous StO_2 (oxygen saturation) traces from the intramuscular near-infrared spectroscopy (NIRS) and intracompartmental pressure (ICP) from the pressure transducer during conditions of compartment syndrome: infusion, steady, extraction (two representative cycles per animal).

We found that muscle oxygenation measured by the intramuscular NIRS device decreased with increasing compartment pressure and then recovered following pressure reduction, consistent with previously published studies.[8,15,23] Additionally, intramuscular NIRS values decreased significantly with decreasing PP.[8,13] Cross-correlation values between the PP and muscle oxygenation were >0.73 indicating concordance between the two measurements. The mean differences between baseline StO_2 and StO_2 when CS began (−7.6%) as well as between baseline and nadir StO_2 (−17.4%) were both easily appreciable and potentially actionable in the context of the StO_2 baseline and observed range of values.

During the 10-min period of steady-state maximum balloon inflation, a small decrease in ICP was observed. This is attributed to stress relaxation of the compartment-defining tissues during this period of supraphysiologic ICP. A corresponding increase in StO_2 was not observed to correspond with this slight ICP reduction, which is expected because the StO_2 was maximally depressed even before the achievement of peak ICP. This period of artefactual asymmetry is one reason that the observed cross-correlations had an absolute value of less than 1.

The strategy of wireless percutaneous intramuscular NIRS monitoring has a conceptual advantage over ICP monitoring because it is a direct measure of muscle oxygenation, whereas ICP is only an indirect measure which estimates the true physiological state of the tissue. Transcutaneous NIRS shares this conceptual advantage but remains practically limited by many factors. Skin pigmentation, skin compromise, and/or bruising may alter the light scattering and absorbance profile of the tissue overlying the muscle compartment, thereby confounding the measurement. Furthermore, excessive skin and adipose tissue thickness or associated hematoma may shield the target muscle tissue from effective penetration of transcutaneously

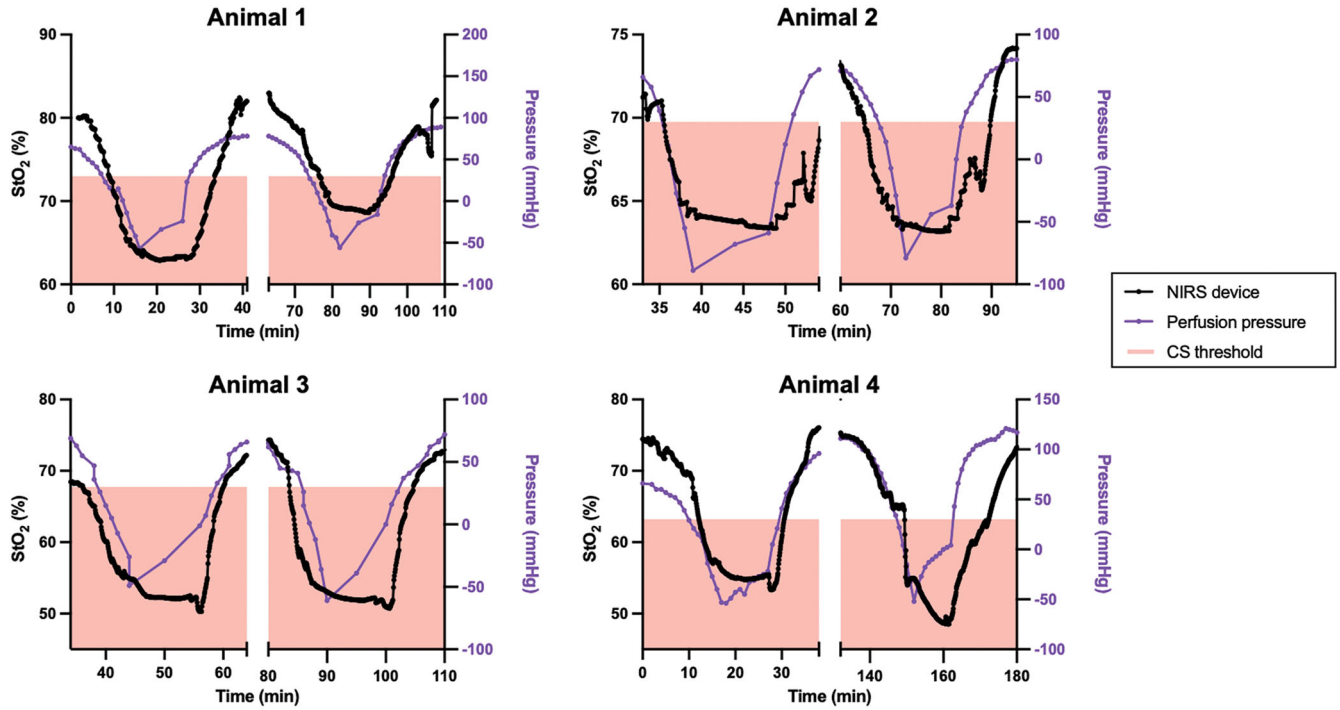

FIGURE 5 For four animals, simultaneous StO_2 traces from the intramuscular NIRS and PP (diastolic pressure – ICP) during conditions of compartment syndrome (2 representative cycles per animal). ICP, intracompartmental pressure; NIRS, near-infrared spectroscopy; PP, perfusion pressure.

applied light and possibly result in unintentional monitoring of extracompartmental tissue.[13,18] Finally, ambient light (and changes thereof) can influence the accuracy of cutaneous NIRS, and if uncontrolled, can lead to spurious changes with no clinical significance. All of these limitations are addressed by placing this novel and easily removable NIRS probe directly into the deep target tissue through a 3-mm percutaneous stab incision.

Beyond its ability to directly measure muscle perfusion within the target compartment, this novel device has several additional advantages over existing systems. Because it is wireless and BLE enabled, an external tether to a bedside monitor is unnecessary. Instead, BLE allows real-time StO_2 transmission to a bedside smartphone or tablet running an app-based display which also supports remote monitoring. Without an external tether, we expect that this device will be minimally susceptible to signal losses and noise caused by patient movement or wire traction. Furthermore, the source material and fabrication cost of a single-use intramuscular NIRS device could be as low as $15, with no capital cost beyond a simple nonproprietary smart device.

This study introduces proof of concept for a new diagnostic technology for CS; however, it has several limitations. Most importantly, this balloon compression model of CS produces elevated ICP in a nonphysiologic manner. This simple model is likely most applicable to a clinical situation of CS caused by pure ischemia followed by the rapid development of intercompartmental hematoma, wherein pressure elevation occurs purely due to a space-occupying lesion. This model is perhaps a less realistic approximation of CS caused by direct limb trauma or ischemia/reperfusion. Further experimentation will be necessary in a model that incorporates skeletal trauma and/or vascular insult to induce CS. Further development could be necessary to cope with instances of fracture hematoma or edema fluid engulfing the device. Furthermore, while we have shown that this device readily detects changes in muscle StO_2, we cannot yet offer a guideline as to what StO_2 value constitutes CS requiring surgical intervention. Conclusions about device sensitivity and/or specificity cannot be drawn without human clinical testing; however, in-human testing must be preceded by animal models demonstrating device efficacy. As such, this study is a necessary preclinical step in the development of this diagnostic technology.

To begin addressing this absence of a clinically relevant context for the measured StO_2 values, we have initiated a series of experiments investigating the changes in muscle histology and gene expression which occur in response to prolonged exposure to variable degrees of pressure-induced intracompartmental muscle deoxygenation. Beyond these avenues, future work will include expansion of this intramuscular platform to measure other muscular metabolic products that may be useful in tracking the development of CS.[24,25]

We report on a wireless, percutaneously introduced intramuscular NIRS device capable of continuous monitoring of muscle StO_2. This novel device successfully measured decreasing intercompartmental muscle oxygenation that corresponded with decreased PP in a live porcine balloon compression model of extremity CS. This study demonstrates the potential suitability for this intramuscular NIRS probe to monitor muscle perfusion in a limb that is developing CS. Additional evaluation and optimization of this technology in animal models are necessary before translating it to the clinical environment.

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CONFLICTS OF INTEREST

Dr. Pet, Dr. MacEwan, and Dr. Rogers have a patent "Novel Wireless Probes for Tissue Perfusion Monitoring" pending. Matthew MacEwan, MD, PhD, holds an equity position in Acera Surgical, Inc. and OsteoVantage, Inc., is a board member at Acera Surgical, Inc. and has received funding from ConductiveBio, Inc. Mitchell A. Pet, MD, has received research funding from Checkpoint Inc. Aside from the issues disclosed above, no authors have any actual or potential conflicts of interest related to the study matter.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX A: BENCHTOP CALIBRATION OF THE DEVICE

The novel device was calibrated against a blood gas analyzer (Abbott i-STAT System Critical Blood Analyzer) on the benchtop. This was accomplished using defibrinated horse blood (100 ml; Fisher Scientific) in a cylindrical reservoir (diameter, 4 cm; height, 30 cm), which was maintained at 37.0 ± 0.1°C. The NIRS probe was introduced into the center of the blood column and oxygenation was monitored continuously. Serial reduction in the ratio of oxygenated to deoxygenated hemoglobin was accomplished by adding small amounts of reducing agent (0.01–0.1 mg of sodium dithionite; MilliporeSigma) to convert a fraction of the oxygenated hemoglobin into deoxygenated hemoglobin. Serial blood gas analysis after every reduction event provided a gold standard measurement of oxygenated hemoglobin concentration, which was used for comparison and fine calibration of the novel device.

A plot of oxygenation over time representing the experiment is shown in Figure A1. In summary, addition of the reducing agent resulted in a stair-step pattern of blood deoxygenation which is visible in the NIRS and blood gas analysis data. All measurements were in agreement ±1.5% oxygenation.

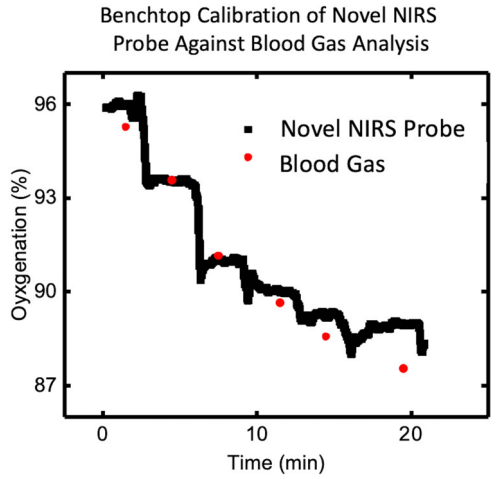

FIGURE A1 Bench top demonstration of accurate calibration of novel near-infrared spectroscopy (NIRS) sensor. The percentage of oxygenated hemoglobin in defibrinated horse blood was serially decreased using a reducing agent. Measurements taken using the novel probe were very similar to those made using gold standard blood gas analysis.

APPENDIX B: PORCINE MODEL VALIDATION OF THE DEVICE

After bringing a pig under general anesthesia, carotid arterial and femoral venous lines were placed via cutdown for the purposes of serial blood draws. A paramedian abdominal incision was used to expose the rectus abdominus muscle and the implantable probe was deployed into a small intramuscular pocket created with a 15 blade. Continuous StO_2 monitoring was initiated. The experiment commenced with the animal breathing 21% oxygen and baseline arterial and venous blood gas (ABG and VBG) measurements were recorded (Nova Biomedical StatPrime Blood Gas Analyzer). Progressive systemic hypoxia was slowly induced (as judged using an ear-mounted commercial pulse-oximetry probe) by decreasing the percentage of inhaled oxygen (and increasing the percentage of inhaled nitrogen). As the pig was progressively deoxygenated, numerous time-stamped ABG/VBG samples were drawn (41 in total) across the spectrum of SpO_2 (mostly between 50% and 100%).

Measured StO_2 from the intramuscular novel NIRS probe was compared to an estimated capillary blood oxygen saturation defined by a 70:30 weighted average of the oxygen saturation measured in the systemic venous and arterial systems. This type of experimental model has previously been used for valuation of an FDA approved cutaneous NIRS device. [26] Correspondence of the values measured by the novel probe and the blood gas analyzer was described using least absolute residuals regression and an R^2 statistic, which was 0.96 (Figure B1). This indicates a strong correlation between modalities of StO_2 measurement.

The finding that there is inexact correspondence between modalities (which would be indicated by a fitted line with a slope of 1 and an intercept of zero, with R^2 equal to 1) is most readily explained by the fact that the 70:30 mathematical mixture of arterial and venous blood gas oxygenation measurements[26] is an imperfect surrogate for the actual muscular blood oxygenation (which cannot reliably be directly measured using blood gas analysis).

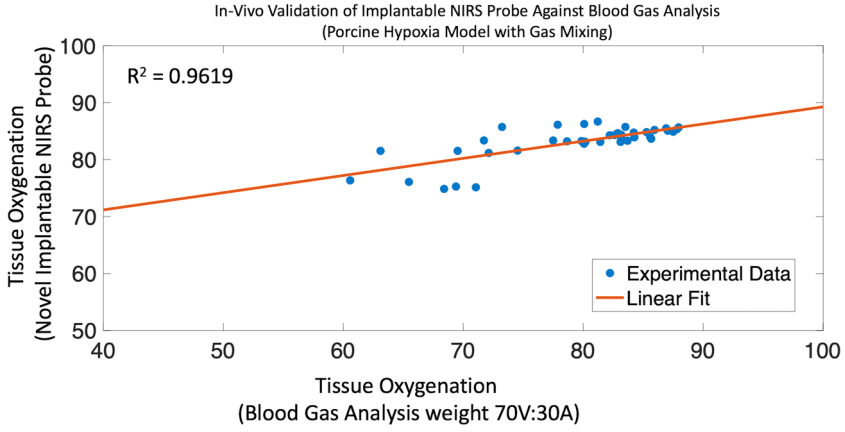

FIGURE B1 In vivo validation of implantable near-infrared spectroscopy (NIRS) probed against gold standard blood gas analysis in a porcine systemic hypoxia model. Variable levels of hypoxia were accomplished by modulating the mix of inhaled oxygen and nitrogen. This graph demonstrates simultaneous measurements of oxygenation determined by the novel probe and also by blood gas analysis.