Breath-Hold Paradigm to Assess Variations in Oxygen Flow in Diabetic Foot Ulcers Using a Noncontact Near-Infrared Optical Scanner

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Objective: Diabetic foot ulcers (DFUs) occur in almost 25% of all patients with diabetes in their lifetime, with oxygen being the key limiting factor in healing. Identifying regions of compromised oxygenated flow can help clinicians cater the wound treatment process, possibly reducing wound healing time. Herein, a handheld, noncontact near-infrared optical scanner (NIROS) was developed and used to measure temporal changes in hemoglobin concentrations in response to a breath-hold (BH) paradigm.

Approach: Noncontact imaging studies were carried out on DFU subjects and control subjects in response to a 20-s BH paradigm. Continuous-wave-based multiwavelength diffused reflective signals were acquired to generate effective oxy-hemoglobin, deoxy-hemoglobin, total hemoglobin, and oxygen saturation concentration maps using modified Beer-Lambert's law. Pearson's correlation analysis was carried out to determine variations in oxygen flow from hemoglobin concentration maps and the extent of variation observed in controls versus DFU subjects.

Results: Temporal changes in hemoglobin concentration maps were observed in controls and DFU subjects. However, the oxygen flow in response to BH varied within 10% in all controls but significantly varied between wound and background regions in subjects with DFUs.

Innovation: A method to assess variations in oxygen supply in and around DFUs was demonstrated using NIROS. This approach has potential to better cater DFU treatment process.

Conclusion: Changes in all hemoglobin parameters due to 20 s of BH was observed. Pearson's analysis indicates that oxy-hemoglobin, deoxy-hemoglobin, and oxygen saturation fluctuations are synchronous in controls. In DFUs, changes are asynchronous with blood flow between the wound region and background region being significantly different.

Keywords: diabetic foot ulcers, tissue oxygenation, breath-hold, Pearson's correlations, near-infrared optical imaging, hemoglobin concentrations

INTRODUCTION

DIABETIC FOOT ULCERATION occurs in almost 25% of patients with diabetes during their lifetime.¹ If left

untreated or poorly managed, diabetic foot ulcers (DFUs) can further lead to amputations. According to the Wound Healing Society, a 50%

reduction in wound size in 4 weeks is used to determine if the wound is healing²; however, even so approximately 50% of DFUs still do not heal by the 12th week.³ In general, the rate of wound healing is impacted by various factors, such as oxygenation, pressure, age, and desiccation (or dryness of the wound). During wound treatment and management in diabetes, healing is clinically assessed from visual changes to the wound in terms of its size, color, and extent of epithelization. Factors such as the extent of oxygenation or pressure to the DFU are not clinically monitored unless they manifest as a visual change to the wound. Physiological assessment of wounds during periodic wound treatment can aid in observing the underlying changes that typically manifest much later as visual changes (the gold-standard wound assessment approach).

Oxygen supply to DFUs is a key limiting factor for successful healing due to increased demand for reparative processes, such as cell proliferation, bacterial defense, angiogenesis, and collagen synthesis.⁴ Prognostic imaging of tissue oxygenation (TO) is essential⁵ to assess the effectiveness of the treatment approach in improving oxygen supply to wounds (and thus enhanced healing rates). Determining the extent of oxygenation to the wound is vital in assessing its potential to heal. Healing can be severely impaired when sufficient amount of oxygen is not provided or reaching the wound area. Prolonged hypoxia or low oxygen conditions prompt poor communication between tissue and cells and can vastly delay treatment. A gold-standard transcutaneous oxygen measurement (TCOM) approach measures partial pressures of oxygen at discrete point locations around the wound to determine if there is enough oxygen reaching the wound.^{6,7} Although TCOM is Food and Drug Administration-approved, the procedure is time consuming (by as much as 45 min), is a contact-based imaging approach,⁷ and provides only information around the wound at discrete points and not across the entire wound area.

TO in and around the wounds can be measured using various imaging tools, such as hyperspectral imaging,^{5,8,9} multispectral imaging,¹⁰ and near-infrared spectroscopy (NIRS).^{11,12} These imaging modalities use wavelengths in the visible and/or near-infrared (NIR) regions to obtain two-dimensional (2D) maps or spectroscopic point measurements of oxy-hemoglobin (HbO), deoxy-hemoglobin (HbR), total hemoglobin (HbT), and/or oxygen saturation (StO₂). The 2D distribution maps of these hemoglobin-based concentration

parameters (HbO, HbR, HbT, and StO₂) are quantitatively determined (as percentages) in and around the wound. Past studies have demonstrated that there is increased HbO in the wound compared with its surroundings at the inflammatory phase of wound healing, and as the wound heals, these concentrations reduce to that of the normal background.¹¹ These observations are possible from periodic imaging of wounds during their weekly or biweekly visits. Apart from physiological assessments performed longitudinally across weeks of treatment, there is a need to understand if any key factors are impacting healing during a given visit. In other words, variations in oxygenation (or oxygen flow) between the wound and surroundings during a given visit are essential. Herein, we developed an NIRS-based imaging approach that can determine the dynamic changes in oxygenation parameters and determine regions, if and where, oxygen flow is compromised in and around the wounds.

Recently, we have developed a handheld NIRS-based near-infrared optical scanner (NIROS) in our laboratory to perform noncontact imaging of wounds as a bedside imaging tool.^{8,12–14} The device has demonstrated the ability to longitudinally measure the hemoglobin concentration changes across the treatment period in DFUs¹² as well as venous leg ulcers.¹⁴ While our previous studies have focused on oxygenation changes across weeks of treatment, the current study focuses on discrete imaging at different time points in response to a breath-hold (BH) paradigm to assess the regions of poor blood flow (or differences in oxygen supply). Venous occlusion tests, which often use a sphygmomanometer to occlude the limb, are commonly used to induce changes in oxygenation.^{15,16} As DFUs are painful many a time and subjects may have vascular issues, a novel BH paradigm has been introduced for the first time to determine if it can detect changes in altered oxygenation flow and if these differences vary between controls and DFU subjects. While BH paradigm is commonly used for cerebral hypoxia studies,¹⁷ it has also been used to observe oxygenation changes in the finger using a pulse oximeter. A decrease in peripheral oxygenation saturation of the finger was observed in response to BH using a pulse oximeter.¹⁸ This infers that BH not only causes a change in the cerebral oxygenation but can also induce similar oxygenation changes from the finger (or skin's surface and subsurface). Hence, a BH-based paradigm has been applied in the current study involving imaging the feet of controls and DFU subjects.

CLINICAL PROBLEM ADDRESSED

The gold-standard approach to assess healing in DFUs is by visual inspection for a reduction in wound size and epithelization. A 50% reduction in wound size across 4 weeks represents a healing DFU. During weekly (or periodic) visit, it is vital for clinicians to assess that oxygen supply to the wound site is not compromised. There is no bedside-based noncontact imaging tool currently available to map the variations in oxygen supply in and around these wounds. Herein, we have developed a BH-based paradigm to quantify the extent of variations in oxygen supply. This is obtained from imaging the hemoglobin concentration changes using a near-infrared optical imaging approach. This approach has potential to provide complementary information of regions with poor oxygenation, which in turn can be used to cater and improve treatment and wound care management.

MATERIALS AND METHODS

Instrumentation

An in-house built continuous wave (CW), hand-held, noncontact NIROS (as shown in Fig. 1) was utilized to image subjects with DFUs and controls (subjects with no ulcers). The NIROS device utilizes four multiwavelength light-emitting diodes (LEDs) with an effective wavelength range from 650 to 950 nm to illuminate the region of interest with NIR light. For this study, each of the multiwavelength LEDs was optimized to operate at 729 and 799 nm wavelengths at an optical power of 7 mW (at the source end before illuminating the tissue surface area) during imaging studies. An LED driver was used to multiplex each source wavelength at 10 Hz and simultaneously illuminate multiple (here 4) LEDs for an increased area of illumination ($\sim 84 \text{ cm}^2$ when located 12.5 cm

from the imaging surface). Diffused reflectance signals from the imaged tissues are filtered through a longpass filter (LP 645, MidOpt) to remove ambient light and focused on an NIR-sensitive CMOS camera (IDS, Germany) through a focusing lens. A custom-developed Matlab-based graphical user interface synchronizes the multiplexing of the source's wavelength and acquisition of the respective wavelength's diffuse reflectance signal by the detector at each of the two wavelengths. A schematic of NIROS and its clinical setup are given in Fig. 1.

Subject recruitment

The current imaging study was carried out at Dr. Mohan's Diabetes Specialities Centre, Chennai, India, with institutional review board approvals from both institutions (Florida International University, Miami, Florida, and Madras Diabetes Research Foundation, India). Written consent was acquired from all participating subjects, along with Health Insurance Portability and Accountability Act authorization (only in DFU subjects) to acquire their diabetes-related medical records. For control subjects, only their (non-)diabetic status was obtained directly from the subject (no access to medical records). For this study, six males and six females between the ages of 21–71 years were recruited, of which eight subjects were DFUs and four subjects were controls (either non-diabetic or managed diabetic subjects without any ulcers). The DFU subjects were those who were being treated by the podiatric surgeon either as an inpatient or as an outpatient at the clinical site. Of the 12 subjects imaged, data from 2 subjects (1 DFU and 1 control) was removed from the study due to increased movement during the BH-based paradigm, thus causing errors in data analysis. One subject, case 10, had a DFU on each foot, leading to a total of 11

Figure 1. (A) Schematic of the NIROS utilized for noncontact imaging of DFUs. Demarcated in the dashed box are all components of the NIROS device. (B) Clinical setup of NIROS during DFU imaging, stabilized in position using an articulating arm. CMOS, complementary metal-oxide semiconductor; DFU, diabetic foot ulcer; LED, light-emitting diode; NIROS, near-infrared optical scanner. Color images are available online.

cases across 10 subjects. Details of the final 10 subjects' diabetes history and wound location are provided in Table 1. Although the DFU subjects had various comorbidities (as described in Table 1), the subjects were not further subgrouped in this preliminary study.

The mean age in the control and DFU groups was 36.3 and 60.1 years, respectively. Additionally, all controls were females, whereas DFU subjects were primarily males (five males and two females). While there is a clear difference between both groups in terms of gender and age, these differences may not be statistically significant due to the small sample size.

Data acquisition

The outpatient DFU subjects were seated by a nurse onto an examination chair and had their foot elevated (if the ulcer was on the sole of the foot) or the foot flat on the floor (if the ulcer was on the dorsal of the foot). All inpatients (cases 8 and 9) had their foot on the bed and were either seated or in the supine position during imaging studies. Based on the location of the ulcer on the foot, the NIROS device's position was adjusted using an articulating arm (as shown in Fig. 1). Before imaging, the wounds were cleaned and debrided, except in cases 4, 6, and 7 (as decided by the podiatric surgeon). In the case of controls (without wounds), the dorsal or sole of the foot (similar to the location of wounds in DFU cases) was imaged by seating them in their comfortable position. The noncontact imaging study consisted of a 90-s paradigm with 20 s of BH, and data were acquired discretely at five time points (0, 10, 30, 60, and 90 s), as illustrated in Fig. 2. Three repeated measurements of diffused reflected data were acquired for each NIR wavelength at each time point along with three repetitions of a calibration image (at each wavelength) acquired using a uniformly diffused sheet.^{9,12} The field of view consisted of the DFU and the sur-

rounding tissue. The distance between NIROS and the imaged tissue was not kept constant in each subject but was varied based on the location of the wound and/or its size. The ability of NIROS to provide similar optical contrast changes, independent of the device location has been demonstrated in the past.¹⁴

Image analysis

TO analysis. Modified Beer–Lambert's law, an empirical description of optical attenuation in highly scattering mediums,^{9,19} was utilized to calculate the relative changes in oxy-hemoglobin (ΔHbO) and deoxy-hemoglobin (ΔHbR) from diffused reflective NIR images acquired at 729 and 799 nm. The change in optical density, $\Delta OD_{t=n}(x, y, \lambda_i)$, at each wavelength was calculated for each spatial coordinate (x, y) at each wavelength λ_i and for each time point $(t=n)$, accounting for the dark noise $[I_D(x, y)]$ and calibration factor, $I_{Cal}(x, y, \lambda_i)$.

$$\Delta OD_{t=n}(x, y, \lambda_i) = -\log \frac{I_{t=n}(\lambda_i, x, y) - I_D(x, y)}{I_{0,t=n}(\lambda_i, x, y) - I_D(x, y)}. \quad (1)$$

Using the extinction coefficient for HbO, $\epsilon_{\text{HbO}}(\lambda_i)$, and HbR, $\epsilon_{\text{HbR}}(\lambda_i)$, the relative changes in HbO and HbR were in turn calculated from the OD using Equations (2) and (3), respectively^{9,19}:

$$\Delta[\text{HbO}](x, y) = \frac{\epsilon_{\text{HbR}}^{\lambda_1} \Delta OD^{\lambda_2} - \epsilon_{\text{HbR}}^{\lambda_2} \Delta OD^{\lambda_1}}{(\epsilon_{\text{HbR}}^{\lambda_1} \epsilon_{\text{HbO}}^{\lambda_2} - \epsilon_{\text{HbR}}^{\lambda_2} \epsilon_{\text{HbO}}^{\lambda_1}) LB}, \quad (2)$$

$$\Delta[\text{HbR}](x, y) = \frac{\epsilon_{\text{HbO}}^{\lambda_2} \Delta OD^{\lambda_1} - \epsilon_{\text{HbO}}^{\lambda_1} \Delta OD^{\lambda_2}}{(\epsilon_{\text{HbR}}^{\lambda_1} \epsilon_{\text{HbO}}^{\lambda_2} - \epsilon_{\text{HbR}}^{\lambda_2} \epsilon_{\text{HbO}}^{\lambda_1}) LB}, \quad (3)$$

where L is the mean free path and B is the path length factor. In this study, ΔHbO and ΔHbR are compared across the time points within a given

Table 1. Medical records of all recruited subjects including subject type, age, gender, medical history, and wound location

Cases	Subject Type	History (Diabetes/DFU)	Wound Location
1	Control (21/F)	Nondiabetic	—
2	Control (40/F)	Diabetic (managed), non-neuropathic	—
3	Control (48/F)	Diabetic (managed), non-neuropathic	—
4	DFU (55/M)	Outpatient, diabetic, severely neuropathic	Sole
5	DFU (60/M)	Outpatient, diabetic, non-neuropathic	Dorsal
6	DFU (60/F)	Outpatient, diabetic, non-neuropathic	Sole
7	DFU (48/F)	Outpatient, diabetic, neuropathic, septic ulcer, osteomyelitis	Dorsal
8	DFU (71/M)	Inpatient, diabetic, neuropathic. Middle toe amputated for low vascularity.	Sole
9	DFU (60/M)	Inpatient, diabetic, neuropathic	Sole
10 (10L)	DFU (67/M), Left foot	Outpatient, diabetic, neuropathic	Dorsal
11 (10R)	DFU (67/M), Right foot	Outpatient, diabetic, neuropathic	Dorsal

DFU, diabetic foot ulcer; F, female; M, male.

Figure 2. Schematic of the 90-s BH paradigm employed in the imaging study, utilized during the study. A 20-s BH was used. Data were acquired at initial rest ($t=0$ s), the beginning of BH ($t=10$ s), the end of BH ($t=30$ s), post-BH rest ($t=60$ s), and final relaxation ($t=90$ s). BH, breath-hold. Color images are available online.

wound (or subject). Hence, L and B are consistent across these time points, allowing for grouping ΔHbO and these factors to obtain an equivalent hemoglobin parameter. In other words, the effective oxy-hemoglobin and deoxy-hemoglobin are used from here, as given by $\Delta\text{HbO}(x,y) = \Delta\text{HbO}(x,y)LB$ and $\Delta\text{HbR}(x,y) = \Delta\text{HbR}(x,y)LB$. The $\Delta\text{HbO}(x,y)$ and $\Delta\text{HbR}(x,y)$ are further normalized (from 0 to 1) and used to obtain the effective changes in total hemoglobin (ΔHbT) and oxygen saturation (ΔStO_2):

$$\Delta\text{HbT} = \Delta\text{HbO} + \Delta\text{HbR} . \quad (4)$$

$$\Delta\text{StO}_2 = \Delta\text{HbO} / \Delta\text{HbT} . \quad (5)$$

During image analysis, regions encompassing the wound and its surroundings are manually demarcated (or segmented) (represented by black border line in Results; see Figures 5 and 6) to avoid background noise. The differences in these hemoglobin concentrations across each time point are compared from pseudo-color maps to determine if 20-s BH caused any change in oxygenation with time (via one or more of these parameters) and across the subject groups.

Dynamic correlation analysis. Pearson's correlation analysis was carried out to identify regions with varied and similar temporal changes in the hemoglobin concentrations (in terms of ΔHbO , ΔHbR , ΔHbT , and ΔStO_2) compared. A 50×50 pixel reference area away from the wound site was randomly picked for each subject case and the changes in the hemoglobin concentration parameters were plotted across the time points. The predominant trend pattern (in each hemoglobin concentration parameter) was chosen to represent the reference trend point, based on which Pearson's correlation coefficient (PCC) analysis was carried out, as given

in Equation (7). In control subjects, these trend lines were synchronous, whereas in DFU cases, they varied even in a reference region away from the wound site.

$$PCC = \frac{\sum_{i=1}^n (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^n (x_i - \bar{x})^2 (y_i - \bar{y})^2}} , \quad (6)$$

where $\bar{x}$ is the mean value of the selected reference region across time, x_i is the value of the reference region at the n th time point, $\bar{y}$ is the mean value of the y th pixel across time, and y_i is the value at the n th time point. The output is a 2D Pearson's correlation map that ranges from -1 to $+1$ (i.e., negatively to positively correlated). Figure 3 illustrates the methodology employed for Pearson's correlation analysis for one sample imaging case.

Contrast in Pearson's correlation. Extent of variation in Pearson's correlation maps of each hemoglobin concentration parameter between the wound and its surroundings (in DFUs) or between any two regions (in controls) was determined via Weber-based contrast analysis. From the chosen reference region (described in the Dynamic correlation analysis section) in each imaged case, contours that are 50 pixels from the reference region (first contour) and 100 pixels apart (in subsequent contours) were drawn to establish three zones: B1 (background-1), B2 (background-2), and W (wound). The first zone, B1, is closest to the reference region and zone 3, W, is farthest away and selected near the wound (as shown in Fig. 4). A 20×20 pixel areas was selected in each of the contour zones (B1, B2, and W), with the last pixel area chosen in the wound site within the third contour zone (in most cases except in case 10R, where the wound was in the second contour zone, due to small field of NIR images). A box plot was used to show

Figure 3. Illustration of Pearson's correlation analysis approach. **(A)** Hemoglobin concentration map (shown here is StO_2 as a sample) at a given time point, from which a 50×50 reference region (shown by a *black box*) is chosen away from the wound site (shown by *red oval*). **(B)** The reference region is binned to 10×10 pixels across time and plotted. **(C)** The trend line that captures the predominant trend pattern is selected. **(D)** Using Equation (6), Pearson's correlation analysis is carried out using the chosen reference point to produce a 2D Pearson's correlation map for each hemoglobin concentration parameter. ΔStO_2 , effective oxygen saturation; 2D, two-dimensional. Color images are available online.

the range and distribution of Pearson's values across all wound and background regions. A Weber-based wound-to-background (W:B) contrast of PCCs was calculated using Equation (7).

$$[W : B] = \frac{W - B}{B} \times 100\%, \quad (7)$$

where W is the averaged Pearson's correlation value of the wound region (W) for a selected tissue parameter in zone 3 and B is the averaged Pearson's correlation value of the background region in zone 1 (B1) for a selected tissue parameter. The W:B Pearson's contrast was used to quantify the differences in correlation between the wound and background (B1), which is further away from the

Figure 4. 2D Pearson's correlation maps with contours drawn from the chosen reference region at fixed distances (50 pixels for first contour and 100 pixels for remaining ones). Regions W, B1, and B2 are three 20×20 pixel areas selected in each contour zone, with W being the one close to the wound. Box plots are produced to show the range of variation in Pearson's correlations from the three chosen regions. Pearson's contrast was quantified using W and B1 that represent the wound and background away from the wound (that represents normal tissue). B1, background-1; B2, background-2. Color images are available online.

wound site. In the case of controls, the same nomenclature was used (W, B1, and B2) for box plots as well as Pearson's contrast values, and W represents another background location unlike in DFUs.

RESULTS

TO maps

Effective ΔHbO , ΔHbR , ΔHbT , and ΔStO_2 maps were determined for each subject across all time points. A sample result for a control case (case 3) and a DFU case (case 5) in terms of ΔStO_2 is presented in Fig. 5. In both the cases, temporal changes in ΔStO_2 concentration maps in response to BH are observed. A visual temporal change can be distinctly observed from the pseudo-color maps of hemoglobin concentration parameter(s) across the entire foot in controls (case 3). In the DFU case (case 5), this change was distinct in and around the wound region (here, big toe) as demarcated by the red circle in Fig. 5. Pseudo-color maps for all hemoglobin concentration parameter maps for cases 3 and 5 are given in Appendix Fig. A1. For cases 3 and 5, temporal changes were clearly visible from ΔStO_2 maps, whereas in some other cases, this visual difference was distinct in other hemoglobin parameters. However, across all subjects (both controls and DFUs), there was change in hemoglobin concentrations in response to a 20-s BH paradigm as observed from the five time-points across the 90-s imaging study. The correlation in the temporal changes of these hemoglobin concen-

tration parameters across the imaged regions is determined from Pearson's correlation analysis.

Pearson's correlation maps

Figure 6 illustrates the differences in Pearson's correlation maps (of ΔStO_2) in a control (case 3) and DFU case (case 10R). In the control subject (case 3), the changes in ΔStO_2 trend lines were identical in all points of the chosen reference region (as shown in Fig. 6B). In general, the trend lines in terms of ΔHbO , ΔHbR , and ΔStO_2 were synchronous (or identical) within all the control cases. Pearson's correlation maps (in terms of ΔHbO , ΔHbR , and ΔStO_2) were highly and positively correlated across the entire foot in case 3, as shown in Fig. 6C (and across all control cases). On the contrary, the trend lines from each pixel of the chosen reference region in DFU case (case 10R) were not identical across the five time points. Furthermore, Pearson's correlation analysis yields a distribution of mixed correlations (i.e., low/uncorrelated and negative). A distinct variation in correlations was observed across all DFU cases and in terms of all hemoglobin concentration maps (ΔHbO , ΔHbR , ΔHbT , and ΔStO_2). Pearson's correlation maps for all hemoglobin concentration parameters and across all subjects (controls and DFUs) are given in Appendix Figs. A2–A4.

Contrast in Pearson's correlation

Box plots of Pearson's contrast values for each hemoglobin concentration parameter from zones

Figure 5. Hemoglobin concentration maps in terms of ΔStO_2 for a control (case 3) and DFU case (case 5), at each of the five time points ($t=0, 10, 30, 60$, and 90 s) across the 90-s BH paradigm. (A–E) and (F–J) correspond to the time points in increasing order in control and DFU case, respectively. Color images are available online.

Figure 6. Hemoglobin concentration maps (in terms of ΔStO_2) for the (A) control subject (case 3) and (D) DFU subject (case 10R). The chosen 50×50 reference region is shown by the *black box* and the respective binned trend lines across the five time points are shown in (B) and (E) for the control and DFU case, respectively. Pseudo-color maps of 2D Pearson's correlations of ΔStO_2 for (C) control case and (F) DFU case are shown. The *black outline* in (A, C, D, and F) are the segmented regions of interest in each case. Pseudo-color maps of only these regions are shown overlapped across the entire field of view during imaging. Blue represents regions with negative correlation (-1) and red represent regions with positive correlation (+1). Color images are available online.

Figure 7. Box plot showcasing the range and distribution of Pearson's correlation values (in terms of ΔHbO , ΔHbR , ΔHbT , and ΔStO_2) obtained from the three locations (W, B1, and B2) in all subject cases (except case 10R that has only two locations (W and B1)). HbO, oxy-hemoglobin; HbR, deoxy-hemoglobin; HbT, total hemoglobin; StO_2 , oxygen saturation. Color images are available online.

B1, B2, and W across all subjects are given in Fig. 7. From these box plots, it can be observed for control cases 1–3, the contrast in ΔHbO -, ΔHbR -, and ΔStO_2 -related Pearson's values was narrow. For DFU cases, the range was wide, often encompassing regions of low to negative values for all parameters.

A Weber-based Pearson's contrast was employed to quantify the differences in parameter changes over time for a wound and background region, and the results are shown in Fig. 8, with exact contrast values provided in Table 2. For the control group, the contrast remained within the 10% interval for ΔHbO , ΔHbR , and ΔStO_2 . For DFU cases, only case 6 remained within the 10% cutoff for ΔHbO and ΔHbT , with all other cases exceeding the 10% range in all parameters. This indicates that in cases where a DFU exists, temporal changes in hemoglobin concentration are distinctly asynchronous or nonuniform (as determined using a BH stimulus) across the imaged region.

DISCUSSION

BH is primarily utilized to induce cerebral hypoxia and was introduced in 1990 by Ratnatunga and Adieseshian²⁰ as a vasodilative stimulus. It has been successfully used in functional magnetic resonance imaging (fMRI) as well as NIRS imaging studies.²¹ A BH of just 10 s was capable of inducing a blood oxygen level dependent signal obtained from an fMRI of the brain.²² In most NIRS imaging

studies, a 20- to 30-s BH was used,¹⁸ and in some cases, it was left for the maximum BH time for each subject.^{23,24} NIRS studies show that within 20–30 s of BH a transient increase in cortical HbO during BH can be observed across all subjects.²³ In some subjects, a vasodilation reaction to carbon dioxide increase was so strong that they observed HbR decrease initially due to enhanced perfusion.²³ In our study among controls, we observed an increase in ΔHbO and a decrease in ΔHbR at the end of the 20-s BH (time point 3) in comparison to before BH (time point 2) in cases 1 and 3 (Fig. 5; Appendix Fig. A1). A similar trend was also observed in cases 5 and 7 of the DFU subjects. Unlike the dynamic NIRS studies of the brain, the current study on wounds was carried out at discrete time points (0, 10, 30, 60, and 90 s). The lack of similar trend across all subjects within the group or across DFUs and controls can be from one of many reasons. These include: (1) No allotted time period for subjects to reach a relaxed state, as is typically carried out in all control studies in NIRS-based brain imaging. (2) The data acquisition was immediately after each set time point, but not precisely at that second, which can impact the value of hemoglobin concentrations that dynamically change. (3) BH involved taking a deep breath before the 20-s hold in the current study, unlike end expiration-based BH paradigms used during brain imaging studies.^{18,21,23,24} Despite these variations, a difference in each of the hemoglobin parameter before and

Figure 8. Pearson's correlation contrast values (in terms of ΔHbO , ΔHbR , ΔHbT , and ΔStO_2) across all subject cases. The blue circles denote control cases and red circles denote DFU cases. The $\pm 10\%$ limits are demarcated by two black horizontal lines. Contrast values beyond $\pm 100\%$ were fixed at $\pm 100\%$ just for plotting purposes (to better scale the figure). Color images are available online.

Table 2. Pearson's contrast values for all hemoglobin concentration parameters and across all subjects cases

Case	ΔHbO (%)	ΔHbR (%)	ΔHbT (%)	ΔStO_2 (%)
1	1.3	1.1	-14.5	0.3
2	3.0	6.2	12.7	4.1
3	-0.3	-0.8	-21.7	-0.8
4	-46.9	-54.6	-86.9	-78.7
5	-124.9	-12.0	-38.6	-20.8
6	-1.8	-76.0	9.9	-19.2
7	-107.7	-781.7	-224.3	15.8
8	-58.9	-93.7	-160.4	-74.5
9	-135.6	-21.1	-340.7	-82.4
10 (10L)	-37.0	-73.8	-46.9	-97.3
11 (10R)	-287.6	78.4	959.5	-458.6

Cases 1 to 3 are controls and the rest are DFU cases. Shaded in gray and bolded are the specific subject and parameters that remained within $\pm 10\%$ difference.

ΔHbO , effective oxy-hemoglobin; ΔHbR , effective deoxy-hemoglobin; ΔHbT , effective total hemoglobin; ΔStO_2 , effective oxygen saturation.

after BH was consistently observed across all subjects in both the groups. Additionally, the distance between NIROS and the imaged tissue varied in each subject, based on the location of the wound and/or its size. This, however, does not impact our results, as the focus was to observe for changes in hemoglobin concentrations across each time point and obtain their dynamic correlations, not to obtain absolute concentrations of these parameters.

The current study was a preliminary approach to validate if a difference in oxygenation (in terms of these hemoglobin concentrations) will be observed in the feet in response to a BH. Future work will involve dynamic imaging every second across the 90 s period to closely map the temporal changes in these parameters and the trend in both subject groups.

Differences in hemoglobin concentration maps were observed in response to BH in both subject groups. The extent of variations in these concentration maps across the time points was determined from Pearson's correlation maps. In all control subjects, these Pearson's correlation maps were consistent across the entire imaged region of the foot. This was observed across all hemoglobin concentration parameters (except in ΔHbT). This is indicative of potential uniformity in the oxygen flow (dorsal or sole) of the foot in control subjects (included individuals with controlled diabetes and no DFUs). On the contrary, a spatial variation in these hemoglobin concentration parameters was clearly observed in all DFU cases. This demonstrates that there is a variation in oxygen flow (or oxygenation) in the feet of subjects with DFUs compared with the controls. When quantified, the variation in Pearson's correlation contrast was within 10% in the controls compared with signifi-

cant variation (much greater than 10%) in the DFU cases, except for case 6. In case 6, the DFU subject's entire foot was swollen, possibly causing a compromise in the entire imaged region, leading to $<10\%$ variation in Pearson's contrast. These results are suggestive of compromised flow across a broader range of the foot, with all DFU subjects failing to stay within the 10% interval for Pearson's contrast, except for ΔHbO and ΔStO_2 in case 6 as described above.

In case 7, the subject had Pearson's contrast values lesser than -200% for ΔHbR and ΔHbT . Medical records for this subject indicated that they had severe osteomyelitis that had resulted in the bone within the foot being worn out, and the big toe in need of amputation. Inspection of the 2D Pearson's maps for this subject showed that changes in the big toe are polarly split with highly positively correlated and negatively correlated regions for ΔHbR (as shown in Fig. 9A) and ΔHbT . It was also observed that the blood vessels were oppositely correlated with respect to its adjacent regions, stating poor oxygen flow to the big toe.

In case 8, there was motion artifact of the foot during the 90-s imaging study. All Pearson's correlation maps for case 8 depicted negatively correlated regions in the middle toe and the wound below it, with the strongest negative correlations occurring in Pearson's ΔHbR map (as shown in Fig. 9B). Clinically, the middle toe was diagnosed as necrotic and amputated the next day. Despite the motion artifact, Pearson's correlation maps demarcated the overall regions of compromised oxygen flow.

Oxygen supply to the foot is crucial toward the wound healing process, and mapping variations in other hemoglobin concentration parameters (including StO_2 or TO) for DFU subjects could potentially allow clinicians to determine the effectiveness of the ongoing treatment and patient compliance to off-loading (if any). In particular, cases 7 and 8 illustrate the potential of the technique to identify regions of differing oxygenated flow in subjects with known complications. It could be seen that regions of poor oxygenation tended to correlate with necrotic regions (case 8) or regions requiring amputation from low vascularity (cases 7 and 8, respectively). Thus, BH stimulus may be useful in observing differences in oxygenated flow in patients with these conditions. Effective assessment of oxygenation can cater better treatment and possibly shorten the healing time by altering the treatment plan (e.g., reduce pressure at wound site, off-load wound region effectively, and appropriate foot wear). In the current study, only dis-

Figure 9. Pearson's correlation maps (in terms of ΔHbR) for DFUs cases (A) 7 and (B) 8. The white circle represents the regions with negative Pearson's correlations that were clinically assessed for potential amputation. ΔHbR , effective deoxy-hemoglobin. Color images are available online.

crete time points were imaged across the 90-s BH paradigm. Our ongoing efforts focus on dynamic imaging at 1 Hz frequency across the 90-s BH paradigm to determine if the trend of changes in these hemoglobin parameters can provide further insight into variations in oxygen supply to the wound and also establish a robust approach that is clinically applicable. NIROS can be maneuvered to image any location of the foot as it is placed on a stable articulating arm. However, excessive movement of the foot during imaging can affect temporal analysis of the foot, resulting in erroneous Pearson's correlations. In our ongoing work, the DFU afflicted foot is positioned sideways on the examination table to improve stability and minimize foot movement. Additionally, the use of appropriate NIR-based longpass filters minimized the effect of ambient light on the acquired NIR signals in the current study. As an ongoing effort, various mathematical image reconstruction approaches using singular value decomposition and principal component analysis are developed in an effort to minimize the surface noise and improve the signal to contrast ratio of the physiological signals acquired from NIR measurements.

INNOVATION

A method to assess variations in oxygen supply in and around DFUs was proposed and demonstrated using a low-cost, noninvasive handheld NIROS. The portable noncontact nature of this device makes it practical for daily clinical use in an outpatient setting. Furthermore, it has potential to provide immediate feedback on the oxygen supply in terms of ΔHbO , ΔHbR , and ΔStO_2 of the wound compared with surrounding tissue to better cater the DFU treatment process.

CONCLUSION

A noncontact, hand-held NIROS was utilized to perform multiwavelength CW-based diffused reflectance imaging in subjects with DFUs. Temporal changes in ΔHbO , ΔHbR , ΔHbT , and ΔStO_2 in response to a 20-s BH paradigm were acquired at five discrete time points (before and after BH). It was observed that all hemoglobin concentration parameters changed in response to the 20-s BH. From the 2D Pearson's correlation maps and Pearson's correlation contrast analysis, it was determined that changes in ΔHbO , ΔHbR , and ΔStO_2 for DFU cases are asynchronous, as opposed to synchronous changes in control cases. This indicates that regions of compromised blood flow exist in the recruited DFU subjects and that the oxygenation flow between the wound site and background region is significantly different. BH in conjunction with Pearson's analysis and W:B contrast has potential as a technique by which clinicians can get immediate feedback of oxygen supply to the foot, which is significant as oxygen is crucial for wound healing. By evaluating temporal variations in oxygen flow to the wound and its surrounding region, clinicians can effectively treat regions with poor flow that can slow the healing process. Ongoing efforts are to develop a dynamic imaging approach during the 90-s BH paradigm to understand the extent of changes in oxygenation continuously and even during the 20-s BH. In parallel, methods to stabilize the foot during imaging to avoid motion artifact are also developed.

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AUTHOR DISCLOSURE AND GHOSTWRITING

There is no conflict of interest with regard to the research in current article. The corresponding author's university (Florida International University) holds patents on the described technology. The content of this article was expressly written by the authors listed. No ghostwriters were used to write this article.

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KEY FINDINGS

- Hemoglobin concentrations change in response to 20-s breath-hold (BH) in the feet of both controls and diabetic foot ulcer (DFU) subjects.
- The hemoglobin concentration changes of the foot (in terms of effective oxy-hemoglobin [ΔHbO], deoxy-hemoglobin [ΔHbR], and oxygen saturation [ΔStO_2]) are synchronous in controls and asynchronous in DFUs, as observed from two-dimensional Pearson's correlation maps and Pearson's correlation contrast.
- Pearson's correlation values are within a narrow range (and close to +1) in controls and varies across a large range (−1 to +1) in DFU cases.
- Pearson's correlation contrast (in terms of ΔHbO , ΔHbR , and ΔStO_2) varies significantly (>10%) between wound and the surroundings in DFUs and less than 10% across any two regions in the controls.

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Abbreviations and Acronyms

ΔHbO	= effective oxy-hemoglobin
ΔHbR	= effective deoxy-hemoglobin
ΔHbT	= effective total hemoglobin
ΔStO_2	= effective oxygen saturation
2D	= two-dimensional
B1	= background-1
B2	= background-2
BH	= breath-hold
CMOS	= complementary metal-oxide semiconductor
CW	= continuous wave
DFU	= diabetic foot ulcer
fMRI	= functional magnetic resonance imaging
HbO	= oxy-hemoglobin
HbR	= deoxy-hemoglobin
HbT	= total hemoglobin
LED	= light-emitting diode
NIR	= near-infrared
NIROS	= near-infrared optical scanner
NIRS	= near-infrared spectroscopy
OD	= optical density
PCC	= Pearson's correlation coefficient
StO_2	= oxygen saturation
TCOM	= transcutaneous oxygen measurement
TO	= tissue oxygenation
W:B	= wound-to-background

Appendix Figure A1. Hemoglobin concentration maps in terms of all parameters (ΔHbO , ΔHbR , ΔHbT , and ΔStO_2) for a control (case 3) and DFU case (case 5), at each of the five time points ($t=0, 10, 30, 60$, and 90 s) across the 90-s BH paradigm denoted by (A–E) in each row. BH, breath-hold; DFU, diabetic foot ulcer; HbO, oxy-hemoglobin; HbR, deoxy-hemoglobin; HbT, total hemoglobin; StO₂, oxygen saturation. Color images are available online.

Appendix Figure A2. Pearson's correlation maps (in terms of ΔHbO , ΔHbR , ΔHbT , and ΔStO_2) for cases 1, 2, 3, and 4. *Blue* represents regions with negative correlation (-1) and *red* represents regions with positive correlation ($+1$). ΔHbO , effective oxy-hemoglobin; ΔHbR , effective deoxy-hemoglobin; ΔHbT , effective total hemoglobin; ΔStO_2 , effective oxygen saturation. Color images are available online.

Appendix Figure A3. Pearson's correlation maps (in terms of ΔHbO , ΔHbR , ΔHbT , and ΔStO_2) for cases 5, 6, 7 and 8. Blue represents regions with negative correlation (-1) and red represents regions with positive correlation ($+1$). Color images are available online.

Appendix Figure A4. Pearson's correlation maps (in terms of ΔHbO , ΔHbR , ΔHbT , and ΔStO_2) for cases 9, 10 (10R), and 11 (10L). Blue represents regions with negative correlation (−1) and red represents regions with positive correlation (+1). Color images are available online.