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***In vivo* assessment of burn depth and wound healing using a handheld terahertz hyperspectral scanner**

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Abbreviations used: THz: Terahertz, THz-TDS: terahertz time-domain spectroscopy, TD: time-domain, ANSI: American National Standards Institute, THz-TDSI, terahertz time-domain spectral imaging, CW: continuous wave, ROI: regions of interest, SA: spectral amplitude, SS spectral slope, BW: bandwidth, IM: intramuscular, HDPE: high density polyethylene, H&E: hematoxylin and eosin, PCA: photoconductive antenna,

ABSTRACT

The accuracy of clinical assessment in partial-thickness burn injuries has remained as low as 50-75%. Depending on the depth and environmental factors in the wound, such as reactive oxygen species, inflammation, and autophagy, partial-thickness burns can heal spontaneously or require surgical intervention. In this study, we demonstrate that Terahertz Time-Domain Spectral Imaging (THz-TDSI) is a promising tool for *in vivo* quantitative assessment of burn injuries. We used a novel handheld THz-TDSI scanner to characterize burn injuries in a porcine scald model with histopathological control. Prior work used THz reflectivity (representation of tissue hydration) as the only source of signal contrast. However, we used the spectral amplitude and the spectral slope of the terahertz electric field to distinguish the different severities of burns, suggesting that the energy loss due to electromagnetic scattering from skin constituents serves an additional metric to quantitatively assess burn injuries. Statistical analysis ($n = 40$) indicates that THz-TDSI can accurately differentiate between partial-thickness and full-thickness burn injuries (1-way ANOVA, $p < 0.05$) and monitor the healing process of partial thickness burns. THz-TDSI has the potential to improve burn care outcomes by helping surgeons to make objective decisions for early excision.

Keywords: Terahertz Time-Domain Spectroscopy, skin tissue hyperspectral imaging, handheld THz-TDS scanner, burn imaging, partial-thickness burn characterization, histology.

INTRODUCTION

Acute burn injuries accounted for approximately 489,000 emergency department visits in 2017¹. At triage, burn injuries are assessed by a clinical evaluation, which is a subjective method that depends on visual and tactile inspection of the injury. A physician will determine the course of treatment based on the perceived depth of thermal damage and the total body surface area that has

sustained thermal injury. Superficial burns affect the epidermis and will heal spontaneously by reepithelialization. Full-thickness burns affect the entire depth of the dermis, can result in hypertrophic or contracted scarring, and will require surgical intervention (i.e. excision and grafting) ². Partial-thickness burns, which affect the epidermis and papillary region, are challenging to assess because they may heal spontaneously or progress into deeper thickness burns. Additionally, wound conversion in partial-thickness burns depends on multiple factors, including perfusion ³, reactive oxygen species ⁴, and mechanisms such as autophagy and inflammation ⁴. Clinical evaluation for determining whether a partial-thickness burn injury will progress into a full-thickness burn has been shown to have an accuracy as low as 50-75% ⁵. This low-accuracy rate often results in delaying diagnosis until the burn declares its nature. On the other hand, early excision and grafting of full thickness burns results in overall better patient outcomes ^{6,7}. Therefore, early and accurate determination of partial-thickness burn injuries can reduce the recovery time and financial burdens that are experienced by burn patients.

Terahertz Time-Domain Spectroscopy (THz-TDS) is a coherent broadband spectral imaging technique, where a pulsed terahertz (THz) electric field (0.1-10 THz) is reflected from a sample and measured in the time-domain (TD) with sub-picosecond resolution. THz radiation has piqued the interest of biomedical researchers because THz-TDS techniques are highly sensitive to the water content of tissue. This is due to the broad absorption of water at THz frequencies that comes from collective intermolecular motions of bound and free water ⁸. Additionally, THz-TDS techniques are coherently detected and are less prone to electromagnetic scattering (compared to smaller wavelengths) ^{9,10}. Many fingerprint spectral resonances of biomolecules in polycrystalline form also exist in THz wavelengths ^{9,10}. These features make THz-TDS a promising imaging modality for diagnosis of burn injuries ^{11,12}. THz waves are non-ionizing and safe for use in humans

and animals. Previous safety studies determined that there are no negative effects *in vivo* and the only thermal effects *in vitro* are a result of extremely high power and long exposure duration of THz radiation ¹³. The American National Standards Institute (ANSI) provides laser safety standards for industrial, military, medical and other applications and their Maximum Exposure Limit of 1 mm wave radiation (> 0.3 THz frequency, or > 1.2 meV photon energy) is 0.1 W/cm^2 ¹⁴. The THz Time-Domain Spectral Imaging (THz-TDSI) scanner introduced in this study is well within this safety standard by using a pulsed system of $< 0.37 \text{ mW/cm}^2$ for 0.35 second scan time per pixel.

Several research groups have approached using THz imaging to measure burn injuries, both *ex vivo* ¹⁵ and *in vivo* ^{11,12,16}. An *ex vivo* THz study of burn wounds on porcine skin samples suggested that the primary contrast mechanism was the water content because there was a reduced reflectivity at the burn site ¹⁵. However, upon the infliction of a burn injury, physiological events, such as increased capillary permeability, increased hydrostatic pressure, and edema, occur and increase the water content ¹⁷. Later THz studies, using *in vivo* rat models, showed that the THz spectra of burns had increased reflectivity 72 hours postburn, which was consistent with overall water content increase due to edema and correlated with the density of structures within the skin ^{11,12,16,18,19}. Moreover, Suen *et al.* showed a practical benefit of using THz radiation over infrared wavelengths because of its ability for transmission and propagation through typical burn wound topical treatments and wound dressings with little attenuation ²⁰, suggesting that THz radiation has the potential to monitor burn injuries throughout the healing process with minimal discomfort to the patient.

Another practical consideration for the utility of the THz-TDS modality is its form factor. Most THz-TDS systems require raster scanning a sample around a fixed optical focus, which will not

be suitable outside research laboratories or small animal studies. To address this issue, a few research groups have developed compact and handheld THz devices. As an example, a single-point spectroscopic handheld device was developed for single-pixel measurement applications²¹, which was also used for measurement dielectric response of human skin²². A handheld single-line scanner was designed for imaging malignant breast tumors, however, it was restricted to scanning along a single dimension²³, with a 2 mm by 15 mm field-of-view (FOV). In contrast to these instruments, we have designed and built a handheld, fiber-coupled, two-dimensional THz-TDSI scanner with a maximum FOV of up to 43×27 mm, and have tested its utility in the first *in vivo* porcine burn study using THz radiation^{24,25}.

In this work, we will present an acute *in vivo* porcine scald study model, established to demonstrate the accuracy of the THz-TDSI modality to assess burn injuries. While most previous *in vivo* THz studies required a challenging alignment setup attached to a cumbersome optical table, we developed the first handheld, alignment-free, and easily deployable THz-TDSI scanner for diagnostic mapping of burn severity in large mammals. We will show that our THz-TDSI device could easily demarcate burned tissue based on the integration of the deconvolved spectral amplitude between the 0.2 to 0.8 THz band, which was within the bandwidth (BW) of our scanner. Also, we will show that using the dual-band hyperspectral slope of the deconvolved THz amplitude, we can differentiate scald burns of different depths of dermal injury against histological assessments as the gold standard ($p < 0.05$) and monitor their progression.

RESULTS

Hyperspectral analysis

Visual images, THz images, and the deconvolved reflectivity, $R(f)$, from the corresponding regions of interest (ROIs) for burn and perilesional skin are shown in Fig. 1. Due to the size of the

wounds (20×20 mm²) and the smaller FOV setting (12×19 mm²), we collected two THz images to include the burn and perilesional skin. THz images were created by integrating $R(f)$ between the frequency bin with the largest amplitude, $f_{R_{max}}$, and nearest bin where the reflectivity, before deconvolution, was below -10.5 dB, defined as $f_{-10.5dB}$. The integrated reflectivity was then normalized by the number of frequency bins, N_{bins} to calculate the spectral amplitude, SA , given by,

$$SA = \frac{1}{N_{bins}} \int_{f_{lower}}^{f_{upper}} R(f) df \quad (1)$$

where

$$f_{upper} = f_{R_{max}} + f_{-10.5dB} , \quad (2)$$

and

$$f_{lower} = f_{R_{max}} - f_{-10.5dB} . \quad (3)$$

Fig. 1. Results from THz imaging. Visual images (a-b), THz images (c-d), where the quantity shown in the colorbar is SA, and representative THz reflectivity of burned (red box) and normal (black box, control) skin within the defined ROI (e-f) (mean $\pm$ SD) are shown for each burn condition on Day 0 and Day 4. There are two side-by-side THz images shown for each $20 \times 20 \text{ mm}^2$ burn and the surrounding normal skin region, because the FOV of the THz scanner was limited to $12 \times 19 \text{ mm}^2$. (Scale bar = 5 mm).

Statistical analysis

The SA parameter alone did not show clear differences between burns severities when compared to histological assessment. Therefore, we calculated the spectral slope, SS , of $R(f)$ by computing the dual-band upper and lower spectral slopes of the linear fit between the frequency bins, f_{lower} to $f_{R_{max}}$ and $f_{R_{max}}$ to f_{upper} , in each pixel of the burned ROI. Our previous work using *in vivo* rodent¹² and porcine¹⁹ models revealed that the THz spectral slopes contain valuable hyperspectral information. An example of the calculation of SS for a single pixel is shown in Fig. 2(a). Because SA is calculated as a sum across a frequency range, this spectroscopic parameter is inherently less sensitive to frequency dependent behavior. SS , however, allows us to quantify frequency dependent changes in each ROI. One example of frequency dependent behavior is electromagnetic scattering, which is wavelength and particle size dependent²⁶ and is likely the source of signal contrast in SS . As a result, the differences in SS between burn depths suggests that physiological and structural changes in the skin constituents postburn can serve as a source of imaging signal contrast because THz wavelengths (3 mm to 10 μ m) are similar in size to some of these adnexal structures.

Fig. 2. THz Hyperspectral and statistical analysis. a) The spectral slopes of $R(f)$ when calculated using the upper and lower BW. b) A boxplot for the Z parameter when compared to histological burn depth assessment is shown for burn depths greater than and less than 50%. 1-way ANOVA showed statistical significance ($p = 0.0016$).

To utilize both the SA and dual-band SS , we optimized a Z parameter, defined as,

$$Z = a * SS_{lower} + b * SS_{upper} + c * SA, \quad (4)$$

where a , b , and c are weighting coefficients for each spectral feature and were optimized between -1 and 1 to equal -0.48, 0.58, and 0.82, respectively. Shown in the boxplot from Fig. 2(b), a one-way ANOVA showed statistical significance ($p = 0.0016$) for distinguishing burns greater than and less than 50%. Spectral measurements contained in the ROI of all THz-TDSI images were included in this analysis.

When adnexal structures are destroyed in a full-thickness wound, reepithelialization must occur from wound margins in full thickness wounds²⁷. In addition to the wound margin, new epithelium in partial thickness wounds originate from hair follicles and apocrine glands in pigs and from pilosebaceous units and apocrine sweat glands in humans^{27,28}. Considering the major role that adnexal structures play in wound healing, utilizing them as a contrast mechanism in the SS parameter in THz-TDSI for non-invasive imaging and monitoring provides a valuable window to monitor burn wound healing, care, assessment and coverage.

Wound progression

In addition to differentiating burns greater than and less than 50%, we used the THz-TDSI scanner to monitor the wound progression over the course of the study. We used the same a , b and c coefficients optimized for the hyperspectral Z parameter on Day 4 to calculate the Z -value for THz measurements obtained on Days 0 to 3. Fig. 3(a, c, e, g) shows the locations where we marked ROI's on each day. To ensure an unbiased comparison between days and account for ROI location

differences, we created 5 randomized subsets of 8 pixels within each ROI and show the standard deviation as the error bar in Fig. 3(b, d, f, h).

Fig. 3. Monitoring wound healing with THz-TDSI. The daily normalized integral of the spectral amplitude, SA, ROI locations (pixel size is $1 \times 1 \text{ mm}^2$) and Z-parameter values (mean $\pm$ SD) are shown for a representative superficial partial thickness injury with 22% burn depth (a-b), superficial partial thickness injury with 37% burn depth (c-d), deep-partial thickness injury with 65% burn depth (e-f), and full thickness injury with 100% burn depth (g-h). When considering all burns in this study, Z parameter increased over time in burns less than 50% depth (i) but remained relatively constant for burns greater than 50% depth (j).

Fig. 3 (a-d) shows a marked increase in Z for two representative superficial partial-thickness burns whose depths were 22% and 37%, respectively. When considering all burns in the study which have less than 50% depth, Fig. 3 (i) shows that Z also had an increasing trend over the course of the study. Fig. 3 (e-h) shows that Z remained relatively constant in a representative deep partial-thickness burn with 65% depth and a full-thickness burn with 100% depth. Similarly, Fig. 3 (j) shows that Z remained constant when considering all burns over 50%. A similar phenomenon is observed in laser doppler imaging, where perfusion increases in superficial burns but remains limited in deeper burns ²⁹. Compromised perfusion is a result of destroyed and damaged microvasculature post-burn and is an important factor for wound healing.

In this work, we used a handheld THz-TDSI scanner to characterize burn injuries in an acute *in vivo* porcine scald model. The scanner was designed and built by our research team and included a custom-fabricated f- θ lens for 2D hyperspectral THz imaging. We showed that, by using the deconvolved spectral amplitude, we could demarcate burned and healthy tissue zones. Including the dual-band spectral slope in the hyperspectral Z -parameter allowed us to differentiate scald burns with depths greater or less than 50% as determined by histological assessment and monitor them over time. Our results suggest that the scattering loss from a THz-TDSI signal can be used to differentiate burn severities and monitor wound healing in partial thickness burns. Further *in vivo* studies will include improved image co-registration and handheld THz instrumentation hardware which has been scaled up to create a larger FOV, scan rate, and enhanced image processing algorithms.

MATERIALS & METHODS

Study protocol

The experimental protocol used in this study was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at Stony Brook University. This study was carried out in compliance with the ARRIVE guidelines and all experiments were conducted in accordance with the protocol approved by IACUC at the Stony Brook University. The animal model was based on 12-weeks of age, female Landrace pigs, weighing approximately 25-30 kg. Female pigs of this size were chosen based on previous works, which demonstrated reproducible animal burn models^{30,31}. Prior to inducing the burns on Day 0, the animal was sedated through an intramuscular (IM) injection of a pre-anesthetic cocktail consisting of ketamine (20 mg/kg), xylazine (2.2 mg/kg), acepromazine (0.1 mg/kg), and atropine (0.02 mg/kg), and then anesthetized with a continuous flow of 0.5 – 5% isoflurane. The pig skin was washed and the hair on its back was removed by trimming and shaving. A stencil was used to mark the locations for burn induction and guide the tattoo margins for tissue identification and image co-registration. Following burn induction, buprenorphine (0.005-0.02 mg/kg) was administered IM and a 72-hour transdermal fentanyl patch (50 µg/kg) was placed proximal to the tail. Additionally, tattoos were placed to note the tissue column and row identification and the location of each burn. These corner tattoos acted as guides for the placement of the handheld scanner and ensured that the device was placed at the same general area every day during the imaging studies. Following the tattoo procedure, the burns were debrided by gently scraping the burn with the blunt end of forceps.

The animal was kept on isoflurane throughout the imaging process and monitored by the veterinary staff of the Division of Laboratory Animal Research at Stony Brook University. After imaging, the burns were bandaged by applying triple antibiotic ointment to the individual injuries and covering the wounds with a transparent Tegaderm™ sheet (3M™, Saint Paul, MN, USA). The midsection of the pig was then wrapped with flexible gauze bandage and adhesive Tensoplast®

(BSN Medical, Hamburg, Germany). On Days 1 to 4, prior to imaging, the animal was sedated and anesthetized with the same procedure as Day 0. After imaging on Day 4, the entire burn and perilesional tissue was collected for histological assessment and the animal was euthanized through intravenous administration of Fatal Plus (100 mg/kg).

Scald burns were created on the dorsal side of the pig using the device described in Fig. 4(a), where a foam wrapped steel pipe contained a hot water inflow tube and a suction tube outlet ³¹. Water was kept at 97° C using an immersion heating circulator and flowed into the device through high-temperature rubber tubing using the circulation feature on the immersion heating device. Water temperature dropped by approximately 2° C as it flowed through the tubing for a final temperature of 95° C at the surface of the skin. Hot water was removed from the device using a vacuum pump (Adafruit Industries, New York, NY, USA) attached to a high-density polyethylene (HDPE) vacuum flask. Varied burn severities were created by exposure to 95° C water for 5, 15, and 25 seconds, using a 20×20 mm² square shaped scald device pipe.

Fig. 4. Animal model. (a) The schematic of the scald burn induction device is shown, where 95° C water would come in direct contact with the skin. (b) Scald condition locations are illustrated where the numbers in each circle represent the heat exposure duration in seconds. (c) Dermal burn depth percentage (normalized by total dermal thickness) are plotted for each experimental condition, as determined by a histopathologist using Day-4 H&E sections.

Burns were systematically distributed ($n = 20$) to minimize variation due to the dorsal location. As illustrated in Fig. 4(b), an equal number of burns in each experimental arm are created in cranial and caudal sections, in proximal and distal locations to the spine. Based on the size of the animal and previous scald models³¹, the 5-second exposure condition was selected to create a superficial burn, whereas the 25-second condition was intended to create a full-thickness burn. The 15-second condition was selected to create partial-thickness burns that would either progress into a full-thickness burn or continue the healing process by Day 4 as a superficial partial-thickness injury.

This dynamic progression effect in the 15-second burns can be seen in Fig. 4(c) by the large variance in the burn depth compared to the 5- and 25-second conditions.

Histology

At the conclusion of the study, a 50×50 mm² tissue block that included perilesional and burned skin was excised and fixed in formalin for 24 hours before storage in alcohol. Punch biopsies (4-mm diameter) were used to obtain tissue samples from various locations and stained with hematoxylin and eosin (H&E). Histological examples of each scald condition are shown in Fig. 5. Blind evaluation was performed by a board-certified histopathologist. The burn depth was assessed by measuring the deepest point of injury, characterized by microvascular occlusion, collagen discoloration, follicular cell necrosis, mesenchymal cell necrosis, and adipocyte necrosis for very deep burns ³². The injury depth was then divided by the total dermal thickness and multiplied by 100 to calculate the percent burn depth.

Fig. 5. Histology results. Images of H&E stained skin samples are shown for each scald condition. Images were captured using a Nikon mm-60 microscope (Nikon Instruments Inc. Tokyo, Japan) with a 5x objective lens. Yellow arrows indicate pyknotic cells (scale bar = 0.2 mm).

Handheld THz-TDSI scanner

THz radiation was generated and detected using photoconductive antennas (PCA) as part of an Asynchronous Optical Sampling (ASOPS, Menlo Systems Inc, Newton, NJ) THz-TDS system. The ASOPS THz-TDS system uses two 1560 nm femtosecond lasers, one used to pump an InGaAs/InAlAs THz PCA emitter and the other to probe a LT InGaAs/InAlAs THz PCA detector. Each laser is set to a slightly offset repetition rate to sample the probe pulse in the time-domain. This is beneficial because, rather than sampling the TD signal in the conventional method that utilizes a motorized stage delay line, we sample the TD signal electronically for much faster data acquisition time while maintaining high signal to noise. The laser repetition rate was set to 100 MHz, with the sampling rate set by the ASOPS difference frequency equal to 100 Hz, which resulted in measurement of a full THz time-domain signal every 0.01 seconds.

THz scans were performed using a Portable HAndheld Spectral Reflection (PHASR) scanner^{24,33}. The design of the PHASR scanner, shown in Fig. 6(a), features a custom HDPE f- θ lens and a mirror mounted on a motorized 2-axis gimbal stage (T-OMG, Zaber Technologies Inc. Vancouver, BC, Canada) in a telecentric configuration^{32,33}. The 12×19 mm² area can be scanned in approximately two minutes with a depth of focus much greater than the penetration depth of the THz beam²⁵. Furthermore, the scanner is “alignment-free” and maintains constant resolution across the entire FOV. Fig.6(b) shows the handheld THz scanner in an operating theatre to image porcine scald burns created in this study. The design of the PHASR scanner was pivotal for this *in vivo* porcine study because large animals cannot easily be raster scanned. Additionally, their physiological movements like respiration would cause significant imaging artifacts and potential misalignment. Using the PHASR instrument allowed for the movement of the scanning optics in tandem with any animal movements, similar to a portable ultrasound scanner, and therefore enabled an alignment-free and fast imaging operation.

Fig. 6. In vivo handheld THz setup. (a) The optical schematic for the handheld THz scanning system. (b) A visual image of the handheld scanner in use.

Further details about the THz handheld scanner can be found in Table 1. When compared to alternative advanced optical imaging techniques such as optical coherence tomography or photoacoustic microscopy, THz systems can increase the FOV by scaling up the hardware while maintaining sub-second pixel scan times. Additionally, THz-TDSI directly measures the optical properties of the wound, while techniques such as laser doppler imaging and thermography rely exclusively on physiological phenomenon, i.e. perfusion, as a contrast mechanism.

Spatial Resolution (mm)	Depth of Focus (mm)	FOV (mm ²)	Scan Time (seconds)	Pixel Size (mm)	THz Intensity (mW/cm ²)
0.76	9.55	up to 43×27	≥ 0.25 per pixel	1	Approx. < 0.37

Table 1. Handheld scanner specifications: Specifications for the handheld THz-TDSI scanner developed for burn diagnostic imaging.

Signal processing

The complete signal processing steps are outlined by the flowchart in Fig. 7. All analysis was performed using the MATLAB software (MathWorks Inc, Natick , MA, USA). The raw TD signal in each pixel was first baseline corrected ³⁵ to remove any inherent signal artifacts. A Gaussian bandpass filter, containing signal in our usable BW (0.05 to 2.5 THz pass band), along with a zero-padded split-Blackman window were applied to the TD signal before applying a Fourier Transform. The complex Fourier-domain representation of the signal, defined as $E_{samp}(f)$, was deconvolved by a reference signal, $E_{ref}(f)$, with the same signal processing steps as the sample. To minimize the impact of noise where the signal-to-noise ratio of the spectra is poor, a Wiener deconvolution algorithm was implemented, where the deconvolved spectral amplitude, $R(f)$, is given by ³⁶

$$R(f) = E_{samp}(f) \frac{E_{ref}^*(f)}{E_{ref}(f)E_{ref}^*(f) + \alpha \langle E \rangle [E_{ref}(f)E_{ref}^*(f)]} \quad (5)$$

The Wiener deconvolution implementation uses a Tikhonov Regularization method ³⁷, where $\langle E \rangle$ is the expected value and α is the regularization constant.

Fig. 7. THz signal processing steps. The signal processing flowchart is shown with selected steps depicted below and noted where analysis is performed in the time or frequency domain.

CONFLICTS OF INTEREST

P: MHA discloses intellectual property owned by the University of Washington, US Patent No. US9295402B1.

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AUTHOR CONTRIBUTIONS

Conceptualization: MHA; Data Curation: ZBH, OBO, JWZ; Formal Analysis: OBO; Funding Acquisition: MHA; Investigation: OBO, JWZ, ZBH; Methodology: MHA, AJS, JWZ; Project Administration: MHA, AJS; Resources: MHA; Software: ZBH, OBO, JWZ, MEK; Supervision: MHA, AJS; Validation: MHA, OBO, AJS; Visualization: OBO; Writing - OBO; Writing - Review and Editing: OBO, MHA, JWZ, ZBH, MEK, AJS.

DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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TABLES AND FIGURE LEGENDS

Fig. 1: Results from THz imaging. Visual images (a-b), THz images (c-d), where the quantity shown in the colorbar is SA , and representative THz reflectivity of burned (red box) and normal (black box, control) skin within the defined ROI (e-f) (mean $\pm$ SD) are shown for each burn condition on Day 0 and Day 4. There are two side-by-side THz images shown for each $20 \times 20 \text{ mm}^2$ burn and the surrounding normal skin region, because the FOV of the THz scanner was limited to $12 \times 19 \text{ mm}^2$. (Scale bar = 5 mm).

Fig. 2: THz Hyperspectral and statistical analysis. a) The spectral slopes of $R(f)$ when calculated using the upper and lower BW. b) A boxplot for the Z parameter when compared to histological burn depth assessment is shown for burn depths greater than and less than 50%. 1-way ANOVA showed statistical significance ($p = 0.0016$).

Fig. 3 Monitoring wound healing with THz-TDSI. The daily normalized integral of the spectral amplitude, SA , ROI locations (pixel size is $1 \times 1 \text{ mm}^2$) and Z -parameter values (mean $\pm$ SD) are shown for a representative superficial partial thickness injury with 22% burn depth (a-b), superficial partial thickness injury with 37% burn depth (c-d), deep-partial thickness injury with 65% burn depth (e-f), and full thickness injury with 100% burn depth (g-h). When considering all burns in this study, Z parameter increased over time in burns less than 50% depth (i) but remained relatively constant for burns greater than 50% depth (j).

Fig. 4: Animal model. (a) The schematic of the scald burn induction device is shown, where 95°C water would come in direct contact with the skin. (b) Scald condition locations are illustrated where the numbers in each circle represent the heat exposure duration in seconds. (c) Dermal burn depth percentage (normalized by total dermal thickness) are plotted for each experimental condition, as determined by a histopathologist using Day-4 H&E sections.

Fig. 5: Histology results. Images of H&E stained skin samples are shown for each scald condition. Images were captured using a Nikon mm-60 microscope (Nikon Instruments Inc. Tokyo, Japan) with a 5x objective lens. Yellow arrows indicate pyknotic cells (scale bar = 0.2 mm).

Fig. 6. In vivo handheld THz setup (a) The optical schematic for the handheld THz scanning system. (b) A visual image of the handheld scanner in use.

Fig. 7 THz signal processing steps. The signal processing flowchart is shown with selected steps depicted below and noted where analysis is performed in the time or frequency domain.

Table 1: Handheld scanner specifications. Specifications for the handheld THz-TDSI scanner developed for burn diagnostic imaging.

Spatial Resolution (mm)	Depth of Focus (mm)	FOV (mm^2)	Scan Time (seconds)	Pixel Size (mm)	THz Intensity (mW/cm^2)
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0.76	9.55	up to 43×27	≥ 0.25 per pixel	1	Approx. < 0.37
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Figures

Figure 1

Results from THz imaging. Visual images (a-b), THz images (c-d), where the quantity shown in the colorbar is SA, and representative THz reflectivity of burned (red box) and normal (black box, control) skin within the defined ROI (e-f) (mean $\pm$ SD) are shown for each burn condition on Day 0 and Day 4. There

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Figure 2

THz Hyperspectral and statistical analysis. a) The spectral slopes of $R(f)$ when calculated using the upper and lower BW. b) A boxplot for the Z parameter when compared to histological burn depth assessment is shown for burn depths greater than and less than 50%. 1-way ANOVA showed statistical significance ($p = 0.0016$).

Figure 3

Monitoring wound healing with THz-TDSI. The daily normalized integral of the spectral amplitude, SA, ROI locations (pixel size is $1 \times 1 \text{ mm}^2$) and Z-parameter values (mean $\pm$ SD) are shown for a representative superficial partial thickness injury with 22% burn depth (a-b), superficial partial thickness injury with 37% burn depth (c-d), deep-partial thickness injury with 65% burn depth (e-f), and full thickness injury with 100% burn depth (g-h). When considering all burns in this study, Z parameter increased over time in burns less than 50% depth (i) but remained relatively constant for burns greater than 50% depth (j).

Figure 4

Animal model. (a) The schematic of the scald burn induction device is shown, where 95° C water would come in direct contact with the skin. (b) Scald condition locations are illustrated where the numbers in each circle represent the heat exposure duration in seconds. (c) Dermal burn depth percentage (normalized by total dermal thickness) are plotted for each experimental condition, as determined by a histopathologist using Day-4 H&E sections.

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