Assessing the Healing of Venous Leg Ulcers Using a Noncontact Near-Infrared Optical Imaging Approach

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Objective: Venous leg ulcers (VLUs) are one of the most common complications in lower extremity wounds. To date, clinicians employ visual inspection of the wound site during its healing process by monitoring surface granulation and reduction in wound size across weeks of treatment. In this study, a handheld near-infrared optical scanner (NIROS) has been developed at the Optical Imaging Laboratory to differentiate healing from nonhealing VLUs based on differences in blood flow to the wound and its surroundings.

Approach: Noncontact near-infrared (NIR) area imaging of 12 VLUs have been carried out at two podiatric clinics. Diffuse reflectance images of the wounds were used to quantify optical contrasts between the wound and its surroundings. The variability in imaging conditions, analysis, and operator dependency were assessed to determine the robustness of the imaging approach.

Results: Optical contrast obtained from diffuse reflectance images of VLUs were distinctly different for healing (positive contrast) and nonhealing (negative contrast) wounds, independent of the varying imaging and data analysis conditions.

Innovation: NIR imaging of wounds to differentiate healing from nonhealing VLUs using a noncontact wide-area imager has been demonstrated for the first time.

Conclusion: The application of a portable handheld imager to assess the healing or nonhealing nature of VLUs during weekly clinical treatment is significant since physiological changes, as observed using NIROS, manifest before visual reduction in wound size during the healing process.

Keywords: venous leg ulcers, lower extremity ulcers, wound healing, near-infrared imaging, optical imaging, handheld, optical scanner

INTRODUCTION

Venous leg ulcers

LOWER EXTREMITY ULCERS have led to devastating complications among wounds and are still unrecognized. Lower extremity ulcers, considered chronic wounds, affect 7 million patients annually and cost over \$25

billion every year in the United States.¹ Diabetic foot ulcers (DFUs) and venous leg ulcers (VLUs) are some common complications in lower extremity wounds, with VLUs accounting for 80% of all leg ulcers.² There are ~1 million people in the United States that develop VLUs. In

addition, there is an annual increase of 600,000 people affected by this type of ulcer.² Every year, U.S. healthcare system spends \$2.5–\$3.5 billion to treat VLUs.³

A wide variety of advanced treatments for lower extremity ulcers, such as dressings, topical antimicrobials, off-loading shoes, hyperbaric oxygen, growth factors, bioengineered products, and matrices have been developed and have proven to improve wound healing.⁴ However, the effectiveness of each treatment is not the same in all patients and often clinicians have to try several different treatments before they find an effective one for a particular wound.^{4,5} Moreover, clinicians employ visual inspection based on experience to assess the healing nature of all cutaneous wound types, including lower extremity ulcers.⁶ Initial assessment constitutes of visual inspection of wound bed size, shape, and depth, as well as a vascular evaluation. In some of the more severely infected ulcers, X-ray and magnetic resonance imaging may be necessary, in spite of their high costs.⁶ Recently, many emerging optical technologies have been developed to potentially allow low cost, noninvasive imaging assessment of wounds.

Near-infrared optical imaging

One of the current optical modalities to assist wound diagnosis is near-infrared (NIR) optical imaging. It is a noninvasive and nonionizing imaging technique, which uses light between 650 and 1,000 nm wavelength to travel through human tissue. In this wavelength, NIR light is minimally absorbed and preferentially scattered by the dominant chromophores (*i.e.*, oxy- and deoxyhemoglobin or HbO and HbR, respectively), allowing deep tissue imaging. Several research groups have implemented NIR optical imaging technology on wound imaging (chronic wounds and DFUs) with animal studies as well as pilot human subject studies.^{5,7–17} A summary of all the studies conducted with implementation of NIRS is listed in Table 1.

Papazoglou's group and Weingarten's group at Drexel University conducted animal studies and a pilot human study to show that NIRS is capable of predicting wound healing in DFUs.^{8–14} Moza *et al.* developed a multiwavelength imager (no contact at the source end, but contact at the detector end) with real-time imaging display and demonstrated that NIRS is capable of imaging perfusion from studies on normal hands.⁷ However, the imaging systems employed by the above two groups (except Anand *et al.*¹⁵) are designed to contact the wound during imaging (either at the source and/or detector end). The contact of the device on wounds

during imaging may not be favored by clinicians and patients, especially in infectious and/or painful wounds.

Recently, a near-infrared optical scanner (NIROS) has been developed at the Optical Imaging Laboratory, which is capable of noncontact whole wound imaging in real time.^{16,17} Preliminary case studies demonstrated its ability to differentiate a healing from nonhealing wound based on differences in the optical contrast in lower extremity ulcers (DFUs and VLUs).^{16–18} However, these preliminary studies were based on qualitative assessment of healing or nonhealing without assessing the effect of varying imaging conditions and image analysis in a clinic setting. The current research focuses on applying NIROS to image VLUs and determine its ability to assess the healing or nonhealing nature consistently, under varying imaging and analysis conditions.

CLINICAL PROBLEM ADDRESSED

The gold-standard approach to assess the healing status of VLUs is through visual inspection of the wound bed size, shape, and depth, as well as surface granulation. Oxygen is vital for the healing of wounds. NIR optical imaging is a noninvasive imaging technique that can assess changes in tissue oxygenation (in terms of changes in HbO and HbR concentrations), through differences in the extent of NIR light absorption by the imaged regions. Since physiological changes in tissue oxygenation manifest before visual changes in the wound bed size, a noncontact optical imager can potentially complement the gold standard clinical assessment of wound healing during weekly treatment studies.

MATERIALS AND METHODS

Instrumentation

The NIROS is a compact setup that consists of a light-emitting diode (LED)-based source and an NIR-sensitive camera CMOS (complementary metal–oxide–semiconductor). The LED-based source is composed of an 830-nm LED, and driven by a custom-developed LED driver. The LED-based source and the camera detector are synchronized through a microcontroller to ensure the image data acquisition. An endoscopic camera is used to record the white light digital image of wounds along with the NIR images acquired using NIROS.^{18,19} A schematic of the NIROS setup is shown in Fig. 1. The whole setup of NIROS is stabilized by an articulating arm to prevent artifacts during imaging of wounds. A MATLAB graphical user interface is developed to

Table 1. Summary of Studies Related to Near-Infrared Optical Imaging of Lower Extremity Ulcers Performed to Date

Reference	Modality	Application	Source/Wavelength	Detector	Measured Parameter	Contact	Population	Conclusion
Papazoglou <i>et al.</i> ¹⁴	NIRS	Chronic wounds	3 laser diodes (690, 785, and 830 nm)	APD	HbO	Yes	20 female hairless rats (SICH-1)	• Total hemoglobin successfully distinguished healing from nonhealing ulcers (Reduction of hemoglobin is indicative of wound healing)
Papazoglou <i>et al.</i> ¹³	NIRS	Chronic wounds	3 laser diodes (685, 785, and 830 nm)	APD	HbR	Yes	20 female hairless rats (SKH-1)	• NIRS may differentiate between healing diabetic and nondiabetic wounds
Papazoglou <i>et al.</i> ¹²	NIRS	Chronic wounds	3 laser diodes (690, 785, and 830 nm)	APD	HbO	Yes	10 control and 10 diabetic rats	• Optical absorption in a healing wound initially diverges from absorption in healthy tissue, and later converges with the absorption of healthy tissue as the wound healing nears completion
Papazoglou <i>et al.</i> ¹¹	NIRS	DFU	4 laser diodes (685, 780, 830, and 950 nm)	APD	HbO	Yes	7 diabetic subjects with chronic wounds	
Papazoglou <i>et al.</i> ¹⁰	NIRS	DFU	3 laser diodes (685, 780, and 830 nm)	APD	ISO ₂	Yes	11 diabetic subjects with chronic wounds	
Weingarten <i>et al.</i> ⁹ , Neidrauer <i>et al.</i> ⁵	NIRS	DFU	3 laser diodes (685, 785, and 830 nm)	APD	HbO	Yes	16 diabetic patients with DFU	
Weingarten <i>et al.</i> ⁸	NIRS	DFU	3 laser diodes (685, 785, and 830 nm)	APD	HbR	Yes	46 diabetic patients with DFU	
Moza <i>et al.</i> ⁷	NIRS	Decubitus ulcers	4 LEDs (660–860 nm) Single LED (650–875 nm)	CMOS sensor	Diffuse reflected intensity	Yes No	Subject's normal hand. No studies on actual ulcer cases.	Allows the penetration of tissue to derive valuable perfusion and oxygen-metric data.
Anand <i>et al.</i> ¹⁵	DRS	DFU	1 tungsten halogen source (400 and 800 nm)	CCD Spectrometer	HbO HbR HbT	No	8 diabetic subjects with 10 foot ulcer sites	DRS can be used to probe the changes in oxygen dynamics, which could serve as a valuable tool for monitoring the wound healing process in foot ulcers.
Godavarty <i>et al.</i> ^{16,17}	NIRS	DFU	LED (710 nm, 830 nm)	CMOS sensor	ISO ₂ Diffuse reflected intensity	No	4 diabetic subjects with foot/leg ulcers	Diffuse reflectance optical contrast differentiated healing from nonhealing wounds.

APD, avalanche photodiode; CMOS, complementary metal-oxide semiconductor; DFU, diabetic foot ulcer; DRS, diffuse reflectance spectroscopy; HbO, oxyhemoglobin; HbR, deoxyhemoglobin; HbT, total hemoglobin; ISO₂, saturated oxygen; LED, light-emitting diode; NIRS, near-infrared spectroscopy.

Figure 1. Schematic of NIROS setup for noncontact imaging of wounds in the clinic. The entire setup is placed on a small portable cart for mobility to patients' examination rooms. LED, light-emitting diode; NIROS, near-infrared optical scanner. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

integrate data acquisition, data processing, and data analysis of NIR images obtained in the clinic.

Subject recruitment

NIR imaging was conducted on volunteer diabetic subjects with VLUs. A total of three subjects between the ages of 61–78 years of age with one or more VLUs, from two local podiatric clinics (Podiatry Care Partners & Wigley Foot and Ankle) were recruited in this Internal Review Board (IRB)-approved study. This was a Florida International University's IRB-approved study, which required written consent forms and HIPAA (Health Insurance Portability and Accountability Act) forms from all the subjects. From an average 7- to 8-week imaging study at the two clinics on various wounds (DFUs¹⁹ and VLUs), only the VLUs were grouped for this study.

Each VLU imaged during each visit (first week and last week's visit were considered) was treated as a new ulcer case, thus totaling to 12 VLUs across

3 subjects (Table 2). Since the physiological nature of the wound changed with treatment across the weeks, these wounds were treated as a new case during each visit. The 12 VLUs included 7 healing and 5 nonhealing cases. Details of the 12 VLUs and their medical diagnosis (whether the wound was healing or nonhealing) provided by the podiatrists before imaging studies each week is given in Table 2. The typical visual inspection of the wound was from surface epithelialization, and measurements of wound size and depth over weeks, and healing assessed from decrease in wound size over time ($\sim 40\%$ in 4 weeks of the treatment).

Data acquisition

A custom-developed MATLAB program was incorporated to acquire static NIR images (using 830-nm wavelength source light) for each of the 12 VLUs (without movement). Before imaging, the wound dressing was removed, the area cleaned, and in most cases the wound was debrided by the surgeon to remove the dead tissue. In some cases, wounds did not require debridement (as determined by the podiatrist), and hence imaging was performed on nondebrided wounds. NIR images were captured at 10 Hz frame rate as a continuous wave for 2 s (a total of 20 frames) by an NIR-sensitive camera, whereas white light images were acquired by an endoscopic camera (Oasis Scientific, Taylors, SC). During imaging, the wound and its surrounding regions were within the field of view. The white light images were used to assist the identification of wound area and its surrounding background during image processing. Images were acquired from up to three angles/locations with respect to the wound, to account for location variability in the quantitative analysis (see Table 2 for details on the number of locations imaged for each

Table 2. Details of Venous Leg Ulcer Subjects Recruited for the Study Provided Along with Their Healing or Nonhealing Status as Diagnosed by the Treating Podiatrist

Subject	Week	Wound	No. of imaged locations	Clinical diagnosis by podiatric surgeon
1. 78-year-old male	1	1	2	Neuropathic, with signs of arterial deficiency. The wounds decreased in size, diagnosed them as healing.
		2	2	
		3	2	
2. 76-year-old female	1	1	2	No signs of neuropathy, with signs of arterial deficiency. Both wounds decreased in size, diagnosed as healing.
	5	2	1	
		1	2	
3. 61-year-old male	8	2	3	No signs of neuropathy, with signs of arterial deficiency. Wounds on the right leg merged, and the left wounds increased in size, diagnosed all as nonhealing.
		3 (bridge between first and second wound)	3	
		4	3	
		5	3	
			5	

The total number of imaging locations is also included for each venous leg ulcer case.

wound). In subject 3, the VLUs were very large and hence images were acquired from three locations, unlike in other VLUs from subjects 1 and 2. In one case (subject 2, week 1, wound 2), only one imaging location data was recorded (due to an error in saving data from a second location).

Data processing: optical contrast

Optical contrast (based on diffuse reflectance images of the wound) was identified to be the biometric for this systematic study. Initially, the detected NIR signals across 20 acquired frames were averaged for each wound as a mean NIR intensity (stated as “intensity” from here on for simplicity). When processing the data and analyzing the optical contrast, a large region of interest (ROI_A in Fig. 2) that encompasses the wound and the background tissue was selected from the raw data. Within this ROI, a region encompassing the wound (ROI_W) was selected based on the wound location observed from the white light image. Optical contrast was determined by obtaining a ratio of the average intensity of the 2D region (ROI_W) of each wound to the average intensity of the periphery or background surrounding the wound (ROI_B). The region of background (ROI_B) was selected three times at different locations from the immediate peripheries of the wound. This process allowed us to obtain three optical contrasts with varying signal strengths. When the selected background region during image processing en-

compassed the wound region (ROI_W), the intensity of ROI_B was determined by excluding the intensity of ROI_W . The nonuniform source illumination and/or specular reflection can cause variations in the imaged wound and background signal. By imaging from various angles/locations of the same wound and its background, the effectiveness of the variability in the source signal strength on the optical contrast could be determined.

The entire data processing was performed by two independent operators to determine if the data analysis showed operator dependency. One operator was present in the clinic and had prior knowledge of image acquisition, whereas the other operator was not present in the clinic, but was presented with the white light images of the VLUs for image processing purposes. Both the operators' selection of region within the VLU (ROI_W) and its immediate peripheries (or background, ROI_B) was subjective, but still remained within the wound and its immediate peripheries, respectively.

Data analysis: effect of varying imaging and analysis conditions

Data analysis was carried out to account for the variability in imaging location, the consistency in background selection, and the dependency in operators. The variability in the choice of imaging location with respect to each wound using NIROS was determined by carrying out a one-way analysis of

Figure 2. (a) White light (digital), (b) and (c) NIR diffuse reflectance images (entire imaged region and chosen ROI_A , respectively) of an example healing and nonhealing VLU. A ROI_A selected from NIR image and W:B ratio processed with ROI_W and ROI_B . Positive optical contrast (28.8%) indicates healing wound, whereas negative (-18.1%) for nonhealing wound. NIR, near-infrared; ROI, region of interest; VLU, venous leg ulcer; W:B wound:background. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

variance (ANOVA) (with optical contrast being the variable). The internal consistency across different selected backgrounds on a given wound at a particular imaging location was measured by performing Cronbach's α test. The dependency in the operators (in their choice of wound and background regions of interest) on the same continuous dependent variable (*i.e.*, optical contrast) was determined from independent samples *t*-test. Data analyzed for this study were the optical contrasts acquired at different imaging locations and processed by two independent operators selecting multiple choices of background region. To determine if there is a statistically significant difference between the optical contrast measurements for healing and nonhealing wounds, two-sample *t*-tests were performed. *Post hoc* power analysis (based on one-sided two-sample *t*-test) showed 80.8% power to detect 15 unit (%) difference in optical contrast measurements between 5 nonhealing and 7 healing wounds (α 0.05).

RESULTS

Optical contrast

Figure 2 shows an example case of a healing and nonhealing VLU, including both its white light and NIR diffuse reflectance images. Markers of known size were placed during imaging for a parallel study on wound size measurements.²⁰ Initially, a ROI_A was selected from the entire imaged wound and then

the optical contrast measured from the selected wound (ROI_W) and background (ROI_B) regions and the contrast values are displayed above the plots. It was observed that healing VLUs typically provided a positive optical contrast and nonhealing wounds provided a negative optical contrast. There is a significant difference between optical contrast measurements for healing and nonhealing wounds ($p < 0.001$) across the 174 combination of processed images. The average optical contrast measurement for nonhealing VLU wounds ($n = 90$ images) was -24.87 (standard deviation [SD]: 12.37), whereas average optical contrast measurement for healing VLU wounds ($n = 84$ images) was 27.52 (SD: 31.50). The difference between the optical contrast for nonhealing and healing wounds was estimated to be -52.39 (95% confidence interval: -59.67 to -45.10).

Effect of variability in imaging and processing parameters

NIR imaging of each wound was acquired from up to three random different locations/angles and the images processed with different chosen regions of interest and by two operators. All these variability studies were carried out to determine if these parameters impact the ability of NIROS in differentiating healing from nonhealing ulcers. An example case of a nonhealing VLU imaged from three different locations (and/or angles) is shown in Fig. 3. Independent of the imaging location and

Figure 3. Example of nonhealing VLUs imaged at various locations/angles; optical contrast is the intensity ratio between wound and background regions; (a) location no. 1 shows an optical contrast of -31.2% ; (b) location no. 2 shows an optical contrast of -25.3% ; (c) location no. 3 shows an optical contrast of -20.2% . To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

the region selected to obtain the optical contrast, all the optical contrasts for this nonhealing VLU were negative. A one-way ANOVA was performed to determine if the optical contrasts obtained across the imaged locations for each wound (for all 12 VLUs) were significantly different or not. From this analysis, it was observed that in 83.3% of the VLU cases, this variability in the optical contrast across the different imaged locations was not significant ($p \geq 0.018$).

The effect of randomly selecting the background regions (ROI_B) around the wound during image analysis of the optical contrast was also assessed. Typically, a nonuniform input light source can cause nonuniformity in the detected NIR signal apart from the variation in this signal from the differences in the physiology of the wound and its background. This was obvious from our imaging studies performed from various angles/locations for each wound (Fig. 3). This nonuniform detected signal can impact the wound:background (W:B) optical contrast light based on the choice of the ROI_B around the wound. Figure 4 shows an example case of the same nonhealing VLU (shown in Figs. 2b and 3) at a given imaged location, but processed by choosing three different background regions (ROI_B) to obtain the optical contrast. Independent of the ROI_B selected, the nonhealing

VLU gave negative optical contrasts. This was similar to most of the wound cases, where healing VLUs showed positive optical contrast and non-healing VLUs showed negative optical contrast, independent of variability in ROI_B selection. A Cronbach's α analysis was performed to measure the internal consistency in data across the different chosen backgrounds. It was observed that 63.2% of all the VLU cases showed internal consistency in the quantitative differentiation of healing from nonhealing wounds with varying selection of ROI_B in each wound. The specular reflection from the nonuniform illuminating source causes a large variation in the intensity of the acquired NIR signals, causing a variability in optical contrast with choice of background (ROI_B) (although all values are either positive or negative for healing and nonhealing VLUs consistently). In future studies, the source illumination distribution (of NIROS) will be calibrated and accounted for, to reduce the 46.8% variability observed from choice of ROI_B in each wound.

Apart from the effect of variability in imaging angle/location and background region selection, the effect of operator dependency in differentiating a wound as healing and nonhealing was also assessed. As described in the Data Processing: Optical Contrast section, two independent operators had performed the complete image analysis of

Figure 4. (a) Example of nonhealing VLU data processed by three different background selections; (b) optical contrast (−18.1%) between wound and a dimmer background, (c) optical contrast (−31.2%) between wound and a brighter background, and (d) optical contrast (−9.0%) between wound and a background encompassing wound region. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

variability in imaging angles/location and background region selection. Independent of the operator processing the data, all the optical contrasts were negative for the shown nonhealing wound in Fig. 3. Similar consistency was observed across all wounds. A *t*-test analysis showed no significant difference between the optical contrasts processed by two independent operators ($p=0.8$), establishing operator independence of NIROS in differentiating healing from nonhealing VLUs.

Each VLU was imaged from up to three different imaging locations, processed by two independent operators, and by selecting three different background regions (during image processing), thus, accounting to a total of 174 combinations of imaging or image processing cases. A scatter plot depicting all these different scenarios for each VLU is shown in Fig. 5, wherein the wounds were categorized as healing or nonhealing based on the podiatrist's diagnosis. From this plot, it was found that positive or negative NIR optical contrast can consistently distinguish healing from nonhealing wounds (correlating well with clinical diagnosis made by the podiatrist).

DISCUSSION

Oxygen supply to VLUs is a key limiting factor for successful healing.²¹ Effective monitoring of tissue oxygenation changes in wound and periwound can assess the oxygen state of VLUs and

their potential to heal in the future.²¹ The extent of absorption or reflection of NIR light is directly proportional to the concentration of the major tissue components (e.g., HbO, HbR, water).²² A normal wound (or ulcer) progresses through various stages of healing, including hemostasis, inflammatory, proliferation, and remodeling.^{23–25} A non-healing VLU remains at the inflammatory phase, during which there is cessation of epidermal growth and migration over the wound surface (that elevate the HbR concentrations at the wound site). On the contrary, a healing wound progresses to other phases, where there is lesser HbR due to cell growth.^{5,9,10} NIR absorption of light at 830-nm increases (or its diffuse reflectance decreases) with increased HbR concentrations, as this wavelength is sensitive to the changes in concentration of HbR.²² Thus, a decreased diffuse reflectance from the wound in comparison to its background causes a negative (diffuse reflectance based) optical contrast in nonhealing wounds.

From the above imaging studies, it was observed that there is a greater absorption (or smaller diffuse reflectance) of NIR light in a nonhealing VLU compared with a healing VLU. This is probably from the increased HbR concentration at the non-healing wound site (from the ulcer's halt at the inflammatory phase), causing an increased absorption or a reduced diffuse reflected signal. This phenomenon was observed by other researchers when imaging DFUs.^{8–14} The reduced diffuse reflectance led to a negative optical contrast in non-healing VLUs. On the contrary, the healing VLU progress to cell proliferation, where oxygen is constantly consumed, causing a decreased absorption of NIR light (or increased diffuse reflected signal). This in turn led to a positive optical contrast in healing VLUs.

The large variations in optical contrast measurements for healing ulcers can be from greater diffuse reflectance (or less absorbance of NIR light from decreased HbR concentrations) in healing cases. This increased diffuse reflectance signal when combined with the specular reflected signal provides an increase in the overall optical contrast. The extent of specular reflection can vary based on the location of the handheld device from the wound (i.e., the imaging location), potentially causing a greater variation in the positive optical contrasts of the healing VLUs as shown in Fig. 5. On the contrary, there is lesser diffuse reflectance (or greater absorbance of NIR light from the halted inflammatory phase with increased HbR concentrations) in nonhealing cases. Hence, the overall reflected signal from the wound site is probably attenuated

Figure 5. Scatter plot of optical contrast versus VLU cases. There is a total of 174 combinations of imaging and image processing of the VLU cases, including optical contrast of all the VLU cases imaged at various locations/angles, each of which processed by two independent operators with three different background selections. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

to a greater extent than the extent of varying specular reflection (that varies with imaging location/angle). This can potentially cause lesser variation in the optical contrast measurements of the nonhealing VLUs. In addition, some of the VLUs were larger than the imager's field of view. This can cause variability in the optical contrast across the different imaged locations (as observed in 16.7% of the cases) as not all imaging locations could encompass the entire wound and its surroundings. In our ongoing studies, the overall field of view of the imager is increased by optimizing the NIR source illumination intensity and distance from the wound.

In the current studies, all the nonhealing VLUs were from subjects that the podiatrist has been treating or assessing for many months to a year. Hence, the clinical baseline was confirmed before correlating the W:B optical contrasts with the healing or nonhealing status of the wounds. Future studies will involve blinded imaging of wounds without a prior knowledge of their status from the clinician, and comparing the effectiveness of NIROS in differentiating them as healing or nonhealing along with a statistical rigor from systematic weekly imaging as well as hemodynamic analysis.

INNOVATION

NIR imaging of wounds to differentiate healing from nonhealing cases using a noncontact optical imaging approach has been demonstrated for the first time on VLUs. The use of the noncontact NIROS to image the entire wound in real time is innovative in comparison to most of the contact-based NIR imaging of lower extremity ulcers (*e.g.*, DFUs) performed in the past.^{7–14} The portable handheld nature of the imager has a potential for clinical use during weekly treatment of VLUs and other lower extremity ulcers during an outpatient visit by ulcer subjects.

CONCLUSION

A noncontact handheld NIROS has been developed to perform real-time imaging of VLUs as an outpatient procedure during weekly wound dressings and treatment. Preliminary studies demonstrated that NIROS was capable of differentiating healing from nonhealing VLUs based on differences in the optical contrast between the wound and its peripheries. In this study, the robustness of NIROS to variations in its location from the wound

KEY FINDINGS

- Noncontact near-infrared imaging approach could assess venous leg ulcers (VLUs) in real time to determine physiological changes beneath the visual wound surface.
- There was a distinct difference between the optical signals of healing and nonhealing VLUs, as observed from the positive and negative optical contrast obtained from diffuse near-infrared (NIR) signals.
- Independent of the variations in the imaging location, choice of background during image analysis, or data processing by two researchers, the ability of the NIR imaging approach to differentiate healing from non-healing wounds was significant.

(during imaging), to the choice of background regions during image analysis, and to operator dependency has been demonstrated from the NIR imaging studies on VLUs. In our ongoing efforts, the device is modified to perform multiwavelength imaging such that the detected NIR signals (from multiple wavelengths) can in turn be used to calculate changes in HbO and HbR of the wound with respect to its peripheries. In future, imaging studies will be carried out using NIROS to assess changes in hemodynamics of the entire wound area across weeks. In addition, with the advantage of noncontact wide-field imaging capabilities of NIROS, the entire area of the wound can be imaged. Hemodynamic imaging of VLUs can potentially assist in predicting healing early on from physiological oxygenation measurements even before visual reduction in wound size.

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AUTHOR DISCLOSURE AND GHOSTWRITING

No competing financial interests exist. The corresponding author's university (Florida International University) holds patents on the described technology.

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Jiali Lei, BS, received her degree in Biomedical Engineering (Honors) from Florida International University (FIU). The current work was part of her BS Honor's Thesis. **Suset Rodriguez, BS**,

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REFERENCES

1. Sen CK, Gordillo GM, Roy S, et al. Human skin wounds: a major and snowballing threat to public health and the economy. *Wound Repair Regen* 2009; 17:763–771.
2. Abbade LP, Lastoria S. Venous ulcer: epidemiology, physiopathology, diagnosis and treatment. *Int J Dermatol* 2005;44:449–456.
3. Fife C, Walker D, Thomson B, et al. Limitations of daily living activities in patients with venous stasis ulcers undergoing compression bandaging: problems with the concept of self-bandaging. *Wounds* 2007;19:255–257.
4. Mulder G, Tenenhaus M, D'Souza GF. Reduction of diabetic foot ulcer healing times through use of advanced treatment modalities. *Int J Low Extrem Wounds* 2014;13:335–346.
5. Neidrauer M, Zubkov L, Weingarten MS, et al. Near infrared wound monitor helps clinical assessment of diabetic foot ulcers. *J Diabetes Sci Technol* 2010;4:792–798.
6. Dereck W, Ghassemi P, Ramella-Roman JC, et al. Noninvasive imaging technologies for cutaneous wound assessment: a review. *Wound Repair Regen* 2015;23:149–162.
7. Moza R, Dimaio JM, Melendez J. Deep-tissue dynamic monitoring of decubitus ulcers: wound care and assessment. *IEEE Eng Med Biol Mag* 2010;29:71–77.
8. Weingarten MS, Samuels JA, Neidrauer M, et al. Diffuse near-infrared spectroscopy prediction of healing in diabetic foot ulcers: a human study and cost analysis. *Wound Repair Regen* 2012;20:911–917.
9. Weingarten MS, Neidrauer M, Mateo A, et al. Prediction of wound healing in human diabetic foot ulcers by diffuse near-infrared spectroscopy: a pilot study. *Wound Repair Regen* 2010;18:180–185.
10. Papazoglou ES, Neidrauer M, Zubkhov L, et al. Noninvasive assessment of diabetic foot ulcers with diffuse photon density wave methodology: pilot human study. *J Biomed Opt* 2009;14:064032.
11. Papazoglou ES, Weingarten MS, Zubkov L, et al. Assessment of diabetic foot ulcers with diffuse near infrared methodology. *Bioinf Bioeng* 2008;8:1–5.
12. Papazoglou ES, Weingarten MS, Zubkov L, et al. Near infrared diffuse optical tomography: improving the quality of care in chronic wounds of patients with diabetes. *Biomed Instrum Technol* 2007;41:83–87.
13. Papazoglou ES, Weingarten MS, Zubkov L, et al. Optical properties of wounds: diabetic versus healthy tissue. *IEEE Trans Biomed Eng* 2006;53: 1047–1055.
14. Papazoglou ES, Zubkov L, Weingarten MS, et al. Monitoring diabetic wound healing by NIR spectroscopy. *Conf Proc IEEE Eng Med Biol Soc* 2005; 4:6662–6664.
15. Anand S, Sujatha N, Narayanamurthy VB, et al. Diffuse reflectance spectroscopy for monitoring diabetic foot ulcer—a pilot study. *Opt Lasers Eng* 2014;53:1–5.
16. Godavarty A, Khandavilli Y, Jung Y-J, et al. Non-contact optical imaging of healing and non-healing diabetic foot ulcers. *Proc SPIE* 2015;9318: 931802.
17. Godavarty A, Rao PN, Khandavilli Y, et al. Diabetic wound imaging using a noncontact near-infrared scanner: a pilot study. *J Diabetes Sci Technol* 2015; 9:1158–1159.
18. Jayachandran M, Rodriguez S, Solis E, et al. Critical review of noninvasive optical technologies for wound imaging. *Adv Wound Care* 2016;5:349–359.
19. Lei J, Rodriguez S, Jayachandran M, et al. Quantitative wound healing studies using a portable, low-cost, hand-held near-infrared optical scanner: preliminary sensitivity and specificity analysis. *Proc SPIE* 2016;9699:96990S.
20. Dadkhah A, Pang X, Solis E, et al. Wound size measurement of lower extremity ulcers using segmentation algorithms. *Proc SPIE* 2016;9703: 97031D.
21. Schreml S, Szeimies RM, Prantl L, et al. Oxygen in acute and chronic wound healing. *Br J Dermatol* 2010;163:257–268.
22. Wray S, Cope M, Delpy DT, Wyatt JS, Reynolds EOR. Characterization of the near infrared absorption spectra of cytochrome aa3 and hemoglobin for the non-invasive monitoring of cerebral oxygenation. *Biochim Biophys Acta* 1988;933: 184–192.
23. Singer A, Clark RA. Cutaneous wound healing. *N Engl J Med* 1999;341:738–746.
24. Gurtner G, Werner S, Barrandon Y, et al. Wound repair and regeneration. *Nature* 2008;453:314–321.
25. Schreml S, Szeimies RM, Prantl L, et al. Wound healing in the 21st century. *J Am Acad Dermatol* 2010;63:866–881.

Abbreviations and Acronyms

ANOVA = analysis of variance
 APD = avalanche photodiode
 CCD = charge-coupled device
 CMOS = complementary metal–oxide–semiconductor
 DFU = diabetic foot ulcer
 DRS = diffuse reflectance spectroscopy
 HbO = oxyhemoglobin
 HbR = deoxyhemoglobin
 HbT = total hemoglobin
 IRB = internal review board
 ISO₂ = saturated oxygen
 LED = light-emitting diode
 NIR = near-infrared
 NIROS = near-infrared optical scanner
 NIRS = near-infrared spectroscopy
 ROI = region of interest
 SD = standard deviation
 VLU = venous leg ulcer
 W:B = wound:background