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*Original Article*

# Rapid tissue viability evaluation using methemoglobin as a biomarker in burns

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**Abstract:** Burns are a frequent cause of traumatic injury, accounting for an average of 1,230 visits to the emergency department every day in the United States. While many of these injuries will heal spontaneously, nearly 1 in 10 are severe enough to require hospitalization or transfer to a specialized burn center. The early surgical management of a severe burn is critical to patient outcome, but few tools exist for triaging viable and non-viable tissue at early time-points post-injury. Without a validated outcome measure, even experienced burn surgeons diagnose tissue viability with an accuracy of only 50-70%, with significant consequences for patient morbidity, mortality and cost to the healthcare system. In this work, we have developed a non-invasive device that uses near-infrared spectroscopy to rapidly assess traumatic burns at the bedside. We report that near-infrared spectroscopy can detect methemoglobin non-invasively, and that this molecule increases in burned tissue immediately following injury in both a porcine model and in humans. Methemoglobin levels are highest in non-viable tissue, and correlate with tissue viability as early as 24 hours post-burn. Methemoglobin is the first reported objective outcome measure for use in the management of traumatic burn injury.

**Keywords:** Burns, spectroscopy, methemoglobin

## Introduction

Emergency departments in the United States treat approximately 890 adult patients and 340 children per day who are suffering from burns [1, 2]. Approximately 1 in 10 of these patients is burned severely enough to warrant hospitalization or transfer to a specialized burn center. Burn triage is usually done in a non-specialized emergency department setting based on 1) total body surface area (%TBSA) of the burn using an estimation technique such as the Wallace Rule-of-9s, and 2) an estimate of burn depth. However, non-specialized personnel overestimate %TBSA in half of all cases and have an accuracy rate of only 60% when assessing burn depth [3]. While acute burn care has improved over the past several decades such that patients can survive with burns to 100% of their bodies [4], delays in the management of patients with difficult-to-diagnose deep partial-thickness injuries can result in extensive scarring and contractures that restrict movement, cause pain, and lower quality of life. Inaccurate determination of burn depth can delay surgery (for those that need it), or lead to unnecessary procedures (for those that do not), and has a significantly negative impact on patient morbidity and mortality. The acute care of these wounds is essential to patient outcomes, but no objective outcome measures exist to differentiate viable from non-viable tissue and classify burns accurately. Even experienced burn clinicians make an incorrect diagnosis 30-40% of the time [5-8].

Several technologies exist to aid in the characterization of burn depth, with limited clinical success (thermography, ultrasonography, nuclear magnetic resonance, laser Doppler, confocal microscopy [9]). Histopathology remains the gold standard, but is invasive for the patient and impractical for the acute care physician, as results often take 4-5 days. A non-invasive, quantitative and real-time measure of burn depth and tissue viability has the potential to

make a significant impact on patient outcomes in the management of burn injury, particularly in the acute care setting.

Our group has focused on using non-invasive near-infrared (NIR) spectroscopy to measure oxygenation, perfusion and edema in burn wounds to assess viability [10-12]. NIR spectroscopy can penetrate a variety of tissues, and is able to assess perfusion using reflection rather than direct transmission between an emitter and receiver pair [13]. Given its contactless and portable nature, it is a technique well suited to the real-time measurement of burn depth. We hypothesized that NIR spectroscopy would be able to differentiate viable from non-viable burn tissue in real time. To test this hypothesis, we characterized burns of varying depth in both a well-established porcine model and in human using NIR imaging. Specifically, our aim was to identify physiological markers that would aid in clinical triage of burn injury in the porcine model, and validating our results in humans. We report the successful identification of a novel marker of tissue viability in both our porcine burn model and its validation in human burn patients using non-invasive and real time NIR imaging. We have used a computer science-based approach to demonstrate the diagnostic accuracy of this marker using artificial neural networks. This work has the potential to revolutionize the acute care and surgical management of burn wounds and has application to the assessment of tissue viability in other areas of wound care including surgical flap viability, traumatic wounds, and chronic wound management.

## Materials and methods

### *Pre-experiment*

Ethics approval was obtained from the National Research Council of Canada's Animal Care Committee. Adult Yorkshire swine (30-45 kg) were acclimatized for 4 days prior to the start of the experiment. Animals were housed in individual cages with water and food ad libitum. The animals were fasted 12 hours, and pre-medicated with midazolam (0.3 mg/kg IM), ketamine 20 mg/kg IM, and atropine 0.02 mg/kg IM prior to anesthesia. Animals were induced by mask at 4-5% isoflurane in 2-3 liters oxygen per minute and intubated with a 7.0-7.5 French endotracheal tube. Isoflurane was delivered at 1.5-2.5% carried by 40-60% oxygen mixed with medical air, with a flow of 2.0-3.0 L/min, via the anesthetic cart (Excel 210, Ohmeda, Mississauga, ON) for the duration of the experiment. Heart rate and oxygen saturation were monitored via a pulse oximeter (PM-9000 Vet by Mindray and 5250 RGM by Ohmeda) positioned on the ear. Body temperature was maintained at 39.0°C ± 0.5°C with a heating blanket placed underneath the animal and monitored via a rectal temperature probe. Animals were placed in a sternal recumbency for the duration of the experiment.

### *Burn wounds*

A pre-fabricated template was used to mark ten (n=10) sites, each 3 cm in diameter, on the dorsal surface of a shaved pig with an indelible pen. Five burn sites were created by holding the brass rod (2 cm diameter) heated to 100°C at constant pressure of 5 pounds for 3, 12, 20, 30, 90 seconds. The control sites (n=5) were created using a brass rod kept at 37.5°C held in situ with constant pressure for 20, 30, 75, 90 and 120 seconds. Varying the time points for the control was important to show that the increasing the length of time of brass rod placement did not produce a crush injury to the tissue and affect the NIR measurements.

The 3 second burn site was treated with polysporin antibacterial ointment and the remaining four burns with silver sulfadiazine (Flamazine™, Smith and Nephew, UK). A custom dressing and swine jacket was placed over the top to keep the dressings clean and prevent the animal from injuring the wounds. Buprenophrine (0.1 mg/kg IV or IM) and carprophen (4.4 mg/kg IM) were given for post-operative pain. The anesthesia was reversed by turning off the Isoflurane. The swine recovered under a heating lamp and all vital signs were monitored before return to the animal housing facility. Pain control was maintained throughout the study by a daily dose of carprophen IM (4.4 mg/kg IM).

### *Near infrared point spectroscopy (NIR point) data collection*

A complete set of NIR spectroscopic measurements was acquired pre-burn, and immediately post burn under anesthesia. Subsequent NIR measurements were acquired at 12, 24, 36, 48

and 96 hours after injury. Medetomidine 0.1 mg/kg IV (Domitor®, Pfizer, London, ON) was utilized to sedate the swine for NIR measurements. The custom swine jacket burn quilt and dressings were removed. Any excess ointment or cream was removed gently with dry gauze as silver may interfere with the Near Infrared data collection. Once the NIR data were collected, the dressings were replaced and sedation reversed with atipamezole hydrochloride IV or IM 0.1 mg/kg (Antisedan®, Pfizer, London, ON).

Euthanasia was the endpoint of the study and the time of last NIR spectroscopic data collection. Biopsies were acquired from each site for histology to confirm the burn depth diagnosis and categorized into superficial partial thickness, deep partial thickness or full thickness burn wounds. Euthanasia time points included 12 hours (n=1), 24 hours (n=1), 36 hours (n=1), 48 hours (n=1), 72 hours (n=1) and 96 hours (n=11). One animal was euthanized at specific time points after burn injury to correlate the histology with the NIR findings. Once the experiment was completed, animals were euthanized by an intra-venous injection of potassium chloride (149 mg/ml, 1-10 ml per animal) or euthanyl (125 mg/kg IV) while under anesthesia.

*Data processing & statistical analysis*

The NIR point spectroscopy device design has been described previously [10]. Briefly, the device is comprised of a fiber optic probe that delivers and collects (detects) light from the tissue. Collected light is dispersed into a spectrum via an imaging spectragraph and a CCD (charge coupled device) records the spectrum over 500 to 1100 nm wavelength range (Sciencetech Inc., London, On, Canada). NIR point spectroscopy data were processed using a modified Beer Lambert relationship. The relative concentrations of methemoglobin were derived over a spectral range of 610-840 nm by a least squares estimate of the extinction coefficients to the measured spectrum. To account for tissue scatter variations in the measured spectrum a linear scatter term (mλ + offset) was added to the Beer-Lambert relationship. All computations were performed using MATLAB Version 2016B (The Mathworks Inc., South Natick, MA).

All statistical analysis was performed using SPSS v. 17 (Chicago, IL). An ANOVA was utilized to evaluate the specific differences between the burn wounds at each time point with statistical significance achieved with a $p$-value <0.05. The results are presented as mean MHB values as a change from the pre-burn measurement. Results are presented for superficial partial thickness (SPT), deep partial thickness injuries (DPT) and full thickness injuries. The DPT and FT burns were collapsed into the category of non-viable burn wounds and the SPT as viable burn wounds for statistical analysis.

*Artificial neural networks (ANN)*

These ANNs were trained using scaled conjugate gradient back propagation (MATLAB 2016B Mathworks, USA), and designed to differentiate between viable and non-viable tissue using histology (porcine model) or time-to-heal (human model) as truth. Samples were randomly selected and divided into three separate groups of data to train, evaluate and test the performance of the ANN. 70% of the data (from 148 burns) were used to train the network. Another 15% of the data (from 31 burns) were used to validate and assess network generalization. This validation data was not used to optimize the conjugate gradient minimization and thus did not have any effect on network learning. The remaining 15% of the data (from 32 burns) were used to test and assess the ANN performance. 12582 values representing reflected light intensity at spectral wavelength sampling points for both burned and control sites were used as input node values. 10 sigmoid hidden nodes were included to facilitate data processing and 2 output nodes, representing either viable or non-viable were created.

Individual ANNs were created to process data for 12, 24, 48, and 72 hours post burn injury. Receiver operator characteristic curves (ROC) were generated and a sensitivity and specificity were calculated based at the maximum Youden's Index from the ROC.

## Results

*Porcine burn model and NIR spectroscopy*

Finding an outcome measure for burn viability determination would be invaluable to acute care management teams, who currently make burn treatment decisions based on their best judgement alone. Towards that end, we chose

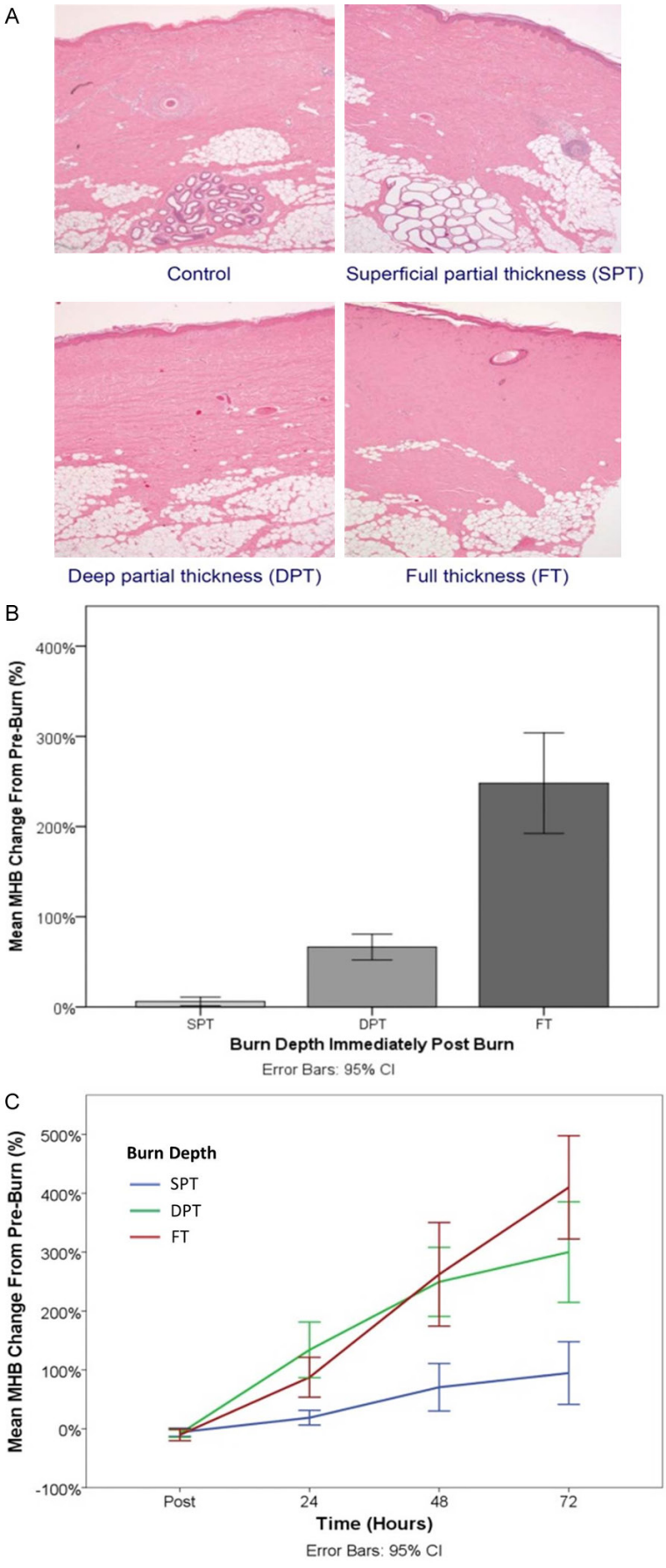


**Figure 1.** Near-infrared evaluation of methemoglobin accumulation in a porcine burn model. (A) Immediately post burn, punch biopsies were taken for histological classification of burns into superficial partial thickness (SPT), deep partial thickness (DPT) and Full thickness (FT) burns. Methemoglobin accumulation at burn sites was then assessed immediately post burn injury (B) and over time (C), and accumulated significantly over time in deep partial thickness and full thickness burns over a 72 hour period. Conversely, methemoglobin levels did not rise significantly above baseline in superficial partial thickness burns over the same time frame.

a validated porcine burn wound model for this study, as swine wound healing and histology is most similar to humans [14]. Punch biopsies and NIR spectral data were collected from burn sites immediately following burn injury and at 12-hour intervals up to 96 hours post-burn. Biopsies were used to classify burns according to depth (**Figure 1**), and spectra were analyzed for ratios of oxy- and deoxyhemoglobin to total hemoglobin, and for the marker of free radical accumulation, methemoglobin (MetHb). Over all time points, the ratio of deoxyhemoglobin to total hemoglobin was low in the superficial burns and increased as the depth of injury increased (results not shown). The opposite was true for oxyhemoglobin, as high levels were found in the superficial wounds and declined as burn depth increased. While these results were not unexpected [9, 15], we also noted an immediate, striking and significant accumulation of MetHb in deep partial thickness and full thickness burns over time (**Figure 1**). To better underst-

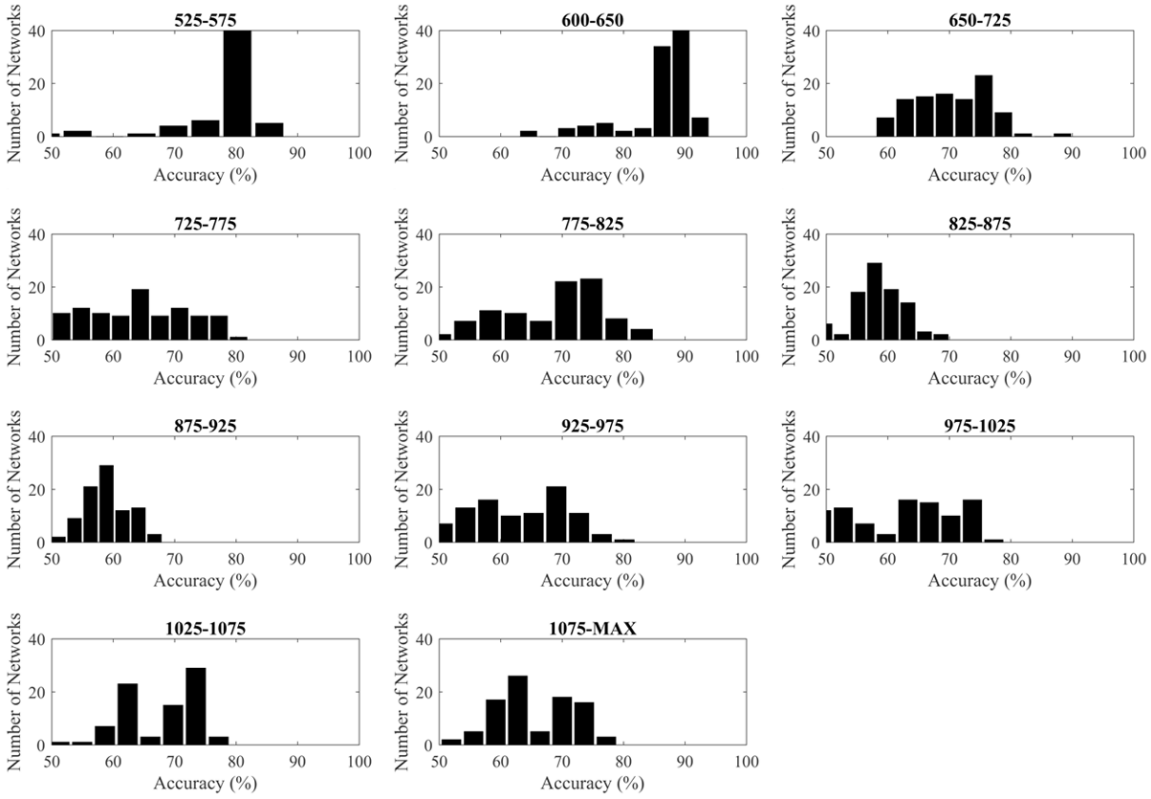


**Figure 2.** Artificial neural networks (ANN) were built *in silico* using NIR spectral data to determine if a computer could automatically differentiate viable from non-viable tissue using NIR in our porcine burn model. The majority of networks we created were able to classify burn depth with an accuracy greater than 91%, using histology and clinical wound healing data as truth. We next trained ANNs on spectra broken down into ranges of 50 nm throughout the spectrum, and found that specific ranges of the NIR spectra had more predictive accuracy than others. Particularly, the range between 600 and 650 nm (corresponding to MetHb) had a very high burn depth diagnostic accuracy, indicating that spectral data in this range is highly accurate for burn depth determination.

and the significance of these findings, we used computer modeling to correlate burn depth to spectral data.

*Methemoglobin can distinguish viable from non-viable tissue: computer modeling*

We constructed artificial neural networks (ANN) using full NIR spectral data and a two layer, feed-forward approach to determine if a computer could automatically differentiate viable from non-viable tissue. We created and trained 100 different ANNs to process the NIR spectral data for 12, 24, 48, and 72 hours post burn injury. Ninety-six out of the 100 networks we created were able to classify burn depth with an accuracy greater than 91%, using histology and clinical wound healing data as truth. This data confirms our hypothesis that NIR spectral analysis leads to robust classification of burn injury. In an attempt to elucidate the features of the spectra that drive network performance, data was broken down into ranges of 50 nm throughout the spectrum. 100 networks were again trained on these sub-spectra and classified (**Figure 2**). We found that specific ranges of the NIR spectra had more predictive accuracy than others. Particularly, the range between 600 and 650 nm had a very high burn depth diagnostic accuracy (81% of the networks were able to classify burn depth with an accuracy of >86% (**Figure 2**)). This region corresponds to the spectral detection range for MetHb. In contrast, regions with few spectral characteristics (725-775 nm, 875-975 nm) performed poorly (only 50% of networks had a classification accuracy >60%). From these results, we conclude that MetHb is a novel marker sufficient to differentiate viable from non-viable burned tissue non-invasively using NIR spectroscopy.

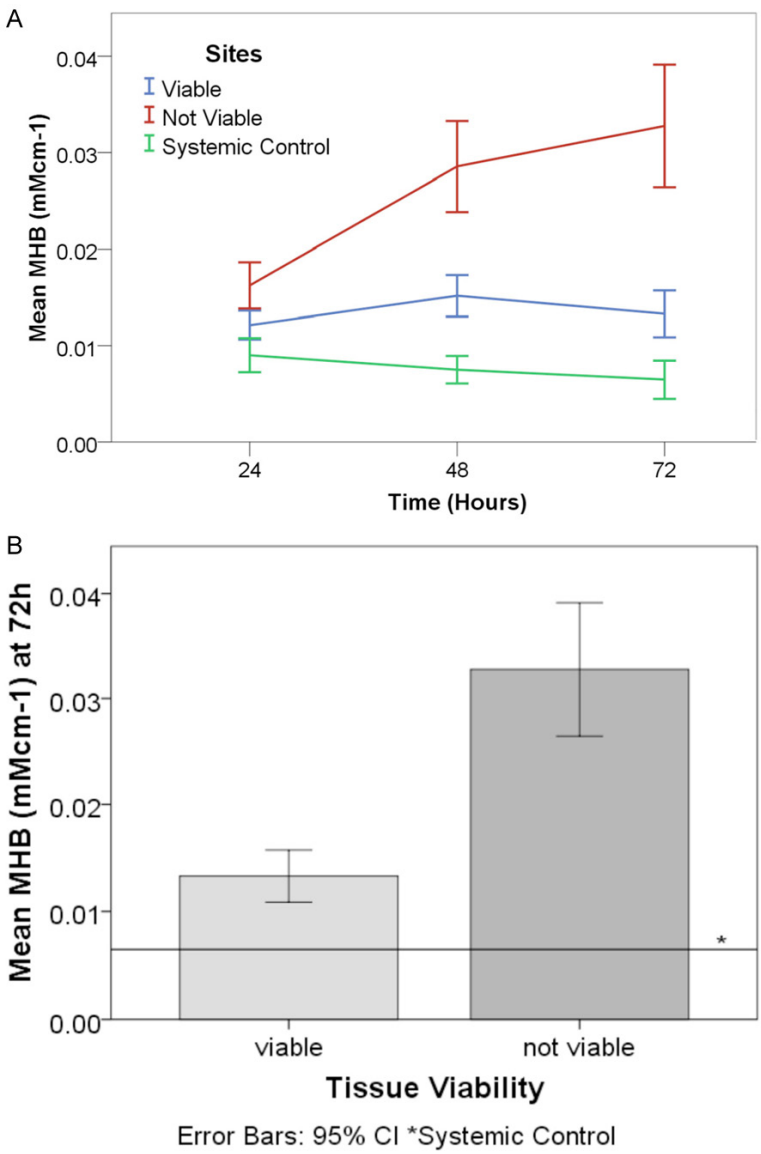


**Figure 3.** MetHb in human burns relative to control sites over time (A) and 72 hrs post-burn (B). Viable burns included superficial and superficial partial burns, and non-viable included deep partial thickness and full thickness burns. Results from our porcine burn model appear to translate well to human burns, and suggest that NIR spectroscopy offers a real time measure of burn viability.

*Methemoglobin is also diagnostic in human burn depth determination*

Seki et al (2014) found a strong correlation between $rSO_2$ values obtained via NIR spectroscopy and regional tissue blood flow assessed by Laser Doppler Imaging, suggesting that NIR spectroscopy is a promising tool for the assessment of burn severity in humans [13]. We set out to verify that the novel marker MetHb was sufficient to differentiate burn depth in humans by assessing 297 burn sites in 26 patients using NIR spectroscopy. At 24 hours post-injury, MetHb levels increased by 25% in non-viable injuries compared to viable wounds, and were 44% greater than that of controls (**Figure 3**). At 48 hours, MetHb levels in non-viable injuries increased to 46% above non-viable injury and 73% greater than control. By 72 hours post-burn, non-viable burns had MHB 80% greater than control (**Figure 3**), suggesting that MetHb is sufficient to differentiate tissue viability in humans. To confirm MetHb is a diagnostic marker in humans, we again constructed artificial neural networks using NIR data from the entire spectrum, and using only the 600-650 nm sub-spectral range. By 24 & 48 hr post-burn, the diagnostic accuracy of ANNs trained on data from the MetHb range was equivalent to the accuracy of an ANN trained over the entire spectra, confirming that MetHb levels alone can predict tissue viability (data not shown).

## Discussion

Clinical assessment is still considered to be the most reliable way to evaluate the viability of a burn wound, despite its 60-70% accuracy rate [8]. However, the misdiagnosis of burn depth has significant consequences for the patient and the healthcare system that range from the physical (infection, pain, mortality) to the financial (longer hospital stays, more complex medical care) [15]. The early and accurate triage of traumatic burns is imperative to improved outcomes, and an objective outcome measure coupled with a portable, non-invasive technology would make a tremendous impact in this area. NIR spectroscopy is well suited to this role, and is already used to assess cerebral and muscle perfusion

along with the determination of end points of resuscitation in trauma patients [16-19]. In this study, we have shown that NIR spectroscopy has the potential to change clinical practice in burn care by identifying a novel marker of burn wound viability. Importantly, we found that levels of the free-radical indicator molecule MetHB can clearly differentiate viable from non-viable burns at early time points in both a porcine model and in human burn patients. In fact, the diagnostic accuracy of our neural networks was the same if they were fed all spectra (including data for oxy- and deoxyhemoglobin) or just the range corresponding to MetHb. These results held true in a human burn population, and we therefore suggest that MetHb is novel outcome measure for tissue viability assessment.

The mechanism or combination of pathways responsible for MetHb production in burn tissue is unknown, but its presence in non-viable tissue fits with our current knowledge of burn wound pathophysiology. Methemoglobin is formed when the heme group is oxidized and may be secondary to the elevated levels of reactive oxygen and nitrogen species in damaged tissue [20]. ROS are generated in burns from neutrophils, the upregulation of xanthine oxidase and hydrogen peroxide [21-23]. Reactive nitrogen species (RNS) like nitric oxide, contribute to methemoglobin formation through interactions with deoxy- and oxy-hemoglobin [24]. In burn patients, nitric oxide and nitrate levels are elevated and can remain high for up to a week post injury (reviewed in [23]). We speculate that MetHb is therefore a surrogate marker for the free radical injury that is known to occur post-burn. Moreover, we suggest that there may be two roles for MetHb in thermally injured skin: 1) Anti-Oxidant Role: MetHb is a free radical scavenger as long as reduction mechanisms can keep pace with MetHb formation. 2) Pro-Oxidant Role: Increased production that exceeds MetHb removal results in lipid peroxidation of the endothelium and the red cell phospholipid membrane, which can cause irreversible damage to the tissue.

The toxicity of MetHb is also related to its ferryl intermediate. The ferryl ($Fe^{4+}$) intermediate, formed by the ferrous hemoglobin interaction with hydrogen peroxide and peroxynitrite, will abstract a hydrogen from unsaturated fatty acids, resulting in heme loss and toxicity to endothelial cells [25]. It can also further react with hydrogen peroxide to produce free iron and porphyrin degradation products [24]. Its formation also produces a reactive globin chain radical which affects the lipid cell membrane resulting in the release of free MetHb and the subsequent release of iron. Reactive globin chain radicals are toxic too as they cannot be reduced by the mechanisms that exist in the red blood cell. Ferrous iron scavenging of hydrogen peroxide or the superoxide anion generates not only MetHb but a highly reactive hydroxyl radical. MetHb is therefore a marker of free radical accumulation and tissue injury, and a cause of irreversible damage through membrane peroxidation effects.

NIR spectroscopy has been available clinically for some time, and has shown promise in the diagnosis of perfusion in trauma care [16-19]. However, NIR technology has not been widely adopted in burn care, largely due to a lack of properly controlled studies, the size of the spectroscopy apparatus required for measurement, and a focus in previous studies on measuring oxygenation and edema alone. This work showing that a narrow spectral range is sufficient to differentiate viable from non-viable burns will permit the development of smaller, more portable technology that can be more easily incorporated into trauma care.

While more work is needed to definitively establish MetHb's genesis and role in burned tissue, our results in both a well validated porcine burn model and in human burn patients confirm that MetHb is a marker of tissue viability. Trained burn surgeons have a success rate of 60-70% in the determination of burn depth, and we have achieved classification accuracies approaching 90% using an objective outcome measure and non-invasive imaging. The use of NIR spectroscopy to evaluate MetHb has the potential to change how burns are triaged and managed in an acute care setting, and has further application in the assessment of tissue viability in surgical flaps, traumatic wounds and in chronic wound care.

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## Disclosure of conflict of interest

None.

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## References

[1] Allareddy V, Lee MK, Rampa S, Nalliah R and Allareddy V. 1587: epidemiology of burn injuries in adults: a burning problem! Crit Care Med 2016; 44: 472.

[2] Allareddy V, Lee MK, Rampa S, Nalliah R and Allareddy V. 1538: epidemiology of burn injuries in children: estimates from the nationwide emergency department sample. Crit Care Med 2016; 44: 460.

[3] Thatcher JE, Squiers JJ, Kanick SC, King DR, Lu Y, Wang Y, Mohan R, Sellke EW and DiMaio JM. Imaging techniques for clinical burn assessment with a focus on multispectral imaging. Adv Wound Care (New Rochelle) 2016; 5: 360-378.

[4] Finnerty CC, Jeschke MG, Branski LK, Barret JP, Dziewulski P and Herndon DN. Hypertrophic scarring: the greatest unmet challenge after burn injury. Lancet 2016; 388: 1427-1436.

[5] Pape SA, Skouras CA and Byrne PO. An audit of the use of laser Doppler imaging (LDI) in the assessment of burns of intermediate depth. Burns 2001; 27: 233-239.

[6] Alsbjorn B, Micheels J and Sorensen B. Laser Doppler flowmetry measurements of superficial dermal, deep dermal and subdermal burns. Scand J Plast Reconstr Surg 1984; 18: 75-79.

[7] Droog EJ, Steenbergen W and Sjoberg F. Measurement of depth of burns by laser Doppler perfusion imaging. Burns 2001; 27: 561-568.

[8] Heimbach DM, Afromowitz MA, Engrav LH, Marvin JA and Perry B. Burn depth estimation--man or machine. J Trauma 1984; 24: 373-378.

[9] Chin MS, Babchenko O, Lujan-Hernandez J, Nobel L, Ignotz R and Lalikos JF. Hyperspectral imaging for burn depth assessment in an animal model. Plast Reconstr Surg Glob Open 2015; 3: e591.

[10] Cross KM, Leonardi L, Gomez M, Freisen JR, Levasseur MA, Schattka BJ, Sowa MG and Fish JS. Noninvasive measurement of edema in partial thickness burn wounds. J Burn Care Res 2009; 30: 807-817.

[11] Cross KM, Leonardi L, Payette JR, Gomez M, Levasseur MA, Schattka BJ, Sowa MG and Fish JS. Clinical utilization of near-infrared spectroscopy devices for burn depth assessment. Wound Repair Regen 2007; 15: 332-340.

[12] Sowa MG, Leonardi L, Payette JR, Fish JS and Mantsch HH. Near infrared spectroscopic assessment of hemodynamic changes in the early post-burn period. Burns 2001; 27: 241-249.

[13] Seki T, Fujioka M, Fukushima H, Matsumori H, Maegawa N, Norimoto K and Okuchi K. Regional tissue oxygen saturation measured by near-infrared spectroscopy to assess the depth of burn injuries. Int J Burns Trauma 2014; 4: 40-44.

[14] Sullivan TP, Eaglstein WH, Davis SC and Mertz P. The pig as a model for human wound healing. Wound Repair Regen 2001; 9: 66-76.

[15] Mazhar A, Saggese S, Pollins AC, Cardwell NL, Nanney L and Cuccia DJ. Noncontact imaging of burn depth and extent in a porcine model using spatial frequency domain imaging. J Biomed Opt 2014; 19: 086019.

[16] Cooper PG, Wilson GJ, Hardman DT, Kawaguchi O, Huang YF, Martinez-Coll A, Carrington RA, Puchert E, Crameri R, Horamand C and Hunyor SN. Blood oxygen desaturation heterogeneity during muscle contraction recorded by near infrared spectroscopy. Adv Exp Med Biol 1997; 428: 285-292.

[17] Cohn SM, Nathens AB, Moore FA, Rhee P, Puyana JC, Moore EE, Beilman GJ; StO2 in Trauma Patients Trial Investigators. Tissue oxygen saturation predicts the development of organ dysfunction during traumatic shock resuscitation. J Trauma 2007; 62: 44-54; discussion 54-45.

[18] Crookes BA, Cohn SM, Bloch S, Amortegui J, Manning R, Li P, Proctor MS, Hallal A, Blackbourne LH, Benjamin R, Soffer D, Habib F, Schulman CI, Duncan R and Proctor KG. Can near-infrared spectroscopy identify the severity of shock in trauma patients? J Trauma 2005; 58: 806-813; discussion 813-806.

[19] Shibata S, Noriyuki T, Ohdan H, Miyata Y, Yoshioka S, Asahara T and Dohi K. Simultaneous estimation of pulmonary edema and tissue oxygenation by near-infrared spectroscopy. Transplant Proc 1999; 31: 178-179.

[20] Horton JW. Free radicals and lipid peroxidation mediated injury in burn trauma: the role of an-

tioxidant therapy. Toxicology 2003; 189: 75-88.
[21] Mulligan MS, Till GO, Smith CW, Anderson DC, Miyasaka M, Tamatani T, Todd RF 3rd, Issekutz TB and Ward PA. Role of leukocyte adhesion molecules in lung and dermal vascular injury after thermal trauma of skin. Am J Pathol 1994; 144: 1008-1015.
[22] Till GO, Guilds LS, Mahrougui M, Friedl HP, Trentz O and Ward PA. Role of xanthine oxidase in thermal injury of skin. Am J Pathol 1989; 135: 195-202.
[23] Parihar A, Parihar MS, Milner S and Bhat S. Oxidative stress and anti-oxidative mobilization in burn injury. Burns 2008; 34: 6-17.
[24] Umbreit J. Methemoglobin--it's not just blue: a concise review. Am J Hematol 2006; 82: 134-144.
[25] McLeod LL and Alayash AI. Detection of a ferrylhemoglobin intermediate in an endothelial cell model after hypoxia-reoxygenation. Am J Physiol 1999; 277: H92-99.