PERSPECTIVE ARTICLE

Noninvasive imaging technologies for cutaneous wound assessment: A review

Dereck W. Paul, MS[1]*; Pejhman Ghassemi, PhD[2]; Jessica C. Ramella-Roman, PhD[3]; Nicholas J. Prindeze, BS[1]; Lauren T. Moffatt, PhD[1]; Abdulnaser Alkhalil, PhD[1]; Jeffrey W. Shupp, MD[1,4]

1. The Firefighters' Burn and Surgical Research Laboratory, MedStar Health Research Institute, Washington, DC
2. Department of Electrical Engineering and Computer Science, The Catholic University of America, Washington, DC
3. Department of Biomedical Engineering and Herbert Wertheim College of Medicine, Florida International University, Miami, Florida
4. Department of Surgery, The Burn Center, MedStar Washington Hospital Center, Washington, DC

Reprint requests:
Jeffrey W. Shupp, The Burn Center 110 Irving Street, NW, Suite 3B-55, Washington, DC 20010. Tel: 202.877.7347 (v); Fax: 202.877.7302 (f); Email: jeffrey.w.shupp@medstar.net

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*Dereck W. Paul and Pejhman Ghassemi contributed equally to this work.

ABSTRACT

The ability to phenotype wounds for the purposes of assessing severity, healing potential and treatment is an important function of evidence-based medicine. A variety of optical technologies are currently in development for noninvasive wound assessment. To varying extents, these optical technologies have the potential to supplement traditional clinical wound evaluation and research, by providing detailed information regarding skin components imperceptible to visual inspection. These assessments are achieved through quantitative optical analysis of tissue characteristics including blood flow, collagen remodeling, hemoglobin content, inflammation, temperature, vascular structure, and water content. Technologies that have, to this date, been applied to wound assessment include: near infrared imaging, thermal imaging, optical coherence tomography, orthogonal polarization spectral imaging, fluorescence imaging, laser Doppler imaging, microscopy, spatial frequency domain imaging, photoacoustic detection, and spectral/hyperspectral imaging. We present a review of the technologies in use or development for these purposes with three aims: (1) providing basic explanations of imaging technology concepts, (2) reviewing the wound imaging literature, and (3) providing insight into areas for further application and exploration. Noninvasive imaging is a promising advancement in wound assessment and all technologies require further validation.

The ability to characterize wounds is of tremendous importance, as wound classification is a critical first step in assessing severity, healing potential, and determining the correct treatment across all wound types. Wound evaluation has traditionally relied on visual assessment by the trained clinician, however, newer technologies are providing objective assessment modalities. Current and emerging techniques include broader evaluation of the wound bed and the quantification of epithelialization, one of the most critical steps in the healing process to prevent dehydration and infection.

Chronic wounds, including diabetic ulcers and pressure ulcers, generate a significant health and economic burden for individual patients as well as the healthcare system. The diabetic ulcer is a major complication of diabetes mellitus, a disease which afflicts 25.8 million in the United States.[1] Worldwide, diabetes afflicts 347 million people and is projected to be the 7th leading cause of death by 2030 according to the World Health Organization.[2] In this population, foot ulceration is the leading cause of hospitalization. Ulceration occurs in 15% of diabetics and precedes 84% of lower limb amputations.[3] Acute cutaneous wounds, including burn wounds, also continue to be a serious health issue in the global community. Nearly 11 million flame burns occur annually and burn deaths rank in the top 15 causes of death for individuals 5–29 years of age. Around 60% of these burn patients heal with debilitating hypertrophic scarring and although burn injuries are considered to be largely preventable, the annual cost of burn care for children alone in the United States in the year 2000 exceeded 211 million dollars.[4]

Assessment of all cutaneous wound types has largely relied on visual inspection by the experienced clinician. Initial assessment of the diabetic foot ulcer involves a visual examination of the wound bed for size, shape, and depth, a neurological evaluation, as well as a vascular evaluation. In more severe or potentially infected ulcers, x-ray and magnetic resonance imaging (MRI) may be ordered.[5] Subsequent assessment of healing progress includes wound size and depth measurements, which are made difficult by significant variation in wound structure and contour.

For thermal injuries, accurate assessment of burn depth is essential to optimize outcomes. Burns of differing depth have different capacities for spontaneous healing and different risks for further burn wound progression (the subsequent cellular death of the tissue in and surrounding the wound bed without proper surgical care), therefore, they must be managed accordingly.[6] Although superficial and superficial partial thickness wounds heal spontaneously, midpartial thickness may require treatment of blisters.

Deep partial thickness burns may heal spontaneously but with a potential for hypertrophic scarring, while full thickness require excision and grafting.[7] The clinician relies on visual examination and experience to assess burn wound depth. Overestimation of burn wound severity may lead to unnecessary surgical procedures, which can increase the risk of infection, scarring, or dyspigmentation as well as increase the cost of care. Underestimation of burn wound severity could allow further burn wound-progression. It is estimated that clinical assessment based on visual inspection is accurate in only 64–76% of cases.[8] To precisely assess burn depth, histological analysis of the tissue remains the gold standard, but the biopsy process is invasive, can be painful, and in some cases can cause additional trauma and worsen scarring.[9,10]

Imaging technologies provide measurements of the optical properties of skin components, which can be analyzed and interpreted to assess wound severity, healing potential and healing progress in a rapid, objective, and noninvasive manner. Various modalities measure scattering and absorption of light by skin components, aiding qualitative and quantitative evaluation of markers such as blood flow, collagen remodeling, hemoglobin content, inflammation, vascular structure, and water content. As optical analysis provides structural, functional and hemodynamic information undetectable to visual assessment, these methods can enhance diagnoses and prognoses as well as elucidate the pathophysiology of cutaneous disease states, aiding cutaneous wound research. Here we review the current application of optical imaging technologies to the assessment of wound types described above; burn wounds and diabetic wounds in addition to vascular wounds, pressure ulcers, and irradiated tissue injury. The noninvasive imaging modalities reviewed include near infrared imaging, thermal imaging, optical coherence tomography (OCT), orthogonal polarization spectral (OPS) imaging, fluorescence imaging, laser Doppler imaging (LDI), laser speckle imaging, microscopy, spatial frequency domain imaging (SFDI), spectral/hyperspectral imaging (HSI), and others, which have been applied to different cutaneous wound types to varying extents. The purpose of this article is to provide basic explanations of optical imaging technology concepts, a review of the wound imaging literature and to provide insight in areas for further application and exploration.

SPECTROSCOPY

Imaging spectroscopy

Spectrophotometric intracutaneous analysis (SIAscope) has been used to image burn wounds by creating calculated maps of chromophores of interest (particularly hemoglobin and melanin) based on spectral analysis of the back reflected light. This imaging device is small, portable, captures images rapidly, and has the potential to provide information on burn depth.[11] SIAscope utilizes a spectrum of light in the range of 400–1,000 nm to probe the skin.

A similar technique, HSI, has also been explored in the evaluation of diabetic wounds[12] and burn wounds.[13] At wavelengths between 500 and 700 nm, HSI can provide information on chromophores oxy-hemoglobin and deoxy-hemoglobin. In any of the spectroscopic imaging systems, a combination of a charge coupled device (CCD) camera and a tunable filter collects backreflected light at different wavelengths. Therefore, the system provides an absorption spectrum for each pixel of the captured image. Using tabulate values of the extinction coefficients of tissue chromphores of interest (oxy- and deoxy-hemoglobin, melanin, etc.) and the mean free path length of light traveling inside the tissue before reflectance, concentration of chromophores may be estimated

In one application of HSI, broadband visible light emitting diodes illuminate tissue from different directions simultaneously. HSI can then generate anatomical tissue oxygen maps that are predictive of the risk of ulceration in the diabetic foot before it occurs.[14] After diabetic ulceration has occurred, HSI has been shown to be predictive of the healing potential.[15] Khaodhiar et al. has developed a healing index based on measurements of oxy-hemoglobin and deoxy-hemoglobin at the wound site and areas adjacent to the ulcer.[12] They found significant differences between tissue oxygenation of healed foot ulcers and ulcers that did not heal, which supports the use of HSI to more widely identify microvasculature abnormality and tissue oxygenation of diabetic foot ulcers.

Although HSI has been demonstrated to provide information that can aid clinical assessment of ischemic wounds, it has not been widely applied. Xu et al. have proposed a digital tissue phantom (DTP) using a hyperspectral image projector that represents functional and spectral properties of biological tissue.[16] They have developed in vitro and in vivo DTPs based on the blood phantom and porcine ischemic skin flap model. A HSI system is utilized to acquire spectral reflectance images from the samples. The abilities of HSI to detect and predict ulceration in the diabetic foot is reviewed by Yudovsky et al. in 2010,[17] who conclude that HSI can optimize therapies by monitoring the diabetic ulcer, predicting the ulceration risk and predicting healing outcomes. Furthermore, measurements of cutaneous oxygenation and perfusion via HSI can both visualize and quantify these characteristics in irradiated skin and provide a possible means of predicting skin reaction immediately after irradiation.[18]

The utility of HSI for burn assessment was recently demonstrated by Calin et al. in a case study. They found that while the application requires more detailed investigation, HSI was able to identify subcutaneous edema in a manner much more quickly than laser Doppler scanning.[13]

Traumatic wounds can also result in cutaneous extravasations known as hematomas. Reflectance spectroscopy has been used to quantify the degradation of hemoglobin in vivo allowing for the determination of the age of the hematoma, although it does require light contact with the skin.[19] Noncontact spectrophotometric analysis can also provide information on which tissue layer has sustained the injury, as hematomas near the surface have a different color impression than those in deeper tissue.[20]

Near-infrared Imaging spectroscopy

Near-infrared (NIR) spectroscopy provides quantitative information on the structural and chemical components of cutaneous tissue, specifically oxygen saturation, hemoglobin content and water content. NIR devices illuminate skin tissue at near infrared wavelengths (700–1,100 nm) and NIR

reflectance is collected via fiber optic probes (or by camera in NIR imaging). Light in the NIR range is absorbed by various skin chromophores specifically water, oxy-hemoglobin, and deoxy-hemoglobin. De-oxyhemoglobin has a maximum absorption at 760 nm, whereas absorption of oxy-hemoglobin is greatest at 900 nm. Water, which may have implications for identifying edema, has a peak of absorption at about 980 nm in this range. While some of the penetrating light will be absorbed by these chromophores, some of the light is scattered and reflected by other skin components. NIR reflectance is collected by a detector and analyzed to provide measurements of chromophore content. The subsequent analysis of this data can be applied to the assessment of wound severity and healing.

NIR has significant potential to aid in burn wound assessment, specifically the classification of burn wound depth and the quantification of edema. Increasing burn severity is accompanied by decreased circulation due to the destruction of the vasculature at the wound bed, which allows variation in burn depth to be correlated with differences in water content, oxygen saturation, and hemoglobin content. Preclinical studies have shown that measurements of oxygen saturation and total hemoglobin alone can differentiate between superficial and full thickness burn wound.[21,22] The addition of water content measurement to the oxygen and hemoglobin data allows further differentiation, distinguishing between superficial, intermediate partial thickness, deep partial thickness, and full thickness wounds.[22] The ability of NIR to measure water content has been explored specifically in the context of partial thickness burns and allows for the monitoring of cutaneous edema over time.[23] This applicability of NIR is particularly important given that the assessment of fluid loss and appropriate resuscitation is a major concern in burn treatment. Although clinical validation is required, these studies reveal NIR to be a promising technology for burn wound assessment.

NIR has also been applied to the study of diabetic ulceration, and is capable of differentiating between diabetic and nondiabetic wounds and is predictive of healing outcomes. Early exploration into these applications in a diabetic rat model, have demonstrated that absorption and scattering coefficients obtained by NIR are increased in diabetic wounds compared to control wounds.[24,25] These increases are thought to be due to decreased perfusion and increased tissue disorganization, both known characteristics of the diabetic ulcer. Similar studies with concurrent histological analysis confirm that the absorption coefficient correlates with blood vessel growth.[26]

Orthogonal polarization spectral imaging

OPS Imaging devices illuminate skin tissue with linearly polarized light, and collect resultant depolarized photons scattered by the tissue components using a polarizer positioned orthogonal to the plane of illuminating light.[27] This technique is based on elimination of single scattered photons, mostly coming from superficial structures. Subsequent analysis allows visualization of hemoglobin in the microcirculation[28] and quantification of the microvasculature.

OPS can differentiate between burn wound depths with limited success. Measurements of polarized light converted to optical density allow OPS to differentiate between superficial and deep burns, although superficial burns and normal skin register with the same optical density.[29,30] Goertz et al. confirm that OPS has utility in differentiating burn depth, but comparison to clinical assessment revealed that OPS was 5% less accurate than the experienced burn surgeon in predicting the outcome of a burn wound.[28] OPS technology currently offers no improvement over the clinically validated LDI devices and is not comparable to clinical assessment by the experienced clinician. Additionally, OPS provides information on microvasculature during cutaneous wound healing,[31] has been applied to the assessment of ischemic wounds[27] and has been used to image the microvasculature in chronic venous insufficiency.[32]

Spatial frequency domain imaging

SFDI involves the projection of sinusoidal patterns of incoherent monochromatic light at a specific frequency and three different phases onto the cutaneous tissue, and the successive measurement of diffused backscattered light by a CCD camera to separate the absorption and reduced scattering coefficient of tissue under examination.[33] The volume fraction of tissue chromophores like oxy-hemoglobin, deoxy-hemoglobin, and water are estimated using tabulated values of absorption coefficient at different wavelengths, similarly to what has been discussed in the imaging spectroscopy sections. These results can be correlated with vascularization and infection of the cutaneous tissue.[34,35]

SFDI has been applied to the imaging of burn wounds. This technology is capable of determining burn wound depth[36] and scar severity[37] and can identify burn wound infection by quantifying changes in optical properties (absorption and reduced scattering), that correlate with bacterial infection.[34] The two-dimensional maps of total hemoglobin and tissue oxygen saturation generated by SFDI can also be used to quantify physiological changes in cutaneous healing after surgical operation and to detect vascular occlusion, which can result in complete flap loss.[35,38] SFDI technology is new to the field of cutaneous wound research but the potential to determine burn wound depth in humans and the identification of burn wound infection is promising. The optical properties measured by SFDI in the studies described above are components of skin that are also altered in diabetic wounds and pressure ulcers, therefore, SFDI may also have utility in the assessment of these wound types.

THERMOGRAPHY

All objects, including skin, emit infrared radiation (IR). IR emission can be measured using infrared detectors and has been found to correlate with temperature, as a product of emissivity. This correlation allows thermographic measurements from skin to be analyzed and processed to create color-coded images that express the relative skin temperature over a defined area.[39–41] This is known as IR thermography or static IR thermography. Active dynamic thermography (ADT) is an advanced thermographic technique that involves recording steady state temperatures using a standard IR camera following thermal excitation of the tissue by a light source, typically halogen lamps. A second set of temperature measurements is then taken following thermal excitation, and subsequent analysis allows the thermal diffusivity of tissue components and wound regions to be quantified.[42,43] Liquid crystal thermography

(LCT) uses a plate of thermochromic liquid crystals to measure the temperature distribution of tissue. The crystals absorb heat radiating from tissue and the plate gives off a spectrum of colors which correlate with temperature readings.[41]

The potential for thermography to aid in the assessment of burn wounds was identified in the early 1960s by Lawson, who used infrared scanning to predict burn depth with an accuracy of 90% as confirmed by histology.[39] These predictions were based in the simple principle that superficial burns would be warmer than uninjured skin due to increased inflammatory processes while deeper burns would be cooler than uninjured skin due to structural damage to the vasculature. Burn depth assessment is critical in determining appropriate treatment for burn injuries and with the relatively low accuracy of clinical assessment, this application of thermography is promising.

Subsequent studies have shown that using thermographic evaluation of burn depth can predict healing outcomes, whether the burn heals spontaneously in 3 weeks or requires excision and grafting, with high accuracy.[40,44] One common problem with this modality is distortion in the image caused by evaporative water loss in the wound bed. Allowing the burn to dry completely eliminates this problem but also delays the timing of the assessment. Immediate assessment by thermography can be achieved with application of a nonpermeable covering to the wound bed, which eliminates the problem of evaporative cooling and allows thermographic measurements to be taken before the burn is dry.[45] This solution is favorable as it has been shown that after a period of time, deeper burns begin to warm and that burns are best assessed within 3 days.[46]

Recent studies have used high-resolution digital infrared thermal imaging cameras to assess burn wound depth with even higher specificity, differentiating between full thickness, deep partial thickness, and superficial partial thickness burns.[43,47,48] Burn depth can also be evaluated using the ADT method described above and studies comparing the ADT method with static IR thermographic methods and histological analysis, conclude that ADT is superior in accuracy, specificity and sensitivity to static IR thermal imaging for the classification of burn depth.[42,49] Still, both static IR thermal imaging and ADT show promise as methods of burn wound depth assessment. These thermographic devices are argued to be less expensive and easier to learn than LDI devices, the only clinically validated burn depth assessment device, and are far more accurate than visual assessment, however further study is required.

Several studies have demonstrated the potential for thermography to aid in the assessment of the diabetic foot. Elevated temperature is a reliable marker of inflammation and can thus be used to predict the site and risk of ulceration as well as risk of amputation and infection. Key studies regarding the application of thermography for detecting inflammation have been most recently reviewed by Bharara et al. in 2012.[50] Although the potential for thermography to aid in diabetic foot assessment is clear, the exact methods by which it is best used are not. The advantages of thermography including its safety, ease and speed of use have largely increased its applications in research studies. Difficulties in patient/camera positioning, visual/thermal image registration, and lack of quantitative tools are reported as limiting factors for clinical routine assessments.

Various methods of analysis for evaluating plantar temperature distributions have been proposed. In 1994 Benbow et al. found that mean foot temperature, determined from eight standard sites on the plantar surface, could be used to assess risk of ulceration and ischemic foot disease.[51] Diabetic patients with a high mean foot temperature were at an increased risk for neuropathic foot ulceration, and those with normal or low mean foot temperature were at risk for ischemic foot disease. Another method of temperature evaluation is to perform an asymmetry analysis, comparing temperature maps (infrared images) of the individuals left foot to their right, in conjunction with a image segmentation algorithm to detect foot ulcer. This method has the advantage of being specific to the patient but is dependent on geometrical symmetry between feet.[52] Nagase et al. have created 20 classifications of thermal distribution to aid in diabetic foot assessment and surgical procedures.[53] An important contribution to the field is the proposal of a wound inflammatory index or temperature index for diabetic foot assessment by Bharara et al.[54] This index takes into account the difference in mean foot temperature and the wound bed, the area of the wound bed, and the area of the isotherm (highest or lowest temperature area), and can be used as tool in diabetic wound assessment.

The way in which the diabetic foot is to be analyzed or assessed by thermography has yet to be solidified and validated, but with its predictive abilities, thermography has the potential to contribute to the prevention and clinical management of diabetic ulcers. In addition to the described methods of detecting inflammation, a case study suggests that thermography may be able to predict osteomyelitis, a serious complication of the diabetic wound, before visible signs of infection are shown.[55] With high and rising rates of diabetic complications, detection of ulceration and osteomyelitis at a time point earlier than visible detection in high risk patients may lead to better wound healing outcomes and help prevent amputations.

The potential for thermography to aid in the classification of pressure ulcers was identified as early as 1973. Barton et al. were able to distinguish between pressure ulcers that were slow to heal and those that were healing normally using thermography.[56] Slow to heal ulcers were characterized by a temperature less than 1 °C different from surrounding skin, while normal healing ulcers had a temperature difference of 2.5 °C. In 1981 Newman and Davis found that thermographic anomalies on admission to a geriatric unit were predictive of sacral pressure sore formation.[57]

More recently, thermography has been used to classify pressure ulcers by their healing potential.[58] Three weeks after ulcer formation, higher temperature at the wound site compared to the surrounding skin implies the presence of factors that may inhibit wound healing. In this way, temperature measurement alerts the clinician to delayed wound healing before it is visually apparent, potentially leading to faster treatment times and better outcomes. A study by Hazenberg et al. also demonstrated that "hot-spots" are indicative of infection, and demonstrated that temperature measurement combined with visual assessment is both sensitive and specific for signs of infection.[59] In addition to classifying ulcers that have already formed, Judy et al. have described the ability of thermography to predict the

precise anatomical location of ulceration before wound formation.[60] This study reveals that thermography is more accurate in predicting pressure sores than the clinical use of the Braden Scale, the most common pressure ulcer prediction tool.

The value of thermographic imaging for pressure ulcers is predictive power; prediction of high-risk sites can lead to better management and outcomes and potentially prevent ulceration altogether if proper action is taken. Thermography has also been used to monitor the wound healing of the closely related venous stasis ulcer,[61] aiding the evaluation of a wound healing therapy and demonstrating its potential in wound care research.

Infrared microscopic imaging has also been used to study lipid migration during cutaneous wound healing in an ex vivo model.[62] In this application IR images captured as a function of postimaging period show the presence of disordered lipid chains in the cutaneous wound healing process, by lipid infrared spectral properties.

PERFUSION IMAGING

Laser Doppler imaging

Laser Doppler Perfusion Imaging (LDI or LDPI) allows for the quantification of microvascular blood flow in a defined region of skin. Relying on the Doppler principle, LDI devices measure changes in the wavelength of electromagnetic radiation after reflection off of moving erythrocytes in the cutaneous tissue. Light penetrating the skin from a source laser is reflected and scattered from the static tissue and moving erythrocytes. While backscattered light from static tissue remains unchanged in wavelength, scattered light from the moving erythrocytes undergoes changes in wavelength that are measured to provide information on the speed and density of moving blood cells. LDI is a further development of laser Doppler flowmetry (LDF) technology which uses a contact probe to access microvasculature over a small area.[63] In contrast, LDI allows for the assessment of a much larger area of tissue and provides measurements in real time.[64] Microvascular blood flow analysis in conjunction with digital photography has been applied to the assessment of burn wounds,[65–76] scars,[37,77–82] pressure ulcers,[83–90] diabetic foot ulcers, and venous leg ulcers.[82,91–98]

Burn severity classifications are marked by characteristic changes in vasculature and blood flow.[66] LDI, providing a quantitative measure of blood flow, can thus be used to predict burn wound outcomes and healing times with accuracy.[66,71,75] Despite this evidence, the classification of burn wounds remains largely limited to visual assessment. Several studies have compared LDI's ability to predict healing outcomes with that of clinical assessment. These studies confirm LDI's utility in assessing burn wound depth and show that it has an increased accuracy over clinical assessment.[67–69,76] LDI has been approved by the Food and Drug Administration for the determination of burn depth[9] and an audit of the use of LDI by Pape et al. recommends the use of LDI to assess all burns of intermediate depth.[76] Additionally, utilization of LDI in burn wound assessment leads to faster decision to graft times in pediatric burns[70] and may lead to faster treatment plans and decrease unnecessary procedures, resulting in projected decreased hospital stay and cost.[74]

Laser Doppler is also able to image scar progression[37,79] and has been used to image vesicant chemical burns[65] and radiation injuries,[72] however, LDI is not a reliable tool in the prediction of clinical outcome in friction burns due to the difference in mechanism of injury compared with thermal injuries.[73] Another is its imaging speed, which has been improved considerably in LDPI based on a complementary metal-oxide semiconductor imaging array.[99]

LDI has been used to a limited extent in the study of the microcirculation and microvascular changes in diabetic wound healing.[91,95,100] LDI measurements have been used in the assessment of microvascular blood flow after treatment with dermal replacement therapy[96] and as marker of wound healing in a study of neurovascular factors of the diabetic foot.[95] The clinical significance of tissue hypoxia measurements in lower extremity wound management are reviewed by Andersen et al.[91] Lower limb perfusion in the diabetic patient is usually evaluated using transcutaneous oxygen measurements and photoplethysmography, here LDI is utilized less frequently, but expected to be of utility.[98]

Pressure ulcers result from sustained and unrelieved pressure during extended cardiovascular, orthopedic and other surgical procedures as well as in other acute and long term care settings and are also candidates for LDI imaging. Heel pressure ulcers and their hyperemic responses to loading and unloading have been studied using LDI.[85,86] The use of LDI in the assessment of stage I pressure ulcers reveals differences in blood perfusion between skin areas of the pressure ulcers and undamaged skin.[84,87,88] A multiparametric system combines LDF and photoplethysmography into a single probe for simultaneous measurement of blood flow at different depths in pressure ulcer tissue.[83]

LDI and capillary microscopy have been combined to image and study ischemic ulcers[92,93] and LDI has been tested for the study of decubitus ulcers after sitting down in healthy[90] and paraplegic patients.[89] In systemic sclerosis with impaired microcirculation, LDPI can find an indirect functional estimation of possible digital artery obstruction.[101] A new device combines LDPI and digital photography to assess skin perfusion and evaluate post operative vascularized grafts or monitoring skin ulcers.[102] Laser Doppler has been used to determine functional impairment in the early stages of arterial disease and venous insufficiency[103] and is used to characterize blood flow in chronic venous disease.[104]

Laser speckle imaging

Laser speckle contrast imaging (LSCI or LSI) is a laser-based imaging technique used to study perfusion. Historically, it has been used to derive full-field, two-dimensional maps of blood velocity. Unlike the one-dimensional raster technique of LDI, LSI expands a laser output to a large 2D shape to examine a full field, rather than a single point. When coherent laser light illuminates a surface with irregularities like natural cutaneous tissue, some photons interfere constructively and some destructively that create a pattern called speckle.[105] When used on tissue, moving blood cells introduce dynamic changes to the speckle pattern. In LSI, these randomly changing patterns are averaged over time to produce what is termed "blur" which are correlates to blood flow. Although both LDI and LSI quantify perfusion, it should be noted that LDI may be

used quantitatively, while LSI is strictly qualitative,[106] with the benefit of rapid, nearly instantaneous image capture. Data acquisition consists of illumination with a coherent source and imaging with a CCD camera.

A laser speckle imager including a 633 nm HeNe laser source has been utilized successfully to study cutaneous deep dermal wounds on a Duroc pig model[105] and scar perfusion over time.[107] It is shown that LSI data is a valuable source of information for wound depth grading. It is inferred from the LSI maps that blood perfusion tends toward lower values as the wound healing process advances.

FLUORESCENCE IMAGING

Fluorescence Imaging can rely either on imaging endogenous fluorescence from skin's natural fluorophores (such as collagen and elastin) or can be used in combination with exogenous fluorescent materials. It generally involves illumination of the skin by laser light at specific excitation wavelengths and the collection and filtering of light with a camera at the emission wavelength of interest. An example of an exogenous process is indocyanine green (ICG) fluorescence imaging, which requires injecting ICG dye into the systemic circulation. Excitation of this fluorescent dye allows fluorescence levels to be correlated with vascularization as well as burn wound depth.[108,109] An example of endogenous fluorescence is fluorescence lifetime imaging (FLIM), in which photon excitation of nicotinamide adenine dinucleotide (NADH) is measured in the wound bed. NADH is involved in oxidative phosphorylation and signals cellular metabolism which can be used as a marker for cutaneous wound healing.[92,109]

Still et al. have indicated that fluorescence imaging can qualitatively measure blood flow in the cutaneous wound as described in a preliminary clinical trial where fluorescence levels were shown to correlate with burn depth assessed by histological and clinical assessment.[108] The major disadvantage to this technique is that it requires an injection of ICG dye to provide blood flow measurements; other techniques can noninvasively quantify blood flow or other markers of microvasculature patency without intravenous injections.

FLIM and second harmonic imaging (SHG) have been combined and utilized in the study of wound healing. Specifically FLIM can monitor cellular metabolism rates by measuring NADH levels, and SHG can map collagen deposition at the site of the wound bed. These methods taken together are comparable with histochemical analysis and may be suitable for study of wound healing supplements and clinical diagnosis.[109] FLIM's allowance for the quantification of cell metabolism may prove to be a useful marker of healing in cutaneous wounds. This marker of tissue viability could be used to supplement other assessment modalities and should be explored further as it contributes to a holistic assessment of cutaneous healing.

MICROSCOPY

Confocal microscopy

Confocal microscopy or confocal laser scanning microscopy (CLSM) is a high-resolution optical detection technique, which provides the ability to monitor specimens in multiple planes of depth by adjusting a specific focal depth.[110–115] In confocal microscopy, a point light source is focused onto a very small volume of a sample. A confocal pinhole detector is designed to collect re-emitted signals originated only from the focal plane. The confocal technique eliminates all out-of-focus photons backscattered from the surrounding material. Since only a small volume of sample is illuminated at each acquiring step, confocal microscopy is not ideal for large fields of view, although recently, tiling processes have been applied to extend the utility of this modality.[116] To cover the entire sample, a mechanical scanning of either sample or light-source and detector is always performed. By changing the focal depth, a series of horizontal sectional images are captured by this technique, which could be stacked vertically to form the final image. CLSM detects reflected light from tissue as described above, after exposure to laser light. CLSM may be used to collect high-resolution information at several different levels of cutaneous wound repair, including cellular, morphological, and architectural information. This allows for visualization of inflammation, blood flow, tissue formation and tissue remodeling, which provide a noninvasive histological analysis over the course of wound healing. Reflectance-mode confocal microscopy (RMCM) is a noninvasive technology operating on similar principles to CLSM. RMCM projects a light source into the cutaneous tissue and provides high-resolution cellular-level images. Light transmittance in cutaneous tissue increases with wavelengths in the NIR region (700–1,400 nm). Most commercially available confocal microscopes utilize NIR light sources.[115]

While CLSM has been used to noninvasively image cutaneous wounds during healing, it is currently limited to superficial epidermal wounds,[110] and has been used to distinguish between normal and abnormal skin morphology[111] The closely related RMCM allows for the simultaneous recording and evaluation of microcirculation, histomorphology and inflammatory cell trafficking, and has been used successfully for the study of burn wounds.[112] The visualization of morphology obtained through this technique allows for real time monitoring of wound repair markers including edema, blood vessel dilation, and extension of keratinocytes and movement of inflammatory cells. A pilot study by Terhorst et al. reviews the abilities of RMCM in studying wound repair and angiogenesis[113] Real-time monitoring of skin morphology with RMCM may provide further insight into mechanisms at work during wound repair and healing.

Multiphoton microscopy

Multiphoton microscopy (MPM) is a high-contrast, high-resolution imaging technique that combines two different imaging modalities, two-photon excited fluorescence (TPEF) and second-harmonic generation (SHG).[117–121] TPEF is a powerful technique for high-resolution imaging of isotropic biological material such as elastin, collagen, keratin and endogenous cellular chromophores. TPEF works by exciting a fluorophore in a small volume of tissue with two photons, each with half the required energy to cause the release of an emission photon. If the absorption of the two photons is simultaneous, the fluorophore will reach its excited state. An important benefit to this technique is the rejection of out-of-focus objects, which is improved greatly from single-photon excitation, as the

probability of receiving two simultaneous photons decreases exponentially outside of the laser spot. The combination of the two techniques in multiphoton microscopy provides better structural understanding of biological tissue components like epidermis, elastin, and collagen. MPM generally consists of an excitation light source, a microscope, and an imaging detector. The excitation wavelength is usually between 700–1,000 nm. MPM utilizes a tunable filter inside the imaging arm to select emitted photons within the specific wavelength range. This MPM configuration allows for the acquisition of high-contrast images of specific tissue components like collagen and elastin, which are known as the dominant scattering structures of skin specifically in the MPM spectral range.

MPM has been applied in combination with OCT to monitor healing following laser exposure on skin-equivalent tissue.[121] In this model MPM effectively identified collagen microstructures, while fibroblast infiltration was simultaneously visualized by TPEF.

Photoacoustic microscopy

Photoacoustic (PA) detection is a method described for cutaneous burn assessment and relies on illumination of tissue with a laser light and measurement of the generated photoacoustic signal reemitted.[122–127] Generally, most PA systems available for burn studies utilize a laser light source with a spectral range of 500–550 nm where the light absorption of blood is considerable. Therefore, the PA device measures total hemoglobin concentration of the biological tissue. Tissue chromophores partially absorb laser light energy delivered by illumination. This process causes thermal elastic expansion that ultimately creates a photoacoustic signal, with subsequent detection by piezoelectric sensor.

PA imaging techniques have been applied to the assessment of burns created on pig and rat skin.[122,124] Shunichi et al. measured PA signal as a function of postburn time and found that the profile of PA signal includes different features depending on the severity of wounds. Hatanaka et al. investigated the validity of PA measurement for tissue growth assessment after wounding in rats[126] using a 532 nm nanosecond-pulsed laser source. Comparison of PA findings with LDI and histological examinations showed a significant correlation between the features of PA signal and histological staining results, as well as LDI measurements. A similar laser light source has also been applied for evaluation of graft adhesion in the early postgrafting period.[123] PA signals indicated the presence of neovascularities 6 hours after grafting which was in agreement with the histological results.

Yamazaki et al. introduced an application of the multiwavelength photoacoustic technique for burn depth profiling in rat skin.[125] PA signals were collected at different wavelengths and the wavelength dependence of PA signals was examined. The authors demonstrated that the PA spectrum is similar to the light absorption spectrum of hemoglobin, confirming the detected PA signals originated from hemoglobin chromophores of the skin.

Videomicroscopy

Videomicroscopy obtains transcutaneous images using a fiber-optic light source and a magnification lens, allowing for direct imaging of the cutaneous microvasculature. Advances in microscopy allow for visualization of cutaneous tissue at the cellular level and can be used for noninvasive histological analysis.

Traditionally, the use of light microscopy in conjunction with histological analysis is the "gold standard" for burn wound depth determination. As the relationship between the integrity of the dermal capillary plexus and burn depth is established, videomicroscopy provides the information necessary to determine burn depth with accuracy comparable to LDI and clinical assessment.[128] McGill et al. states the advantages of videomicroscopy over LDI as increased portability, reduced cost, and the fact that it is unaffected by patient movement, skin curvature or ambient light reflection. Videomicroscopy in the assessment of burn wounds does require some contact with the patient, and while the possibilities of patient discomfort and infection are minimal, the need for contact remains a disadvantage for videomicroscopy.[128]

OPTICAL COHERENCE TOMOGRAPHY

OCT provides high-resolution cross-sectional images of tissue microstructure, and is based on low-coherence interferometry.[129] Utilizing a broad-band NIR light source and an interferometer, OCT produces a two-dimensional cross-sectional image of the tissue, based on the backscattering of laser light from the sample. A beam of laser light is split into two separate paths, one is focused into the tissue and the other is guided into a reference arm. The reflected light beams from both arms are recombined to generate an interference pattern.[129–131] Only if the length difference of the light pathways is equal to the coherence length of the light source, is the coherence signal imaged. Therefore, the imaged signal originates from a specific depth of sample. Image reconstruction is achieved by repeating axial (depth) measurements taken by the optical beam across the tissue. Polarization sensitive OCT (PS-OCT), a modification of OCT, incorporates the measurement of polarization state changes of polarized illumination, induced by skin from birefringent materials, namely collagen.

OCT has been utilized in the assessment of burn wound severity in multiple capacities. PS-OCT can determine burn depth with accuracy comparable to histological analysis.[131–134] This assessment is achieved via a correlation between burn depth and the reductions in collagen birefringence, measured as changes in the polarization of light reflected from the tissue. OCT is further improved for burn imaging by an extension of PS-OCT, Muell matrix OCT. Jiao et al. demonstrated that Mueller matrix OCT, which separates phase-based polarization contrast from amplitude based contrast and allows for more detailed information to be gathered from tissue which is also applicable to burn depth assessment.[135] Todorovic et al. further improved Mueller matrix OCT for burn wound imaging through the use of continuous source polarization modulation.[136]

In addition to analysis of collagen denaturation, PS-OCT can provide information on the microvasculature of burn wounds. A combination of microvascular measurements and birefringence data has been used to characterize burn depth in humans by Kim et al. They argue that PS-OCT may allow for earlier burn depth detection than current standards, and point to cost savings that may be made using such methods.[137] Additionally, microvascular

Table 1. Wound types assessed by noninvasive imaging

Noninvasive imaging modality	Wound type(s) assessed
Fluorescence imaging	Burn,[108] excisional[109]
Laser Doppler imaging/flowmetry	Diabetic,[91,95,96,98,100,102] burn,[9,36,65–71,73,74,76,79,99] pressure,[83–88] vascular[78,89,90,92–94,101–104] pressure ulcer,[83] radiation[72,102]
Luminescence imaging	Normal skin[150]
Capillary microscopy	Vascular[78,93,103]
Confocal microscopy	Burn,[112,113] normal skin,[111] wound variety[110]
Multiphoton microscopy	Skin analog[121]
Photoacoustic microscopy	Burn[124]
Videomicroscopy	Burn[128]
Optical coherence tomography	Burn,[132–138] traumatic,[140] skin analog,[121,139] radiation,[141] excisional,[142–144] diabetic excisional[141]
Thermal imaging/thermography	Diabetic,[50,52,54] burn,[39,44,45,48,49] radiation,[151] pressure ulcer[56,58]
Near-infrared spectroscopy	Diabetic,[24–26] burn[21–23]
Spatial frequency domain imaging	Burn,[36,75] surgical[35,38]
Orthogonal polarization spectral imaging	Burn[28,30]
Spectral imaging (including hyperspectral and multispectral)	Diabetic ulcer,[12,14,15,17] radiationchin,[18] traumatic,[19,20] vascular,[152] burn[11]
Ultrasonography	Diabetic[91]

measurements obtained by OCT can also be used clinically to monitor scar progression following burn injury.[138] OCT for burn wound imaging is a relatively new application with significant potential. PS-OCT is particularly promising, but like NIR, OCT must be able to differentiate burn wound depths with high accuracy and repeatability to be of use to the clinician.

Combining OCT with multiphoton microscopy has allowed for the monitoring of tissue in a skin-equivalent model.[121] OCT results show a decrease in the image intensity due to the collagen loss in the thermally injured region. High resolution PS-OCT has been used to evaluate tissue morphology and structure of skin equivalents with accuracy comparable to histology.[139] Swept source-OCT also produces measurements with histological correlation suitable for the monitoring of lower limb traumatic wound healing.[140] Ultrahigh Resolution OCT allows for identification of wound size, epidermal migration, dermal-epidermal junction formation, and wound composition, lending itself as a detailed, noninvasive metric of wound healing.[141] Additionally, PS-OCT has also been demonstrated to have utility in the study of abnormal wound healing and the evaluation of wound treatments by quantifying healing through measurements of collagen content and fiber orientation.[142]

Clinically, OCT can be used to monitor wound reepithelization, as it accurately differentiates between the epidermal and dermal layers.[143] OCT has also found use in the differentiation of collagen implants from epidermal and dermal tissue. Additionally, measurements of wound size, inflammation, reepithelization, and early reabsorption aid the monitoring of assisted wound healing with these collagen implants.[144] OCT may prove to be a useful tool for the study of chronic wounds as well, where epidermal and dermal structure may be compromised and reepithelialization is problematic.[145] Quantitative data on epithelization, collagen deposition, and inflammation can be obtained noninvasively with accuracy comparable to histology, which is preferable to invasive biopsies that may interfere with the healing processes under study.

3D PROFILOMETRY

3D laser imaging can determine volume, perimeter and area of wounds.[146,147] A novel 3D laser scanner has been developed for the primary application of leg ulcer profilometry.[147] The system contains a laser displacement sensor capable of measuring the distance to an object using a triangulation principle. Two computer-controlled servomotors scan the wound in a raster pattern, and a color-coded map of wound contour is generated by processing the data. Changes in the wound contour are detected using the laser scanner and the volume of wound is estimated by applying a specific algorithm. A comprehensive understanding of the status of the healing process is achieved by taking several postinjury measurements. A laser-based 3D profilometer has been successfully used for diagnosis of venous leg ulcers.[146,148] This 3D laser scanning system consists of two major parts; a 670 nm laser multiple line projector and a digital camera. Wound healing has been estimated by presenting the change of wound area over time.

Fringe projection profilometry, a separate 3D profilometry method, has also been applied for wound healing studies, specifically for chronic and pressure wounds.[149] This modality consists of four sinusoidal patterns of visible light with the same frequency but different phases (0, $\pi/2$, π, $3\pi/2$)

Table 2. Biological characteristics assessed by noninvasive imaging

Noninvasive imaging modality	Cutaneous characteristic assessed
Fluorescence imaging	Oxygen saturation,[150] blood flow,[108] collagen deposition,[109] cellular metabolism[109]
Laser Doppler imaging/flowmetry	Blood flow[9,64–71,74–97,100–104,128]
Luminescence imaging	Oxygen saturation,[150] pH[150]
Capillary microscopy	Blood flow[78,93,103]
Confocal microscopy	Blood flow,[112] basal layer thickness,[112] inflammation,[110,112,113] vascular structure,[110,113,128] reepithelization,[110] skin morphology,[112,113] water content[113]
Multiphoton microscopy	Collagen deposition,[121] fibroblast migration[121]
Photoacoustic microscopy	Hemoglobin content[124]
Videomicroscopy	Vascular structure[128]
Optical coherence tomography	Collagen denaturation,[121,132–137,141] water content,[141] vascular structure,[137,138] reepithelization,[143,144] inflammation,[144] pigmentation,[140] wound size,[141,144] epidermal migration,[141,143] collagen deposition[142]
Thermal imaging/thermography	Inflammation,[50,52,54] temperature,[39–42,45–52] thermal time constant,[42,49] thermal effusivity[151]
Near infrared spectroscopy	Hemoglobin content,[21,22,24,26] water content[22,23,26]
Spatial frequency domain imaging	Oxygen saturation,[34–36,38] water content,[34–36] hemoglobin content,[36] lipid content,[36] blood volume[34]
Orthogonal polarization spectral imaging	Blood flow,[27–32] vascular structure,[27–32] capillary density[27,31,32]
Spectroscopy/hyperspectral imaging	Oxygen saturation,[12,14,15,17,18] color measurement,[19,20] melanin,[11,17] epidermal thickness,[17] blood volume,[17] tissue birefringence,[11,17] hemoglobin content,[12,15,19] granulation,[152] reepithelization,[152] microcirculation[153]
Orthogonal polarization spectral imaging	Blood flow,[27–32] vascular structure,[27–32]
Ultrasonography	Blood flow,[91] epidermal thickness[82]

projected onto the skin surface. A reflectance image at each step is captured by a CCD camera. Topography of the object's surface is generated by applying an algorithm on recorded images. In this algorithm, a phase shift map caused by object topography is formed and consequently a surface height distribution map is generated. An evaluation of wound healing may be inferred by wound depth analysis using the generated topographic maps.

In Conclusion, Optical technologies offer improved assessment of wound severity and healing potential, and the ability to monitor healing progress over time. Noninvasive optical technologies have enormous potential to supplement clinical assessment and aid research in the wide field of cutaneous healing and regeneration. The potential benefits in terms of patient outcome and cost reductions through development and implementation of these modalities are significant. We encourage the further development of already existing technologies in the application to wound assessment and suggest that the technologies available can be combined and modulated to provide a holistic picture of wound healing. We have included Tables 1 and 2 to characterize the literature reviewed herein and delineate the type of wound assessed and the biological characteristic examined in each study. Lastly, awareness that some technologies have been advanced in specific wound types invites experimentation of those technologies in other cutaneous pathophysiology with related healing markers. Noninvasive imaging technologies collectively have potential to improve wound assessment but all modalities require further investigation.

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