EVALUATION OF DIABETIC FOOT ULCER HEALING WITH HYPERSPECTRAL IMAGING OF OXYHEMOGLOBIN AND DEOXYHEMOGLOBIN

Running Title: Hyperspectral imaging of diabetic foot ulcers

Aksone Nouvong DPM\textsuperscript{a}, Byron Hoogwerf MD\textsuperscript{b},\textsuperscript{*}, Emile Mohler MD\textsuperscript{c}, Brian Davis PhD\textsuperscript{b}, Azita Tajaddini PhD\textsuperscript{b}, Elizabeth Medenilla MD\textsuperscript{c}

\textsuperscript{a} UCLA/Olive View Medical Center, Los Angeles, CA; \textsuperscript{b} Cleveland Clinic, Cleveland, OH; \textsuperscript{c} University of Pennsylvania School of Medicine, Philadelphia, PA. *Current affiliation: Lilly USA, LLC, Indianapolis, IN.

Corresponding Author:
Aksone Nouvong, DPM
anouvong@ucla.edu

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Objective- Foot ulceration remains a major health problem for diabetic patients and has a major impact on cost of diabetes treatment. We tested a hyperspectral imaging technology that quantifies cutaneous tissue hemoglobin oxygenation and generated anatomically relevant tissue oxygenation maps to assess the healing potential of diabetic foot ulcers (DFU).

Research Design and Methods- Prospective single arm blinded study that enrolled 66 patients with type 1 and 2 diabetes mellitus (DM) and followed them over a 24-week period. Clinical, medical and diabetes histories were collected. Transcutaneous oxygen tension was measured at the ankles. Superficial tissue oxyhemoglobin and deoxyhemoglobin were measured with hyperspectral imaging from intact tissue bordering the ulcer. A healing index, derived from oxyhemoglobin and deoxyhemoglobin values was used to assess the potential for healing.

Results- Fifty-four patients with 73 ulcers completed the study; 54 ulcers healed while 19 ulcers did not heal at 24 weeks. When using the healing index to predict healing, the sensitivity was 80% (43/54), the specificity was 74% (14/19), and the positive predictive value was 90% (43/48). The sensitivity, specificity and positive predictive values increase to 86%, 88% and 96% respectively, when removing three false positive osteomyelitis cases and four false negative cases due to measurements on a callus. The results indicate that cutaneous tissue oxygenation correlate with wound healing in DM patients.

Conclusion- Hyperspectral imaging of tissue oxyhemoglobin and deoxyhemoglobin may predict healing of DFUs with high sensitivity and specificity based on information obtained from a single visit.
Diabetes mellitus (DM) is a major global disease which affects 194 million people worldwide and is expected to increase in prevalence to 344 million by the year 2030 (1). One of the major complications of DM is foot ulceration which occurs in as many as 15-25% of type 1 and 2 patients over their lifetime (2-4). Studies show that between 2-6% of DM patients will develop a foot ulcer every year (5, 6). The feet of patients with DM are at risk for ulceration due to a wide range of pathological conditions, the major three being peripheral neuropathy, foot deformity and trauma which may be exacerbated by comorbid peripheral vascular disease (4, 7). If left untreated, foot ulcers will lead to infection and deep tissue necrosis (8).

Foot pathology is a major source of morbidity in patients with DM and is a leading cause of hospitalization. Infected and/or ischemic diabetic foot ulcers (DFU) account for about 25% of all hospital days among patients with DM. Previous studies have shown that a DFU precedes roughly 85% of all lower extremity amputations in patients with DM (10, 11) and more than 88,000 amputations are performed annually on diabetic patients (12). The cost to manage foot disorders is estimated at several billion dollars annually (5, 9). Successful clinical management of DFU has not only the potential to reduce the cost of caring for these patients but also improves quality of life by reducing comorbidities.

Current treatment options for DFU’s may include offloading to reduce pressure on the wound, wound care to prevent infections, and wound debridement to remove necrotic debris and restimulate the wound healing process (12-14). Even with these measures, some fraction of wounds fail to heal. Having a means to assess healing potential may help triage wounds earlier to more aggressive therapies, thereby avoiding infections and amputations.

Clinical measurements of microvascular function may be an important part of DFU assessment (15-17). Hyperspectral imaging (HSI) was developed as a novel non-invasive diagnostic tool to quantify tissue oxygenation and generate anatomically relevant maps of microcirculatory changes seen in DM patients (18). HSI generates a map of regions of interest based on local molecular composition. With proper wavelength selection, spatial maps of molecules such as oxyhemoglobin and deoxyhemoglobin can be acquired.

A pilot study of ten type 1 DM patients with 21 DFU sites showed that HSI identified changes in tissue oxygenation in the diabetic foot that were predictive of ulcer healing (18). The sensitivity, specificity, and positive predictive value of the healing index was 93, 86, and 93%, respectively. The goal of the current study was to test the accuracy of HSI in evaluating the healing potential of DFU’s in a large number of type 1 and 2 DM patients.

**RESEARCH DESIGN AND METHODS**

This was a prospective observational study conducted at three centers: UCLA/Olive View Medical Center, Cleveland Clinic and University of Pennsylvania School of Medicine. Patients were followed for 11 visits over 24 weeks. The primary endpoint was to establish the effectiveness of hyperspectral tissue oxygenation mapping (HTOM) for predicting whether DFU’s in both type 1 and 2 DM patients would heal. Healing was defined as complete re-epithelialization at 24-weeks. The study was designed based on the preliminary data obtained from the pilot study (18).

**Inclusion criteria:** Patients age 21 to 85 diagnosed with type 1 or 2 DM with at least one DFU were eligible. The diagnosis
of type 1 and 2 DM was established according to the recommendations of the American Diabetes Association Expert Committee (19).

**Exclusion criteria:** Exclusion criteria included heart failure with consequent lower-extremity edema, stroke or ischemic attack with residual nerve dysfunction, uncontrolled hypertension, end stage renal disease/renal transplant, peripheral arterial disease that was severe enough to require surgery, severe peripheral edema, any other serious chronic disease that can affect wound healing, treatment with antineoplastic drugs or glucocorticoids, and pregnant or lactating women.

**Data Collection:** Studies were performed according to a uniformed study protocol that was approved by Institutional Review Boards at each center. After describing the protocol and answering questions, all patients agreeing to participate signed an approved informed consent form. Medical and family history was collected from each patient. Clinical evaluation included age, gender, ethnicity/race, weight, height, BMI, systolic and diastolic blood pressure, ankle brachial index (ABI), HbA1c, DM type and duration. Neuropathy was graded according to the Neuropathic Symptom Score (NSS) and Neuropathy Disability Scores (NDS) (20, 21).

Transcutaneous oxygen tension (tcPO\(_2\)) was measured at the ankle of both legs using a transcutaneous oxygen monitor (Model PF-5000, Perimed, Inc., North Royalton, OH). The oxygen monitoring electrodes were coupled to skin using adhesive fixation rings, an electrolytic solution and set to maintain a temperature of 44°C. The solution was allowed to equilibrate for 15 minutes prior to recording.

Hyperspectral tissue oxygenation mapping (HTOM) and skin temperature at the center of the image were collected with a commercial HSI system (OxyVu, HyperMed Inc., Burlington MA). The HSI system obtains multiple images at discrete wavelengths, providing a diffuse reflectance spectrum for each pixel in the image. The system uses wavelengths between 500 and 660 nm to include oxyhemoglobin (oxy) and deoxyhemoglobin (deoxy) absorption peaks. Tissue oxygenation images or maps were constructed from oxy and deoxy values determined from each pixel in the image. Skin temperature was monitored with an infrared remote temperature sensor.

Prior to imaging, the system was calibrated to a reflectance card. Patients were imaged supine on a standard examination table or reclining chair and were allowed to rest for 10 minutes to minimize systemic vascular effects. Dorsal foot and periwound tissue were imaged. A fiducial target was placed to facilitate image realignment correcting for patient movement. Image registration, processing and quality assessment were conducted following the procedure. For wounds larger than 1 cm in diameter, mean oxy and deoxy values were extracted from a 1-cm radial border consisting of intact skin in the periwound region, while avoiding any hyperkeratotic tissue. For wounds less than 1 cm in diameter, a 0.5 cm border was used.

Spectral decomposition was used to extract relative values of tissue oxy and deoxyhemoglobin from the diffuse reflectance spectra by comparing to standard transmission spectra from solutions (22). Oxy and deoxy units represent relative concentrations of oxyhemoglobin and deoxyhemoglobin found in the tissue volume measured by the HSI system (approximately the effective pixel size at the object, 0.1 mm x 0.1 mm, multiplied by the penetration depth of light into tissue, about 1 to 2 mm in this wavelength range). Tissue oxygen saturation (StO\(_2\)), the fraction of oxygenated hemoglobin in superficial (predominantly subpapillary plexus) blood vessels, was calculated as the percentage of oxy over the sum of oxy and deoxy.
Ulcers were classified into one of two groups: ulcers that healed within 24 weeks or ulcers that did not heal within 24 weeks. Ulcers with complete re-epithelialization and no exudates at the last visit (~24 weeks) were classified as healed. A healing index was then derived to best separate healed from non-healed ulcers. The healing index was calculated as the distance between the point defined by the oxy and deoxy value, and the linear discriminant decision line that best separated healed ulcers from non-healed ulcers. A positive healing index was more likely to heal while a negative healing index was more likely not to heal.

Patients received regular care by their doctors which included offloading and debridement when required. The treating physicians were blinded to the HSI data. No criteria for wound size or duration were used to select patients. Clinical and HSI data were captured on case report forms and uploaded into a central data base and central file server, respectively.

Statistics: Statistical analyses were performed by an independent biostatistician. The data were analyzed to detect differences between patients whose DFU’s healed and those DFU’s that did not heal. For categorical factors such as gender, differences between the healed and non-healed proportions were compared with the Chi-square test. For continuous factors such as oxy, deoxy and StO$_2$, differences between the means of the two groups were compared with Student's t-test using the more conservative test assuming unequal variances. Values are reported as means and SD. A p-value < 0.05 was considered significant. Sensitivity, specificity and positive predictive values for healing were calculated using standard definitions. Linear discriminant analysis was used to develop the threshold for separating the healed and not healed groups.

RESULTS

A total of 66 type 1 and 2 DM patients with DFU’s were enrolled in the study. The mean age was 50 (range 25 to 68) and consisted of 58 males and 8 females. Twelve patients were excluded from the study because they either failed to complete the study (n=10) or required amputation prior to the 24-week visit (n=2). The 54 patients who completed the study presented with 73 ulcers which comprise the data in this report. Fifty-four ulcers healed while 19 ulcers did not heal.

No significant differences in demographics or clinical characteristics were detected when comparing patients with DFU’s that healed (n=38) and patients with any DFU’s that did not heal (n=16). Both groups were found to be well matched for gender, age and other patient demographics and clinical characteristics as shown in Table 1. No differences were seen in the level of neuropathy when comparing feet with DFU’s that healed and feet with any DFU’s that did not heal. The mean neuropathy disability score (NDS) was 6.7±4.9 and 7.7±3.4, respectively (p=0.39).

The ankle brachial index (ABI) was similar for limbs with healed and non-healed ulcers (Table 2). All ABIs were 0.78 or greater which is consistent with at most mild peripheral arterial disease in a few subjects. As expected for a high-risk diabetic group, a large fraction of limbs (47%) were noncompressible, having an ABI greater than 1.2. Mean tcPO$_2$ at the ankle was 48±15 mmHg and 46±18 mmHg, respectively (p=0.61). In addition, no differences were seen in the level of tissue oxygenation. Mean StO$_2$ taken from the dorsal foot had mean values of 53±13% for feet with DFU’s that healed and 47±12% for feet with any DFU’s that did not heal (p=0.16).

When evaluating mean HTOM values in the periwound area, significant differences were observed when comparing DFU’s that healed and DFU’s that did not heal (Table 2).
Higher oxy values were noted around DFU’s that healed, 85±21, versus 64±22, (p=0.0013). Mean StO₂ was also higher in healing ulcer: 66±9% versus 60±10%, p=0.024. The temperature around the ulcer was not found to be different. A difference in ulcer size was detected when comparing DFU’s that did not heal to DFU’s that healed. The mean areas were 5.8±6.2 cm² and 3.2±3.9 cm², respectively. However, based on the more conservative Student’s t-test using unequal variances, this difference was not significant (p < 0.10).

No major difference was seen when comparing anatomical site distribution for the healed and non-healed ulcers. Twenty-nine ulcers were located on the plantar metatarsal phalangeal joints; 20 healed, 9 did not heal. Fifteen ulcers were located on the plantar phalangeal joints; 10 healed, 5 did not heal. Eight ulcers were located on the plantar arch; 7 healed; 1 did not heal. Seven ulcers were located on the dorsal metatarsal phalangeal joints; 5 healed, 2 did not heal. The remaining ulcers were located on the heel (n=5), lateral foot (n=4), dorsal phalangeal joints (n=3) and distal ankle (n=2).

**HTOM as a predictor for DFU healing:** Hyperspectral and visual images from a patient at baseline with a DFU that healed and corresponding images from a patient with a DFU that did not heal are shown in Figure 1. In the healed DFU case, the mean values for oxy, deoxy and StO₂ were 75, 34 and 69% respectively; while in the non-healed case, tissue oxygenation was lower with values of 60, 53 and 53%, respectively.

A scatter plot of mean oxy and deoxy values, measured from periwound tissue for all ulcers during the initial visit, is shown in Figure 2A. The data were grouped according to the healing status at 24 weeks. The linear discriminant decision line best separating healed ulcers from non-healed ulcers is shown (diagonal solid line). HTOM points on the left of the decision line best represent ulcers that do not heal and would have a negative healing index while points on the right of the decision line best represent ulcers that heal and would have a positive healing index. The mean healing index for ulcers that did not heal in 24-weeks is significantly different compared to ulcers that healed within 24-weeks (-0.15 vs. 0.15, p<0.0001).

A plot of the healing index is shown in Figure 2B. The healing index indicated that the sensitivity for healing was 80% (43 of 54), the specificity was 74% (14 of 19), and the predictive positive value (PPV) was 90% (43 of 48). The sigmoidal line represents the probability of healing based on the healing index from the two groups. Of the 5 non-healed ulcers that had a positive healing index, three had underlying osteomyelitis and one refused to wear proper foot gear. The remaining 70 ulcer sites did not have any clinical signs of osteomyelitis. Of the 10 healed ulcers that had a negative healing index, the tissue surrounding 4 of the ulcers was callused.

When selecting borders around the wound, the thickness of the border was important. The ability to separate healing from nonhealing ulcers was optimal when using a border thickness between 0.5 and 1cm. Reduce discrimination was observed when increasing the border thickness to 2cm.

**CONCLUSIONS**

In this multi-center 24-week study, we show that HTOM provides a local assessment of microvascular oxygenation status that is predictive of ulcer healing in type 1 and 2 DM patients. HTOM offer high sensitivity (81%) and specificity (74%) in determining healing potential. Additionally, the data shows that HSI can assess healing capacity with a 90% positive predictive value. Higher ulcer healing predictions are possible if care is taken to avoid evaluating ulcers with underlying osteomyelitis and ulcers with
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overlying callus. The sensitivity, specificity and positive predictive values increase to 86%, 88% and 96% respectively, when removing three false positive osteomyelitis cases and four false negative cases due to measurements on a callus.

The results of this study not only confirm preliminary results obtained in an earlier pilot study (18), but firmly establish that HTOM serve as a clinically relevant technique for predicting DFU healing, based on an evaluation of 54 patients and 73 ulcers. Because data are collected and assessed from the first visit, HTOM allows the physician to identify DFU’s at risk of not healing much earlier, i.e., without having to wait for 6 months. These results demonstrate that HSI can measure tissue oxygenation with spatial resolutions of 100 microns, without coming into contact with the patient’s foot.

Adequate tissue microvascular perfusion is an essential element for wound healing. The HSI system used in this study was designed to evaluate tissue oxygenation at the superficial microvascular level. HTOM values predicted healing status better when evaluating tissue close to the wound margins, thus demonstrating periwound changes in microvessels that can be used for assessing the healing capacity of a DFU. Healed DFU’s demonstrate increased microvascular oxygenation as evidenced by an increase in oxy and StO2 when compared to non-healed DFU’s. A similar increase in StO2 has been noted previously with another independently established technique (23).

Based on the neuropathy disability score, 65% of the patients had moderate to severe foot neuropathy (NDS ≥ 5). HTOM taken from the dorsal foot showed that 93% of patients had a StO2 that was greater than 30%, while 87% of patients had a tcPO2 at the ankle that was greater than 30 mmHg. Both oxygenation techniques indicate the foot was reasonably well oxygenated in the basal state and differences between healing and non-healing ulcers only surface once the wound is present and the tissue is either able to or not able to respond to the injury.

In summary, HTOM can accurately predict wound healing in advance, e.g., several months before the wound heals. Physicians are now able to identify which DFUs are at risk for delayed healing based on reduced oxygenation levels and thereby can elect to triage to therapies designed to increase oxygen delivery to tissue. Based on these results assessing microvascular perfusion, HTOM may be used in three main areas of patient care: DFU management, surgical planning, and monitoring of therapy. The addition of quantitative oxygenation information to the current available tools and treatment could allow for more targeted therapy, and could potentially accelerate current case resolutions.

Ultimately, HTOM has the potential to screen for lower extremity complications due to DM. This is based on its ability to quantify risk in a specific area of tissue and the ability to observe ischemic complications before they would be visible to the naked eye. HTOM provide the DM caregiver with information necessary to treat and monitor foot complications faster and more specifically than would be possible with currently available methods which lack adequate sensitivity, specificity and adequate spatial localization to assess the microvascular status of the foot.

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REFERENCES
Table 1: Patient demographics and clinical characteristics

<table>
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<tr>
<th></th>
<th>Patients with Healed Ulcers</th>
<th>Patients with any non-Healed Ulcers</th>
<th>P-value</th>
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<tr>
<td>No. of Subjects</td>
<td>38</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Age (range)</td>
<td>51 (34 - 68)</td>
<td>52 (25 - 63)</td>
<td>0.73</td>
</tr>
<tr>
<td>Gender (m/f)</td>
<td>35 / 3</td>
<td>14 / 2</td>
<td>0.34</td>
</tr>
<tr>
<td>DM (type 1/ type 2)</td>
<td>15 / 23</td>
<td>8 / 8</td>
<td>0.19</td>
</tr>
<tr>
<td>DM Duration (yr)</td>
<td>13 ± 10</td>
<td>12 ± 8</td>
<td>0.77</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>9.7 ± 2.6</td>
<td>9.5 ± 2.4</td>
<td>0.83</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>34 ± 10</td>
<td>31 ± 12</td>
<td>0.41</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>135 ± 24</td>
<td>142 ± 21</td>
<td>0.28</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>76 ± 13</td>
<td>79 ± 9</td>
<td>0.38</td>
</tr>
<tr>
<td>NSS</td>
<td>5.3 ± 3.3</td>
<td>4.9 ± 3.0</td>
<td>0.65</td>
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<tr>
<td>NDS</td>
<td>7.7 ± 3.4</td>
<td>6.7 ± 4.9</td>
<td>0.39</td>
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</tbody>
</table>

Data are means ± SD unless otherwise indicated. NSS = neuropathy symptom score. NDS = neuropathy disability score.

Table 2: Lower limb assessment

<table>
<thead>
<tr>
<th></th>
<th>Foot with non-Healed ulcers (N=19)</th>
<th>Foot with Healed ulcers (N=54)</th>
<th>P-values</th>
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</thead>
<tbody>
<tr>
<td><strong>At the ankle</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N (ABI &lt; 0.4)</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>N (ABI 0.4 – 0.69)</td>
<td>0</td>
<td>0</td>
<td>---</td>
</tr>
<tr>
<td>N (ABI 0.70 – 0.89)</td>
<td>3</td>
<td>4</td>
<td>---</td>
</tr>
<tr>
<td>N (ABI 0.90 – 1.19)</td>
<td>6</td>
<td>16</td>
<td>---</td>
</tr>
<tr>
<td>N (ABI &gt; 1.2)</td>
<td>5</td>
<td>19</td>
<td>---</td>
</tr>
<tr>
<td>N (ABI, NR)</td>
<td>5</td>
<td>15</td>
<td>---</td>
</tr>
<tr>
<td>tcPO^2 (mmHg)</td>
<td>46 ± 16</td>
<td>48 ± 15</td>
<td>0.61</td>
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<tr>
<td><strong>At the foot</strong></td>
<td></td>
<td></td>
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<tr>
<td>Oxy (dorsum, au)</td>
<td>42 ± 18</td>
<td>44 ± 19</td>
<td>0.72</td>
</tr>
<tr>
<td>Deoxy (dorsum, au)</td>
<td>44 ± 13</td>
<td>37 ± 13</td>
<td>0.081</td>
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<tr>
<td>StO_2 (dorsum, %)</td>
<td>47 ± 12</td>
<td>53 ± 13</td>
<td>0.16</td>
</tr>
<tr>
<td><strong>At the ulcer border</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Ulcer size (cm^2)</td>
<td>5.8 ± 6.2</td>
<td>3.2 ± 3.9</td>
<td>0.10</td>
</tr>
<tr>
<td>Oxy (au)</td>
<td>64 ± 22</td>
<td>85 ± 21</td>
<td>0.0013</td>
</tr>
<tr>
<td>Deoxy (au)</td>
<td>41 ± 12</td>
<td>44 ± 14</td>
<td>0.47</td>
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<tr>
<td>StO_2 (%)</td>
<td>60 ± 10</td>
<td>66 ± 9</td>
<td>0.024</td>
</tr>
<tr>
<td>Healing Index</td>
<td>-0.15 ± 18</td>
<td>0.15 ± 19</td>
<td>&lt;0.0001</td>
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<tr>
<td>Temperature (°C)</td>
<td>33 ± 3</td>
<td>33 ± 3</td>
<td>0.83</td>
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</table>

Data are means ± SD unless otherwise indicated. Sample numbers are shown in parentheses. ABI = ankle brachial index.
Figure Legends

**Figure 1:** Visible and hyperspectral image of a healing diabetic foot ulcer taken with the HTOM system. *(Top row)* Healed DFU case. HTOM values are 75, 34, and 69% for oxy, deoxy and StO₂, respectively. *(Bottom row)* Non-healed DFU case. HTOM values are 60, 53, and 53% for oxy, deoxy and StO₂, respectively. Tissue oxygenation is higher in the healed ulcer as seen by the more purplish tone compared to the more cyan/green tone. Mean oxy and deoxy values were determined for each ulcer from an approximate 1 cm thick band drawn within the periwound area.

**Figure 2(A):** Oxy and deoxy values for healed and non-healed DFU’s. The diagonal solid line represents the decision line for a healing algorithm based on oxy and deoxy values. Ninety percent of points lying to the right of the line healed. The diagonal dashed line represents a second decision line where 87% of the points (7 of 8 excluding calluses) lying to the left of the line do not heal. *(B)* Probability of healing based on HTOM healing index for healed and non-healed DFU’s. An ulcer with a positive healing index has a higher likelihood to heal. Open diamonds represent ulcers with underlying osteomyelitis that did not heal. Open circles represent ulcers that healed that were surrounded by callus.
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(A) Scatter plot showing the correlation between Oxy (arb units) and Deoxy (arb units) for healed and non-healed wounds at 24 weeks.

(B) Graph depicting the probability of healing (%) against the DFU Healing Index, with sample number indicated.