Imaging of the Chronic Wound and the Emerging Role of Fluorescence Microangiography

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Techniques for imaging pathological tissue are critical components of medicine and surgery practices across many fields. While computed tomography (CT), magnetic resonance imaging (MRI), ultrasound (US), and standard X-rays are occasionally used for anatomic assessment in patients with chronic wounds, there has been no specific modality for functional imaging in wound care until recently. Functional imaging provides wound care practitioners information on parameters related to the ability for a wound to heal, and helps to guide clinical decision-making by directing treatment based on individual patient needs.

Angiogenesis, the growth of new blood vessels, is a critical process in wound healing. New vessels contribute to forming granulation tissue and deliver oxygen, micronutrients, and paracrine survival factors to proliferating tissue after injury. Notably, angiogenesis is compromised in virtually all wounds with delayed healing. Defective angiogenesis is observed in diabetic foot ulcers (DFUs), venous insufficiency ulceration, and in ischemic and pressure ulcers. In people with diabetes, deficient growth factors, impaired vascular endothelial cell response, and decreased vascular stem cell recruitment lead to insufficient angiogenesis and wound healing. In contrast, venous leg ulcers (VLUs) exhibit morphologically abnormal microvessels that are stuck in the glomeruloid phase of microvascular development. Ischemic wounds seen in severe peripheral arterial disease (PAD) are unable to mount an adequate angiogenic response. In pressure ulcers, prolonged mechanical pressure prevents the completion of normal healing processes, including angiogenesis, granulation, and epithelialization. For these reasons, many advanced modalities have been developed and are in clinical use to promote angiogenesis in the wound to accelerate healing.

Advanced interventions that actively stimulate wound angiogenesis include:
- Recombinant growth factor (becaplermin)
- Platelet rich plasma (PRP)
- Living skin equivalents (Apligraf, Dermagraft)
- Amnion/chorion membrane (EpiFix, AmnioFix)
- Negative pressure wound therapy
- Non-contact low frequency ultrasound (MIST)
- Hyperbaric oxygen therapy (HBOT)

Despite a growing list of modalities, wound care providers have been limited to evaluating the wound and its response to treatment though superficial visual assessment. The identification of biomarkers of wound angiogenesis is therefore an important goal. While genomic profiling of wound biopsies and protein determination of wound fluid are promising, imaging wound angiogenesis — using indocyanine green (ICG) — is a pragmatic and clinically useful approach.

Indocyanine Green Dye and its Clinical Applications

Indocyanine Green (ICG) is a fluorescent dye originally created by the Eastman Kodak Company for use in making a cyan-colored layer in color photographic and movie film. Adapted for medical use in the 1950s as a biocompatible dye detectable in blood, it became FDA approved in 1956 for use by cardiologists to measure cardiac output. As the dye is excreted exclusively by the liver, it was used for hepatic angiography as well. Ophthalmologists began using ICG angiography to assess the choroidal vasculature in the eye. An ICG angiographic system called SPY was developed in 1999 to assess blood flow through vascular grafts and myocardial perfusion before, during, and after cardiac bypass grafting. By 2007, SPY technology was used in multiple medical specialties ranging from plastic surgery to gastrointestinal surgery. In 2013, wound specialists began using ICG angiography to assess the microcirculation of both acute and chronic wounds.

Over its history, ICG has demonstrated excellent safety due to its short (3-5 minute) half-life, and its clearance by the liver. It is safe for patients with compromised renal function. The only contraindication for ICG is in patients with a history of sensitivity to iodides and iodinated contrast agents.
LUNA Imaging of Wounds

LUNA is a fluorescence imaging system used in wound care for perfusion assessment, consisting of a near-infrared laser, high-definition camera, a central processing unit, an operator touchscreen, and a patient viewing screen. The system can be moved easily to various exam rooms. There is an articulating arm that houses the camera head and light source. The camera head is positioned over the area being imaged and transmits the data to the CPU, which is displayed on the monitors in real-time following intravenous injection of ICG.

Wound clinics use LUNA fluorescence microangiography to obtain real-time visualization of tissue perfusion in patients with diabetic ulcers, venous ulcers, ischemic ulcers, and other types of non-healing wounds. Its specific advantage is providing visual as well as quantitative assessment of wound and periwound perfusion in a manner that is not possible with TcPO2 measurements or calculation of the ankle brachial index (ABI). LUNA can directly assess the wound, aid in precision-guided therapy, quantify the impact of various wound healing techniques in driving the wound perfusion back to tissue baseline over time, and assist physicians in determining when it is appropriate to continue or switch treatments to optimize healing outcomes.

Procedure and Generation of Wound Data

ICG microangiography can be easily performed in the outpatient wound clinic with the patient fully awake. Clinicians place a peripheral intravenous catheter and inject 2.5-3.0 mL of ICG intravenously followed by a saline flush injection. ICG enters the blood stream painlessly, is rapidly bound to plasma proteins, and enters the microcirculation around and within the wound bed. The resulting fluorescence is captured by the HD video camera in real-time. Greater density of microcirculation leads to increased fluorescence compared to the background. Lesser density of microcirculation results in a darker region within the image. The wound microcirculation is analyzed by the LUNA software and depicted on viewing screens. The entire process takes 10 to 15 minutes per sequence.

Hyperfluorescence in Chronic Wounds and its Resolution with Proper Therapy

The observation of hyperfluorescence is often described with LUNA images of chronic wounds. This phenomenon is typically asymmetrical and seen around the wound periphery. Initially, hyperfluorescence was confusing to clinicians who expected less fluorescence (hypofluorescence) in a poorly healing wound due to deficient angiogenesis and granulation. However, this phenomenon is easily explained by the biology of activated microvasculature in chronic wounds.

During normal wound healing, angiogenesis is stimulated by a variety of growth factors delivered by platelets and inflammatory cells. These factors activate receptors on vascular endothelial cells and stimulate new blood vessel growth. One of them, vascular endothelial growth factor (VEGF), is also a potent vascular permeability factor. Accordingly, early stages of wound healing angiogenesis exhibit leaky blood vessels with an increased vessel density. As inflammation subsides in normal wound healing, the permeability decreases as inflammatory cells release less VEGF and tissue oxygenation improves. As the wound granulates and epithelial-
izes, the neovasculature becomes pruned to near basal levels, and eventually the activated blood vessels become quiescent.\textsuperscript{13}

Chronic wounds are characterized by persistent, sustained inflammation.\textsuperscript{14} The activated vessels surrounding the wound bed become stuck in the inflammatory, hyperpermeable stage of healing. Wound inflammation can be exacerbated by infection, ischemia, repeated trauma, and a host of other etiologies associated with chronic wounds. Persistent inflammation leads to excessive and persistent expression of VEGF and thus leakiness. In the chronic wound setting, ICG tends to collect in the activated blood vessels around the wound perimeter and leak out due to their inappropriate sustained permeability, resulting in hyperfluorescence.\textsuperscript{12}

As proper wound care — sharp debridement, infection control, minimized proteases, maintenance of a moist wound environment — is instituted, the inflammatory stimuli are lowered. Initiation of an effective advanced wound therapy will actively promote cell proliferation and migration that pushes the wound from its dormant stage to continue through and complete the orderly sequence of healing.

Accordingly, with the initiation of proper wound care, the hyperfluorescence seen by LUNA may transiently increase as more angiogenic vessels are stimulated to develop in the wound bed. With progressive healing, the wound area microcirculation becomes less permeable and decreases in density so the LUNA image will show progressively less fluorescence. Newly sprouted angiogenic blood vessels are gradually pruned back to more normal physiological levels.\textsuperscript{15} The resulting fluorescent signal decreases to more closely approximate non-wounded tissue such as the contralateral limb reference site.

**Clinical Scenarios**

Three common scenarios in which wounds are imaged using the LUNA system will be described with emphasis given to the expected fluorescence image findings. Clinical case examples of these three common scenarios are illustrated in figures 1-7.

**Figure 4.** Clinical case example 2: The initial photo (left) of a dorsal left foot shotgun entry wound. The wound base extends to include deeper tissue layers and has disrupted arterial and venous flow. Color contrast applied to the LUNA image (right) highlights areas of similar signal intensity. A reference marker has been applied to proximal non-wounded tissue and the central wound defect has been selected for further analysis. Comparing the reference marker to the selected region, LUNA shows a wound defect with 18% signal intensity, confirming perfusion deficit.

**Figure 5.** Clinical case example 2: A follow-up photo of a shotgun wound (left) after one month of hyperbaric oxygen therapy. The central portion of the wound remains open with uncertain perfusion status due to magnitude of injury. The follow-up LUNA image (right) shows fluorescent signal increasing from 18% to 70%, confirming effective angiogenesis.

**Figure 6.** Clinical case example 3: The initial postoperative photo (left) of a diabetic foot. LUNA imaging (right) confirms a suture line problem and identifies decreased right second toe perfusion.

**Figure 7.** Clinical case example 3: The follow-up clinic photo (left) shows a dehisced amputation site. One week following percutaneous transluminal angioplasty and stent placement within the right tibioperoneal trunk, LUNA image (right) shows signal intensity increasing from 41% to 94% with reference to the left great toe.
1. Chronic wound. Following injection of ICG, LUNA imaging may demonstrate hyperfluorescence, compared to non-wounded tissue, in an asymmetrical pattern around the wound perimeter. Within the wound perimeter, there may be a hypofluorescence in regions that lack perfusion and require angiogenesis for healing. Following the initiation of good standard wound care, with or without an advanced modality, the healing wound may exhibit even greater fluorescence, reflecting increasing angiogenesis. Subsequent LUNA imaging should reveal declining fluorescence as the tissue heals and as the microcirculation is pruned back to baseline levels (Figures 1-3).

2. Acute wound. Fluorescent signal behavior depends largely on the mechanism, duration, and extent of the injury. Soon after injury, ICG penetration is limited by capillary bed injury, causing affected areas to appear hypofluorescent. These areas of relatively low signal are associated with compromised microcirculation and may be non-viable. With appropriate treatment, the return of microcirculation is seen by LUNA as increased fluorescence, which will then subside to basal levels as the wound heals (Figures 4 and 5).

3. Evaluating vascular interventions. A low fluorescent signal adjacent to the wound or injury suggests a proximal vascular defect. LUNA imaging defines areas of concern while establishing a baseline, allowing for efficient point-of-care monitoring before and after intervention. Percutaneous improvements are verified by increasing signal intensity of specific areas. Importantly, improved large vessel hemodynamics noted on the arteriogram may not translate into improved microvascular status, thus the value of visualizing functional microcirculation at the wound level (Figures 6 and 7).

Clinical Application of ICG Fluorescence Microangiography

Chronic wounds
Obtain a baseline LUNA image of the wound and surrounding area. For reference purposes, image the contralateral limb or similar area. Perform SPY-Q AutoView analysis of image sequences. Monitor therapy via serial monthly analysis. Effective therapy is confirmed as AutoView metrics move toward reference values.

Acute wound, injury, or post-intervention
Obtain a LUNA image sequence of the wound, injury, or poorly perfused area. Repeat the LUNA sequence following intervention. Select the area of interest using the SPY-Q region analysis tool. Increasing ingress and/or egress values indicate improved status. Note that vascular intervention effectiveness may be visualized immediately and may continue to improve over 1 to 2 weeks.

Summary
Wound bioimaging using ICG fluorescence microangiography is a new technique that permits visualization and qualitative and quantitative assessment of microcirculation surrounding and within the wound bed. For chronic wounds, LUNA imaging is used to assess the baseline status of the wound microcirculation and monitor changes reflecting therapeutic angiogenesis, improved perfusion, and return to normal physiological vascularity. Hyperfluorescence is often observed asymetrically in the wound perimeter, reflecting an inadequate and abnormal angiogenic response where vessels are activated and stuck in a hyperpermeable state. This form of hyperpermeability is associated with chronic inflammation and increased production of VEGF. Successful treatment of chronic wounds may lead to a transient increase in hyperfluorescence followed by a reduction in fluorescence as the microcirculation normalizes and returns to the physiological baseline. Therefore, fluorescence microangiography represents a major advance for wound care by providing the first visual biomarker to assess wound microvasculature at presentation, allowing for the selection and precise application of advanced therapies and enabling longitudinal follow-up of this biomarker until complete healing. Future wound interventions integrating LUNA imaging during clinical trials may discover a companion system for wound care by providing the first visual biomarker to assess wound microvasculature at presentation, allowing for the selection and precise application of advanced therapies and enabling longitudinal follow-up of this biomarker until complete healing. Future wound interventions integrating LUNA imaging during clinical trials may discover a companion system for...