Noninvasive assessment of burn wound severity using optical technology: A review of current and future modalities

Meghann Kaiser, Amr Yafi, Marianne Cinat, Bernard Choi, Anthony J. Durkin

Department of Surgery, Division of Trauma, Burns, Critical Care and Acute Care Surgery, University of California, Irvine, Orange, CA 92806, United States

Beckman Laser Institute and Medical Clinic, University of California, Irvine, Orange, CA 92806, United States

Abstract

Clinical examination alone is not always sufficient to determine which burn wounds will heal spontaneously and which will require surgical intervention for optimal outcome. We present a review of optical modalities currently in clinical use and under development to assist burn surgeons in assessing burn wound severity, including conventional histology/light microscopy, laser Doppler imaging, indocyanine green videoangiography, near-infrared spectroscopy and spectral imaging, in vivo capillary microscopy, orthogonal polarization spectral imaging, reflectance-mode confocal microscopy, laser speckle imaging, spatial frequency domain imaging, photoacoustic microscopy, and polarization-sensitive optical coherence tomography.

© 2010 Elsevier Ltd and ISBI. All rights reserved.

Keywords:
Burn
Optical
Laser
Perfusion
Collagen
Partial-thickness
Laser Doppler imaging
Near-infrared
Indocyanine green
Capillary microscopy
Orthogonal polarization spectral imaging
Reflectance-mode confocal microscopy
Laser speckle imaging
Spatial frequency domain imaging
Photoacoustic microscopy
Polarization-sensitive optical coherence tomography

Contents

1. Introduction .......................................................... 378
2. In vitro light microscopy ............................................. 378
3. Macroscopic imaging ............................................... 378
1. Introduction

A half century ago, Dr. Zora Janzekovic and others demonstrated that timely tangential excision and grafting of appropriately deep burns prevented sepsis-related morbidity, diminished the development of hypertrophic burn scars, and radically improved cosmetic and functional outcomes [1–3]. Early excision and grafting of burn wounds thus revolutionized the field of burn surgery and has become a central tenet of the field today [4,5].

The difficulty lies in determining which burn wounds will most benefit from early excision and grafting. Red, painful, non-blistering superficial burns—which do not require excision—are immediately apparent to most clinicians. Likewise, most physicians can identify the pale, leathery, insensate deep burns that must be excised. The gray zone that lies between these extremes consists of “partial-thickness” burns. Some, termed “superficial partial-thickness,” will heal spontaneously in less than two weeks, with minimal or no scarring. Others, categorized as “deep partial-thickness,” will require prompt excision and grafting, without which patients suffer prolonged expensive hospitalizations, painful repetitive dressing changes, and complications such as infections and exacerbated scarring [6–8]. This is the daily challenge of the burn surgeon. Overestimating burn severity could mean unnecessary surgery, underestimation could be just as detrimental.

The stakes are high, and picking the ideal course of treatment is not easy. Most clinicians make their determination on the basis of clinical exam alone. But without additional objective measurements, the judgment alone of even experienced burn surgeons correlates with histology and eventual outcome only about three-quarters of the time [9–13]. A sa...
e.g., less than 14 days, 14–21 days, >21 days (Moor Instruments, UK). Thus, similar to light microscopy, LDI cites patent blood vessels as the primary determinant of burn depth, although the focus is on flow rather than the vessels themselves. Not surprisingly then, LDI correlates with burn wound histology and need for surgical excision and grafting about 95% of the time [11–13].

LDI offers obvious advantages over in vitro light microscopy. LDI is noninvasive, and in fact can be performed at distances >1 m from the subject with no physical contact whatsoever. The laser energy emitted is harmless. Moreover, a large area can be evaluated, allowing for different management of areas within the same wound [21]. A fairly large body of literature on the subject of LDI speaks to a general comfort level with the device.

LDI is not, however, without its own imperfections. The current commercial device is expensive, large and difficult to position [22]. In our personal experience, between positioning, calibrating, and scanning, evaluation of an area 50 cm × 50 cm may take several minutes, during which the patient must remain motionless to avoid artifact. This can be difficult for anxious or shivering patients and young children in particular, necessitating sedation. The most commonly used commercial device is not promoted for use during the 1st 48 h after injury, secondary to reactive vasoconstriction, and more than 5 days after injury, secondary to the proliferation of granulation tissue [23,24]. Errors can result from vasomotor reactivity and blood pooling in response to second-to-second changes in ambient temperature, patient positioning and emotional states [25] (see Fig. 1). The interpretation of LDI blood flow maps is likewise unclear in the presence of anemia, cellulitis, or peripheral vascular disease. Surface moisture, topical medications, and transparent dressings can also all alter LDI measurements [26]. Where the tissue surface slopes, artifacts may result, making recorded signals difficult to interpret. It is unclear to what extent all this affects the accuracy of LDI, and which wounds are most appropriate for assessment with this modality. Nonetheless, LDI remains an extremely valuable clinical modality for the assessment of burn wounds.

3.2. Indocyanine green (ICG) videoangiography

Indocyanine green is a non-toxic, protein-bound dye that is retained within the vasculature after intravenous injection for several minutes until rapid clearance by the liver. As a diagnostic pharmaceutical, ICG has been in clinical use for decades in the determination of cardiac output. ICG absorbs and fluoresces within the near-infrared spectrum, which has excellent skin penetration, making deeper dermal vasculature visible using this dye [27]. Fluorescence can then be detected, quantified, and digitally translated into color-coded regions of relative perfusion for ease of interpretation, similar to the LDI device (Fig. 2) [28]. Relative to the subject’s normal skin, ICG fluorescence is markedly higher in spontaneously healing wounds, and markedly lower in those that required surgery [29–31]. In a few small human studies, ICG videoangiography findings correlated with histology and/or clinical outcome approaching 100% [30,31].

ICG videoangiography is capable of producing rapid, macroscopic, easily interpreted scans very reminiscent of LDI, on a more compact, less expensive device. In animal studies, it can distinguish deep and deep partial wounds very early, within the first few hours following injury [27]. Its utility has been clearly demonstrated even in the presence of other microvascular pathologies, such as diabetes and heart failure.

Fig. 1 – On the left, LDI-generated blood flow map of a superficial partial thickness burn on a patient’s face. High blood flow is manifest as a predominantly bright red region. On the right, the same area on the same patient minutes later, showing significantly diminished perfusion, as suggested by the now dark blue facial area. Shortly after the second scan, the patient experienced a near-syncopal episode. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)
The primary, obvious drawback of ICG videoangiography is the need for intravascular dye injection. While actually an extremely safe compound, ICG is nonetheless associated with headache, pruritis, urticaria, diaphoresis, and the ever-present risk of life-threatening anaphylactic reaction. Safety has not been well-established in pediatric, pregnant or lactating patients. Additionally, measurements obtained via ICG videoangiography are relative to normal skin controls, and anatomically equivalent regions of normal skin are not always available in burn subjects. The differentiation between normal, decreased, and increased fluorescence can be a very fine line on the order of a few percent making assessment less than clear-cut. Finally, relative to LDI, the body of literature on ICG videoangiography is somewhat small, although a modality with such promise certainly warrants further investigation.

3.3. Near infrared spectroscopy (NIRS)

As mentioned above, near-infrared light can penetrate further into tissue than the visible spectrum, and several biochemically crucial constituents of the dermis absorb wavelengths within the near-infrared spectrum (650–1100 nm). De-oxy hemoglobin has a maximum absorption at 760 nm, whereas absorption of oxy-hemoglobin is greatest at 900 nm. Water, has peak absorption at 980 nm and may have implications for identifying edema. Near-infrared spectroscopy contact probes, termed “point probes” can be geometrically tailored to target specific depths of up to several centimeters in soft tissue. Noncontact near-infrared spectral imaging, which currently represents the majority of NIRS burns research, can generate a map depicting the relative prevalence of these compounds (Fig. 3). Based on the unique reflectance spectra of these compounds, the relative oxygenation, and edema status of burns can be inferred
produced by different degrees of injury, models can be derived
to significantly predict the presence of superficial partial
versus deep partial wounds with an accuracy of 87% in animal
models [34].

The ability of NIRS to differentiate between oxygenated and
deoxygenated blood confers a distinct theoretical advantage
to this modality. LDI detects active blood flow through patent
vessels, and assumes that the absence of such blood flow
correlates with thrombosed vessels and necrosis. As previously
discussed, however, many physiologic states other than
thrombosis can temporarily alter blood flow. Deoxy-hemoglo-
bin—present even in thrombosed vessels—may be pivotal in
discerning thrombosis from reactive vasoconstriction. More-
over, NIRS spectra reflect not just blood flow, but also the
extraction of oxygen from this blood flow. Alterations in
cellular metabolism may manifest much sooner after an insult
than blood vessel injury and thrombosis, potentially allowing
for dramatically earlier injury grading and treatment, on the
order of hours rather than days [14,35,36]. Even the degree of
inflammation—manifest as edema secondary to capillary
leakage—may differ subtly between superficial partial and
deep partial wounds [37]. Finally, some NIRS devices have the
potential to detect denatured collagen as light scattering [38].
Integrating data on the prevalence of all these molecules—
onxy-hemoglobin, de-oxyhemoglobin, water, and denatured
collagen—improves the predictive reliability of NIRS [35].

On the other hand, like LDI, scans may take several minutes
to complete. Scanning may entail physical probe contact with
the wound, causing discomfort for some patients, although
noncontact imaging is now commonly used for burn purposes
[34,37]. To date, NIRS has reliably differentiated only superfi-
cial from full thickness burns in human subjects, not
superficial partial from deep partial, which is a much finer
line [39]. And, while the variety of device designs, wave-
lengths, and analysis algorithms possible allow for an enviable
degree of customization, there is also a distinct lack of
standardization. The full capacity of NIRS in the diagnosis of
burn injury is promising but as yet, incompletely elucidated.

4. Microscopic imaging

4.1. Capillary microscopy

Like all the above modalities, transcutaneous in vivo capillary
videomicroscopy estimates burn wound depth based on the
presence of functioning dermal vasculature. The ability of
capillary microscopy to accurately assess dermal capillaries
has been verified in a number of other disease states affecting
skin circulation, including diabetes, chronic venous insuffi-
ciency, and psoriasis [40]. In the case of burn injuries, a 200×
 lens is applied to a small (≤1 mm²) area of interest and the
superficial dermal capillary plexus—filled with red, oxygenat-
ed blood cells—easily examined under the visible blue-green
spectrum. Intravascular injection of sodium fluorescein dye,
and examination under a conventional fluorescence filter with
excitation 450–500 nm, may serve to further accentuate the
presence or absence of viable vessels [40,41]. The presence of
Orthogonal polarization spectral imaging is a specialized form of in vivo transcutaneous videomicroscopy. Polarized light of around 548 nm (well absorbed by hemoglobin) is directed at the tissue, and reflected light is gathered through a second polarization filter perpendicular to the first. Any light permitted through the second filter must encounter multiple scattering surfaces, rendering it no longer at right angles to the filter. Thus superficial structures are eliminated from the image and blood vessels around 3 mm deep—highlighted by the presence of red blood cells absorbing the incident light—are very apparent. With this technology, resolution is sufficient to image the movement of a single red blood cell transversing a capillary in real-time. Circulatory patterns distinct for healing and nonhealing burn wounds can be observed [43]. OPSI visualizes both the form and function of dermal capillaries to determine an index of “functional capillary density” (FCD), or the length of perfused vessels in cm per cm² of wound examined [44,45]. In one study, setting the threshold for deep partial thickness burns at an FCD of 100 cm/cm² detected need for operative intervention with a sensitivity of 93% [45].

Like transcutaneous videomicroscopy, OPSI is relatively inexpensive, portable, unaffected by skin curvature and does not require the subject to remain completely still [45]. FCD is arguably a much more objective and reproducible measure of the capillary plexus than the relative grading system used for transcutaneous videomicroscopy. Given the unique optics of this technology, the injection of fluorescein—with any small but real associated risks—is not necessary to attain an impressive level of contrast. Thrombosis can be physically distinguished from vasoconstriction [42], allowing different causes of low-flow states to be discerned. Capillaries can be differentiated from larger venules and arterioles, theoretically adding a level of precision to the microscopic determinations of burn wound depth.

However, it is not at all clear that such detail and precision is necessary, and the extra information adds layers of complexity to data analysis. Fields of view are small (=1 mm²). Since not every functioning capillary will contain a red blood cell at any given time, thorough examinations take around 15 min to complete, and thereafter must be replayed and meticulously reviewed to accurately assess [45]. FCD improves dramatically between 1 and 4 days post-injury, and the ideal measurement interval has not been established [42,45]. The probe is in direct contact with the burn causing potential discomfort. One can assume that OPSI is affected by comorbidities such as anemia, infection and diabetes [43,44], although it has not been extensively studied in a variety of patient populations. Most importantly, while sensitivity for deep partial and deep burns is high, specificity is low at only around 45% in one study [45]. Furthermore, in this same study, 20% of wounds could not be accurately evaluated due to the presence of edema. Presumably, the extraordinary resolution of OPSI is also its downfall: even slight variations in pressure,
Afterwards, review of the images requires even more time, as although subjects need not be completely still for the duration, results in a relatively protracted exam of around 10 min. Requires direct contact with the wound. Miniscule fields of other forms of transcutaneous videomicroscopy, RMCM yields a precise determination of the injury’s extent. However, like OPSI, RMCM raises some fascinating possibilities but is far from ready for widespread routine clinical use.

4.3. Reflectance-mode confocal microscopy (RMCM)

Reflectance-mode confocal microscopy is yet another variation on the transcutaneous videomicroscopy theme. In this version, light from a near-infrared laser is projected at an area of interest, and reflected light received through an aperture of specific diameter. This aperture selects for a specific focal depth by screening out non-focused light and is responsible for a unique feature of RMCM: the ability to view tissue in multiple planes of depth to a maximum of about 350 μm [47]. By combining multiple planes, or “optical sections,” a three-dimensional map of the burn wound can be obtained [47,48]. Dermal vessels appear as dark spaces through which bright erythrocytes pass in real time. Other structures helpful in burn wound assessment can also be visualized. Melanin pigment increases reflection and adds contrast, allowing for rapid identification of the epidermal–dermal junction, assuming it is still present post-burn. White blood cells, which proliferate considerably more in deep and deep partial wounds, can be discerned and quantified [46,49]. Also apparent are dermal appendages such as hair follicles, from which epidermal cells regenerate and migrate in the healing wounds [47,48]. Many of these features are significantly different when comparing spontaneously healing and non-healing wounds (Fig. 6) [46,49].

RMCM is a true means of “optical biopsy.” The degree of histologic detail possible may add to the accuracy of depth determination. Serially adjusting the depth of focus allows for a precise determination of the injury’s extent. However, like other forms of transcutaneous videomicroscopy, RMCM requires direct contact with the wound. Miniscule fields (500 μm × 500 μm) are imaged, requiring repetitive measurements at multiple tissue levels to establish a truly representative sampling of the wound—80 fields in one study [46]. This results in a relatively protracted exam of around 10 min, although subjects need not be completely still for the duration. Afterwards, review of the images requires even more time, as well as considerable expertise. Optical sectioning in a plane parallel to the skin surface, as opposed to the perpendicular sectioning of traditional biopsies, makes interpretation more difficult. RMCM is expensive, at around $130,000 for a commercially developed device, almost half again the cost of LDI. Most importantly, this modality has not been extensively studied in human burn subjects. No absolute thresholds for differentiating superficial partial thickness burns from deep partial thickness burns are yet established, only relative changes over time in blood flow and white cells. Accuracy, sensitivity and specificity are unknown, undermining its practical applications in decision-making for the average burn surgeon. Like OPSI, RMCM raises some fascinating possibilities but is far from ready for widespread routine clinical use.

4.4. Future modalities

Many other promising optical techniques for the assessment of burn wound depth await human trials, and though clinical data is sparse at this time, it behooves dedicated burn surgeons to familiarize themselves with emerging modalities of such potential. Some represent conceptual improvement on current modalities that measure tissue perfusion. Laser speckle imaging (LSI) creates a speckle image with laser light reflecting off wound bed structures. By capturing two images within milliseconds of one another, speckles appear to “smear” in a manner analogous to increasing exposure time on a conventional camera. The degree of smearing corresponds to the speed and volume of red blood cells. As a result, LSI creates a color-coded map similar to LDI without the prolonged scanning time. LSI assessment of microvasculature has been documented in port-wine stains and other pathologic and physiologic states [50–52]. Our group is currently investigating the use of LSI in human subjects with burns (Fig. 7).

Spatial frequency domain imaging (SFDI) represents the next generation of NIRS. “Spatial frequencies” refer to different patterns of near-infrared light used to illuminate the tissue and specify particular depths of penetration, analogous to the aperture of confocal microscopy. Using these patterns, absorbance of certain relevant wavelengths,
such as the peak absorbance of oxy-hemoglobin, deoxy-hemoglobin, and water, can be measured at different depths, creating a three-dimensional map of both the perfusion and metabolic activity within a tissue. Changes in scattering that result from the denaturation of collagen can also be quantified. SFDI can be adjusted to measure areas ranging from 1 cm² to in excess of 100 cm² and, unlike many other NIRS-based devices, does not require any physical contact with the wound. Animal studies with SFDI show clear distinction between superficial and deep wounds [36,53]. A study of SFDI now underway in human burn wounds has produced preliminary findings echoing those of animals (Fig. 8).

Fig. 7 – On the left, a digital photograph of a hot oil burn to the hand with areas of deep (white) and partial (pink) thickness. On the right, the laser speckle image (LSI) of the same burn. Areas of high perfusion are represented by brighter colors (red, yellow, green, in order of decreasing perfusion) and lower perfusion is represented by blue. Deep thickness regions on the proximal second, third, and fourth digits correspond to light blue areas on the speckle image, whereas the dorsum of the hand overlying the metacarpals is partial thickness on clinical exam green/yellow/red on the speckle image. Uninjured, non-inflamed skin is blue. (Patient permission was obtained to publish photograph.) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Fig. 8 – In the upper left corner, a digital photograph of mechanical burn (road rash) to the forearm with areas of full (oval), deep partial (dashed oval), and superficial partial (circle) thickness injury. Regional maps of hemoglobin, oxygen saturation, and light scattering can differentiate between different depths within the wound as shown (patient permission was obtained to publish photograph).
Photoacoustic microscopy (PAM) is based on the principal that objects reflecting light energy vibrate as the energy undergoes conversion to heat, emitting sounds waves. These sounds waves can be picked up by an ultrasonic detector. By projecting a wavelength of light at hemoglobin’s peak absorption, inflamed, hyperemic tissue appears dark (hypoechogenic) on PAM, while surrounding tissues reflecting such light waves appear bright (hypoechoic). The result is an image very similar to conventional sound-based ultrasonography, but on a microscopic scale proportional to the much shorter wavelength of light [54]. In an experimental model of burns, PAM was able to distinguish different durations of thermal exposure within minutes of injury [55].

Collagen in its native state is birefringent, or capable of splitting light into two rays polarized perpendicular to one another. When collagen is denatured by thermal injury, it loses this property [56]. Polarization-sensitive optical coherence tomography (PSOCT) quantifies tissue damage according to the degree of polarization in reflected light, detected as phase retardance. The phase retardance of tissues at different tissue levels can be quantified to demarcate injured from uninjured tissues. Animal studies show a statistically strong mathematical correlation between PSOCT measurements and absolute burn depth as determined by histology [57,58]. This last modality thus differs from the previous modalities discussed in that it is entirely dependent on the structure of collagen, not the microvasculature, for burn depth assessment.

5. Conclusion

The ability to predict which burn wounds will heal spontaneously and which will require surgical intervention is a critical component of clinical treatment algorithms. Gross clinical exam alone is accurate only about three-quarters of the time. Current optical techniques to complement clinical exam operate on the premise that functioning blood vessels are retained in viable tissue. Both macroscopic and microscopic modalities are available, offering different advantages and disadvantages. While many questions remain to be answered, experience to date indicates that the field of optics will contribute an invaluable degree of accuracy and insight to the field of burn assessment.

Acknowledgements

Drs. Durkin and Yafi acknowledge salary support provided by the NIH NCRR Laser and Medical Microbeam Program, (LAMMP: SP-41RR01192); the Military Photomedicine Program, (AFOSR Grant # FA9550-08-1-0384), the Beckman Foundation and the Hazem Chehabi BLI Research Fellowship.

Conflict of interest

Dr. Anthony Durkin has a financial interest in Modulated Imaging Inc., a company with interests related to spatial field domain imaging (SFDI). Dr. Durkin is a cofounder of the company and owns equity interests in Modulated Imaging.

REFERENCES


