Assessment of Diabetic Foot Ulcers with Diffuse Near Infrared Methodology

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Abstract— **A pilot human study was conducted in order to demonstrate the efficacy of diffuse near infrared (NIR) methodology for *in vivo* assessment of diabetic foot ulcers. Nine patients were evaluated using diffuse NIR methodology to assess their wound healing process over time. Absorption and scattering coefficients of the wound site were determined over time and compared to those of a control site. The results validate the model wound healing developed during our animal studies of wound healing with NIR, and indicate that differences in optical properties can be seen between wounds with different healing behaviors.**

I. Introduction

Chronic wounds are a growing problem as the world's population ages and the prevalence of diabetes increases [1]. It is estimated that diabetic foot ulcers are the underlying cause of 85% of all non-traumatic amputations [2]. Approximately 15% of patients with diabetes will develop diabetic foot ulcers during their lifetimes, and 14% of diabetic ulcers lead to amputation [3]. Mortality during the 5-year period following amputation ranges from 39%-80% [2].

Chronic wound treatments such as topical growth factor and hyperbaric oxygen therapy are costly yet are often administered ineffectively because clinicians are unable to objectively and accurately measure the progress of wound healing [4]. Currently, the standard method for monitoring wound healing progress is to measure the dimensions of a wound on each visit to the physician [5]. This technique is highly inaccurate and makes it difficult for a physician to understand the effectiveness of a prescribed wound therapy until late in the treatment cycle.

Tissue oxygenation and blood volume are important indicators of the wound healing process. Angiogenesis, collagen synthesis, and epithelialization are all critical processes in wound healing, and all require high concentrations of oxygen [6]. Additionally, studies have shown that the inflammatory cells present in wounds consume high amounts of oxygen in the production of bacteria-killing oxidants [7]. It is well established that poor oxygenation and tissue perfusion are linked to impaired wound healing [6-9], therefore tissue oxygenation and blood volume in the wound environment would be good indicators of wound healing potential.

Diffuse near infrared (NIR) methods have the potential to provide a quantitative measure of wound healing that would allow physicians to better predict the healing potential of a wound, and therefore the effectiveness of a prescribed treatment. Frequency-domain diffuse near infrared methodology measures the amplitude and phase shift of sinusoidally modulated NIR light as it travels through tissue, and uses a diffusion-based model of light propagation in tissue to calculate the absorption and reduced scattering coefficients (μ_a and μ_s', respectively). In living tissue, oxyhemoglobin and deoxyhemoglobin are the primary chromophores in the near infrared wavelength region (650nm – 850nm); therefore measurements of NIR absorption directly reflect the tissue oxygenation and blood volume of probed tissue [10]. Furthermore, the absorption of light in the NIR spectrum is relatively low compared to visible and infrared wavelengths, allowing the measurement of tissue oxygenation and blood volume several millimeters beneath the surface of a wound [11].

We hypothesize that by measuring oxygenated hemoglobin, deoxygenated hemoglobin, and blood volume with diffuse NIR technology it would be possible to predict wound healing in diabetic foot ulcers, earlier and with greater accuracy than current clinical methods. In this paper, we describe a model of the expected optical changes to be observed during wound healing and the preliminary results of an ongoing clinical study to validate our model.

II. Theoretical Background

Diffuse Near Infrared Spectroscopy (NIR) is a non-invasive technology which can analyze tissue by measuring

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its optical properties (absorption and scattering coefficient). The optical properties of tissue at NIR wavelengths are determined mostly by the levels of oxygenated (HbO_2) and deoxygenated hemoglobin (Hb). The traditional optical method of determining concentration of components in a multi-component medium is the measurement of its absorption spectrum. If the spectrum of each chromophore is known, its concentration can be calculated using the following equation, for each wavelength (□) of incident light.

$$\mu_a(\lambda) = \sum_i^n \varepsilon_i(\lambda) * c_i, \qquad (1)$$

where μ_a is the measured absorption coefficient, ε_i(□) is the wavelength-dependent extinction coefficient of the *i*-th chomophore, c_i is the concentration of the *i*-th chromophore, and n is the total number of different chromophores in the medium. Data on extinction coefficients of various chromophores in tissue are available from http://omlc.ogi.edu. The simple Beer – Lambert law cannot describe light propagation in tissue because it is a very dense medium with inhomogeneous structure and very strong light scattering. The best model currently used to describe light propagation in tissue is the diffusion approximation; closed solutions of the diffusion equation allow determination of tissue optical properties.

Currently three methodologies are used in the NIR range to measure absorption and reduced scattering coefficients (μ_a and μ_s') in multiply scattering tissues. The difference between these techniques lies in the source of the incident light. Constant power lasers are used for continuous wave devices [12, 13], where the reduction in light scattering as a function of the source –detector separation ρ is measured. In this case difficulties emerge when one tries to distinguish contributions from absorption and scattering effects in the attenuation of light intensity. On the other hand, time resolution spectroscopy instruments (TRS) observe the very informative broadening of short NIR light pulses after propagation in tissue [14, 15], allowing a distinction to be made between absorption and scattering effects. However, this method is complex and expensive.

An approach that gives adequate information to assess independently both absorption and scattering, while maintaining low cost and small size is that of the frequency domain instruments where incident light is modulated by Radio Frequencies (RF) [10, 16]. Using frequency domain technology, the intensity and phase shift of multiply scattered light are used to determine μ_a and μ_s'. In the case of biological tissue the time – dependent diffusion equation adequately describes the propagation of light in biological tissue since a) the radiance is quasi-isotropic; b) $\mu_a << \mu_s'$; and c) the modulation frequency of laser emission is less than the frequency of photon collision. Closed analytical solutions to the diffusion equation can be obtained for special cases of the boundary conditions. For semi – infinite geometries typical of noninvasive tissue measurements, sources and detectors are placed on an air –tissue interface and the optical fiber source is modeled as an isotropic, point light source. The final equations describing the absorption and scattering coefficients as a function of the measured light intensity and phase shift are included in reference [10].

The probe configuration can be designed to accommodate the tissue where measurements need to occur. The advantage of NIR wavelengths is their ability to penetrate deeper into tissues. However, even relatively superficial depths can be probed. The transport length l^* represents the distance of propagation of a collimated beam of light before it becomes effectively isotropic. It is possible to use the diffusion approximation to calculate absorption and scattering of tissue from the intensity and phase shift of scattered NIR light using a probe where the minimum distance between source and detector fibers ρ is greater than three transport lengths ($\rho > l^*$). In particular, after propagating more than two or three transport lengths most photons have undergone multiple light scattering (i.e. they are now at a different orientation from their incident direction) and may be described as diffuse [17, 18]. For most human tissues μ_s' is approximately 10 cm^{-1}, therefore the transport length l^* is approximately 1 mm, since l^* is the inverse of the reduced scattering coefficient μ_s'. This suggests that the smallest source-detector distance that can be used in probe design is 3 mm, for the diffusion approximation to be valid [19]. The probe used in this research had a minimum distance between source and detector fibers of 4 mm to be safely within the diffusion approximation regime (Figure 1). A rule of thumb for depth penetration in tissue is between 1/3-1/2 of the source-detector distance [20, 21].

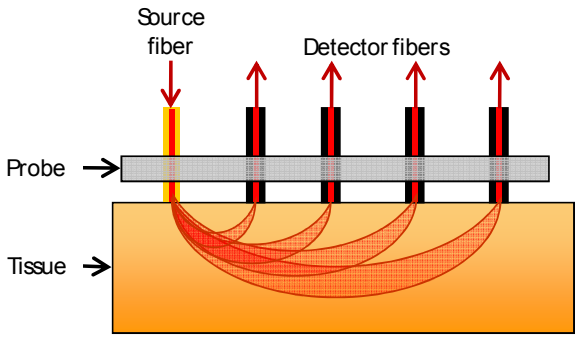


Figure 1: Schematic of probe and approximation of photon propagation pathways

III. Model of Wound Healing

In our studies to this date on the use of diffuse near infrared methodology to assess healing of wounds in various animal models, we developed a hypothesis on the expected behavior of near infrared absorption coefficients during the course of healing. This model of healing is based on the physiological changes related to vascularization that were verified by histopathology and immunohistochemistry along with optical measurements [22]. The clinical success of any

optical device would depend on the validity of this hypothesis in human studies.

Our results from independent animal studies [10, 22] demonstrate increased optical absorption as blood volume increases in healing wounds (Figure 2, solid red line). If the animal healing curve is projected forward, a decrease in optical absorption during the remodeling phase of wound healing is expected as vessel density/volume decreases to normal levels (Figure 2, dashed red lines). It needs to be noted that the time dependence of NIR optical absorption for human patients is different than that observed during the animal studies. Human patients are first seen when they have already developed wounds, corresponding to a constant absorption level (non-healing) in our model of healing. Any progress in healing manifests itself by a decrease in the absorption coefficient, and a convergence to the values of non-wound tissue (Figure 2, dashed red lines). In wounds that do not heal, the level is expected to stay constant (Figure 2, blue markers).

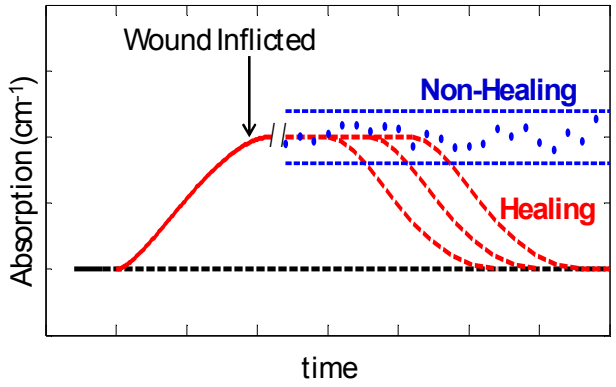


Figure 2: Hypothesized wound healing curve. The dashed black line represents normal (non-wound) tissue. The solid red line represents the results of our animal studies. The dashed red lines represent the hypothesized curve for healing wounds. The blue markers represent measurements on non-healing wounds. The black dashed line represents a control (non-wound) measurement.

The human studies necessary to validate use of our method in chronic wounds need to occur during a period of at least 12 weeks with weekly measurements to follow any changes in a statistically significant manner. Compliance can become a problem and in this paper we report our initial human data. The case studies presented validate our model, and we fully understand the need for more data to make it clinically relevant.

In the course of our measurements we realized that the value of our method lies in its ability to penetrate tissue at a depth of several mm. Wounds on feet of diabetic patients are on non-flat surfaces, with callous tissue and simple visual observation or surface optical measurements do not reveal information about the subsurface processes of healing. Therefore, misdiagnosis may occur or treatment may not be altered in a timely fashion. This has direct implications on the quality and cost of care for chronic wounds [4, 5].

IV. Methods

A. Near Infrared Instrumentation

Details of the frequency domain near infrared instrument have been described previously [10]. Briefly, an optical fiber was used to deliver intensity modulated light (70MHz) to the tissue from four diode lasers (λ = 685, 780, 830, and 950 nm). Four optical fibers were used to deliver backscattered light from the tissue to the instrument. A Teflon probe was used to hold the fibers in place, with the four detector fibers fixed at ρ = 4, 8, 12, and 16 mm from the source fiber. Using the diffusion approximation, it is possible to calculate the optical absorption and reduced scattering coefficients (μ_a and μ'_s) from the amplitude and phase shift of backscattered light at each detector position.

B. Human Subjects

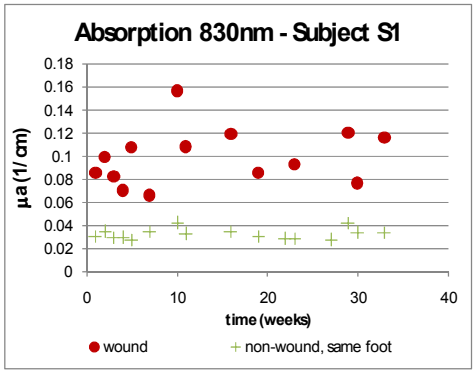
Seven subjects with diabetes and chronic wounds were recruited from the Drexel University Wound Healing Center in Philadelphia, PA. At the time this manuscript was written, two of the nine wounds have healed, while the remaining five wounds remain unhealed. Data for only two of the five non-healing wounds are presented here, because similar trends were observed for non-healing wounds. All patients were between 18 and 65 years of age, had documented diabetes mellitus for at least 6 months, and had an ankle or foot wound with a minimum surface area of 1 cm^2 that was secondary to the complications of diabetes, including vascular disease and/or neuropathy. All patients received standard wound care, which included weekly or biweekly debridement, treatment with moist wound healing protocols, and offloading when appropriate. In some patients, active wound healing agents such as topical hydrogels, growth factors, and hyperbaric oxygen were employed.

All optical measurements were conducted prior to wound debridement on a weekly or biweekly basis. During each measurement session, the wounds of each patient were interrogated using the NIR instrument in up to ten different locations. Measurement locations were chosen based on the geometry and size of each wound, and can be classified into four general locations: (1) directly on the wound, (2) intact skin at the edge of the wound, (3) non-wound tissue on the contralateral limb symmetric to the wound location if possible, (4) non-wound tissue on the wounded limb at a distance of at least 2 cm from the wound. Tegaderm transparent sterile dressing (3M Health Care) was used to cover the fiber optic probe during all measurements.

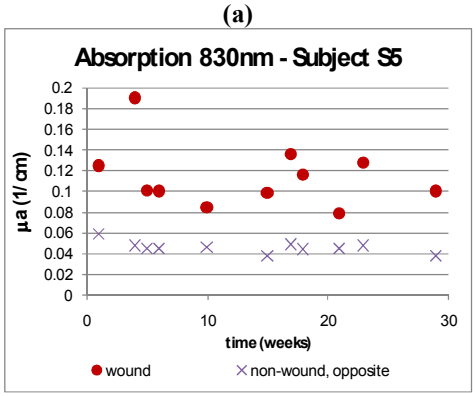
Wounds were digitally photographed during each measurement session using cross-polarization to reduce surface reflection. Wound areas were calculated from the photographs using image analysis code developed with Matlab (Mathworks, Inc.) software.

V. Results and Discussion

The values of absorption coefficient (μ_a) at each measurement time point are shown for two representative subjects with non-healing wounds in Figure 3. Similar results were observed for all non-healing wounds. The subjects presented with diabetic ulcers on the plantar aspect of their feet measuring 6.3 cm^2 (figure 3a) and 27.5 cm^2 (figure 3b) in area, as determined by image analysis of digital photographs. After 28 and 33 weeks of treatment, the wound areas were 5.5 cm^2 and 23.8 cm^2, respectively. The quality of new granulation tissue was poor as assessed by the clinician. Each wound was interrogated with diffuse NIR each time the patients came for routine wound cleaning procedures. During each measurement session, the centers of open wounds (Fig 3, solid circles) and non-wounded tissue on the plantar aspects of the same or opposite feet (Fig 3, + and x symbols) were measured. Optical absorption at the non-wound measurement locations was constant over the measurement period, and optical absorption at the wound locations did not establish a dynamic trend.



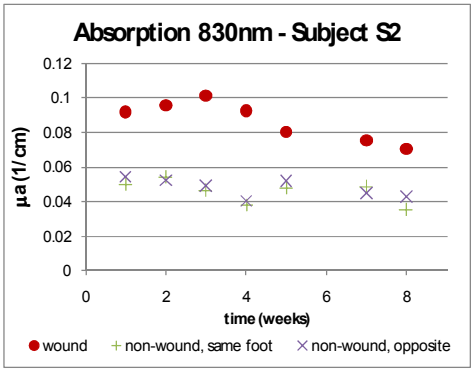
(a)



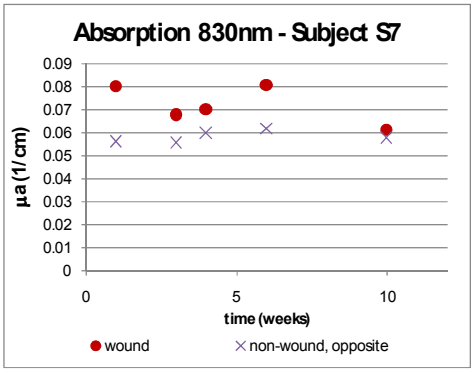
(b)

Figure 3: Optical absorption coefficient (μa) at 830 nm for two wounds that showed little or no clinical healing for the duration of the study

Figure 4 shows the values of absorption coefficient (μ_a) for two subjects with healing wounds. The subjects presented with diabetic wounds measuring 11.1cm^2 (figure 4a) and 1.98 cm^2 (figure 4b) in area, as determined by image analysis of digital photographs. After 8 and 10 weeks of treatment, the wounds were nearly closed, with calculated areas of 0.26 cm^2, and 0.14 cm^2, respectively. The quality of new tissue was good, as assessed by the clinician. Each wound was interrogated with diffuse NIR each time the patients came for routine wound cleaning procedures. During each measurement session, the centers of open wounds (Fig 4, solid circles) and non-wounded tissue on the plantar aspects of the same or opposite feet (Fig 4, + and x symbols) were measured. Optical absorption at the non-wound measurement locations were constant over the measurement period, while optical absorption at the wound locations established a dynamic trends by decreasing toward the optical properties of normal tissue as wound healing progressed.



(a)



(b)

Figure 4: Optical absorption coefficient (μa) at 830 nm for two wounds that healed during the study

The non-wound data in Figures 3 and 4 demonstrate constancy of optical properties of tissue in healthy locations on patient feet. The within-patient variance of μ_a across all time points was less than 15% of the mean for non-wound tissue. The variance of μ_a in optical phantoms measured during the same time period was less than 4% of the mean; therefore much of the variance in human subjects can be attributed to physiological changes in tissue over time. Comparison of "non-wound, same foot" data to "non-wound, opposite foot" data in Figure 4 demonstrates that stable data can be obtained from both limbs. This is an important finding because many diabetic patients do not have

contralateral limbs due to amputation; therefore they might not have a location symmetric to the wound to use for comparison.

In summary, the preliminary results from this pilot study support our hypothesis that changes in optical properties of wounds reflect wound healing status. 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. Such dynamic changes of optical properties were not observed in the non-healing wound, indicating the possibility of using diffuse near infrared technology to assess the healing of wounds in a clinical setting. Analysis of the existing data may be enhanced by calculating physiological parameters such as oxyhemoglobin and deoxyhemoglobin concentration from the optical absorption coefficients and observing their trends in healing and non-healing wounds. This study is ongoing; however, it may be necessary to expand to multiple wound centers in order to recruit a patient population large enough to provide statistically significant results.

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