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Wound assessment paradigm shift: incorporating point-of-care bacterial fluorescence imaging into standard of care

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ABSTRACT

The clinical signs and symptoms of infection in acute and chronic wounds are unreliable. Similarly, swab cultures are inaccurate in this population. Tissue biopsies and polymerase chain reaction (PCR) are more accurate but the results require several days to obtain. As a result, the clinician is forced to treat patients empirically. This has led to the overuse of antibiotics and the failure of advanced therapies due to unrecognized infection. To address this problem a point-of-care diagnostic was developed to identify bacteria in acute and chronic wounds. The MolecuLight procedure (MiX) exposes the wound bed to violet light at 405 nm. Bacterial fluorophores absorb the light. In turn they fluoresce at specific wavelengths: porphyrins (red) and pyoverdines (cyan). The device detects bacteria in the wound bed at a level greater than 10^4 by measuring the amounts of red and or cyan fluorescence. A robust body of literature has demonstrated that elevated bacterial levels impede wound healing. The MiX can detect elevated bacteria burden in a wound allowing the clinician to address the infection. In addition, the device can guide advanced wound therapies such as antibiofilm agents, negative wound pressure therapy and preparation of the wound bed for grafting.

Keywords: bacterial fluorescence imaging, MolecuLight, wound assessment, wound infection

1. INTRODUCTION

Wound infection currently costs Medicare/Medicaid an estimated $28 billion per year[1] in the US alone, and affects all health care settings (in-patient and out-patient in hospitals, wound care and burn centers, long-term care, etc.). Wound infection occurs when bacteria or other microbes move deeper within the wound tissue and proliferate at a rate that evokes the host response[2]. This results in damage to local tissue and prevention of wound healing[3]. Bacteria in wounds is present in both acute and chronic wounds and presents a clinical challenge that delays or prevents wound healing at loads of 10^4 CFU/g or higher[4-6]. Presence of clinically significant bacterial loads requires a targeted treatment plan to optimize the wound for healing. Despite advances in wound healing technology (i.e. skin substitutes, negative pressure and other advanced therapies) over the past decades, the percentage of wounds that heal within 12 weeks remains at 40%[7], prolonging and exacerbating wound care costs and patient trauma. An area of advancement that has lagged behind other areas of wound care is the diagnosis of infection. Standard of care is highly subjective as clinicians typically look for classic signs and symptoms in wound tissue (i.e. friable granulation tissue, slough, redness, edema, purulent exudate, pain or presence of necrotic tissue) that are visible to the naked eye[8]. However, bacteria in wounds are not visible and wound infection is often grossly under-detected as concerning levels of bacteria and infection are often asymptomatic[9]. Furthermore, detection of these clinical signs and symptoms is highly subjective and varies widely from patient to patient[9-11]. Methods to measure and verify presence of bacterial load in wounds include wound sampling techniques (swabs, biopsies) and microbiological culture analysis. These methods are limited by the delay in time to acquire results (often up to a week) and the costs involved[12]. Furthermore, these methods are prone to erroneous results; thus many clinicians opt not to sample the majority of wounds[12] and wound diagnosis is subject to high false negative rates.

Diagnostic imaging provides objective evidence and documentation that can be used to guide timely decisions and interventions, which is essential since wound care treatment often happens in the same visit as the diagnosis. Bed-side diagnosis of the presence and degree of bacterial burden in real-time can inform appropriate treatment of wounds just as ultrasound, computed tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography (PET) have done for the fields of oncology and cardiology. The position of wounds on the surface of skin, where they can undergo non-invasive illumination with an excitation light, makes them ideally suited for optical imaging. Technologies

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that enable real-time visualization of bacteria in wounds provide an effective solution to encourage a paradigm shift in wound care, whereby clinicians can use objective imaging information obtained in real-time to facilitate evidence-based treatment of wounds.

2. DETECTION OF BACTERIAL LOAD AND LOCATION USING THE MOLECULIGHT *I:X* IMAGING DEVICE

The MolecuLight *i:X* imaging device (MolecuLight Inc., Canada) is a handheld point-of-care diagnostic tool designed to detect the load (>10^4 CFU/g) and location of bacteria in wounds to guide wound care. The imaging device excites and captures fluorescence signals by shining a violet excitation light (405 nm) on the field of view (i.e. wound) using built-in light-emitting diodes (LEDs) (Figure 1). Unlike ultraviolet light, which is phototoxic, the low intensity visible spectrum violet light is entirely safe for clinical use[13]. The autofluorescence wavelengths emitted by the 405-nm excited wound components are typically between 420-700 nm in the visible spectrum. Various components of the wound fluoresce in different wavelengths; bacteria that colonize wounds produce red or cyan fluorescent signatures[13, 14]. A customized optical filter retains signals only from wavelengths associated with bacterial fluorescence and a narrow range of tissue autofluorescence (for anatomical context). The optical filter narrows the spectrum of emitted wavelengths to allow fluorescence between 501-542.5 nm (green in color) and 601-664 nm (red in color). The specific optical transmission and emission bands of the filter also blocks the 405 nm excitation light from being captured during fluorescence imaging. This occurs in real-time without the need for any digital processing. As a result, an image with red, cyan and/or green fluorescence is produced. Multiple clinical trials and clinical studies have demonstrated that red fluorescence is associated with bacterial loads >10^4 CFU/g (moderate to heavy growth), with a positive predictive value of 95-100%[13, 15, 16].

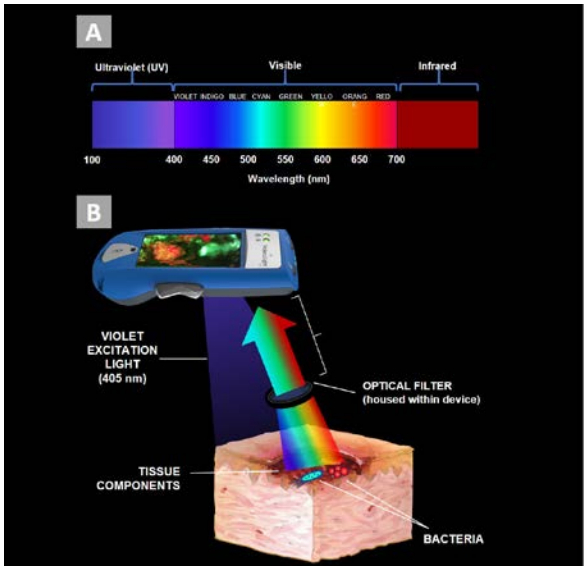

Figure 1. Excitation of tissues and bacteria with 405 nm violet light results in fluorescence signals that span the spectrum of visible light (A). Optical filters enable signals from information-rich wavelength bands and prevent violet light from contaminating image without any digital processing (B). Figure adapted from[17].

2.1 Bacterial and Tissue Fluorescence

Most bacterial pathogens produce red fluorescing porphyrins, which are endogenously produced in the bacterial heme pathway[18]. Synthesis progresses through a number of intermediates including red fluorescent porphyrins (protoporphyrin IX, coproporphyrins, uroporphyrin) before incorporation of iron, resulting in heme[18, 19]. An *in vitro* study of the *i:X* device's ability to detect certain bacterial species detected fluorescence signals from 35 common bacterial wound pathogens, 31 of which produced readily visible red fluorescence[20]. Red fluorescence was observed from the most common bacterial species found in wounds *including Staphylococcus aureus*, *Enterococcus faecalis*, and *Escherichia coli*[20], gram positive bacteria, gram negative bacteria, aerobes, and anaerobes. Pseudomonads (e.g. *Pseudomonas aeruginosa*) uniquely produce pyoverdines resulting in a cyan (blue/green) fluorescent signature[21]. Most tissues appear green on fluorescence images due to fluorescence of collagen and elastin within the skin[22, 23]. The MolecuLight *i:X* imaging device can detect a diverse variety of planktonic bacteria as well as bacteria housed within a biofilm. Biofilm, an extracellular polymeric substance that encapsulates bacteria and protects it against host defenses and some antibiotics and other antimicrobial agents[24, 25] is found in the majority of chronic wounds[25, 26]- estimates range from 50 to 90% - and certainly contributes to wound chronicity. Compelling *in vitro* data demonstrate the utility of the Moleculight *i:X* imaging device in detecting bacterial fluorescence in biofilms, both monomicrobial and polymicrobial in nature[20]. These data clearly show the ability of both the violet light and the resulting fluorescence signals to penetrate the biofilm matrix and visualize the bacteria within.

2.2 Acquiring Fluorescence Images and Measuring Wound Area Using the MolecuLight *i:X*

Capturing images of bacterial fluorescence and measuring wound area are non-invasive procedures that can be obtained in real-time without need for contrast agents (**Figure 2**). To measure wound area, specially designed WoundStickers™ are first placed adjacent to the wound and opposite to one another for calibration. The *i:X* device is positioned 8-12 cm away from and parallel to the wound. The device's "range finder" light turns green when the device is placed within this range. The device automatically focuses on the wound (or the clinician can touch the screen to focus the image) to capture an image. Wound measurement is obtained by either manually outlining the wound border or using the device's software to automatically detect the WoundStickers and the wound border. Using the handheld device, >95% accuracy of wound area, length and width can be achieved when measurements are overlaid on an image of the wound[27]. The lights in the room are switched off before performing fluorescence imaging to ensure minimal light contamination. Alternatively, a disposable DarkDrape™ accessory could be used to achieve an optimal level of darkness to capture the image. The violet light is then turned on and fluorescence information from the wound instantly appears on the screen. The presence of red or cyan fluorescence in the image indicates regions of moderate-to-heavy bacterial loads [13, 16, 17] in real-time. A video or image is used to document this fluorescence information.

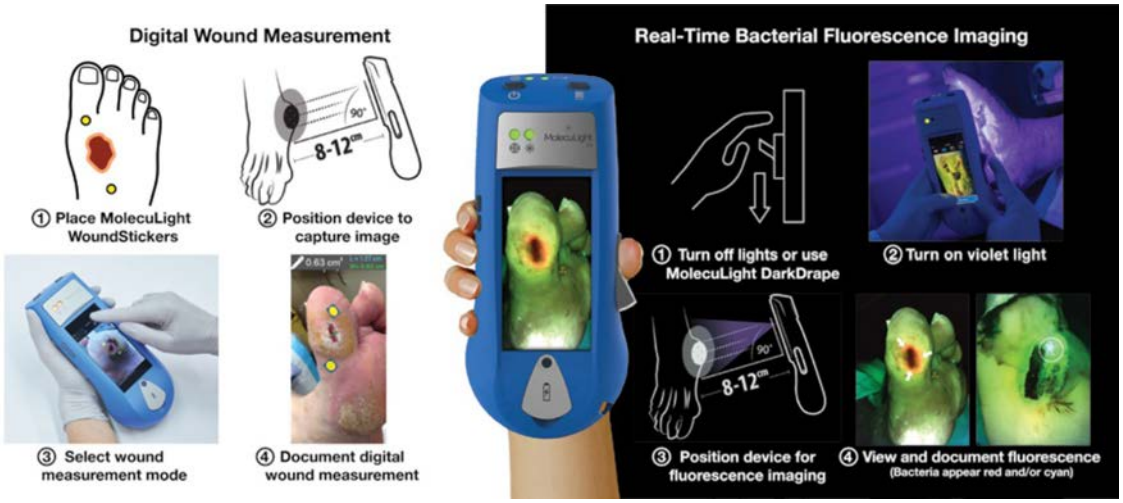

Figure 2. Acquisition of wound measurement and fluorescence images using the MolecuLight *i:X* imaging device. Digital wound measurement is obtained under standard lighting. Fluorescence images are captured in darkness. Figure adapted from [27]

3. USING REAL-TIME FLUORESCENCE IMAGING INFORMATION IN WOUND CARE

Many studies have demonstrated that the presence of bacterial fluorescence in wounds is indicative of bacterial loads $>10^4$ CFU/g[13, 16, 28-30]. These levels of bacteria are known to be detrimental to wound healing[4]. Use of the MolecuLight *i:X* fluorescence imaging enables visualization and localization of these potentially harmful levels of bacteria, enabling evidence-based decision making to enhance patient care. The utility of fluorescence imaging information when used as part of standard wound assessment is highlighted in a recent clinical trial consisting of 19 adult patients with a variety of chronic wounds (e.g. pressure ulcers, venous leg ulcers, diabetic foot ulcers, and surgical wounds). Fluorescence imaging identified wounds with bacterial loads $>10^4$ CFU/g that were missed by clinical signs and symptom (CSS) assessment alone. Furthermore, in 95% of wounds assessed, fluorescence imaging was found to guide care as it identified the locations of those bacterial loads so that localized treatment could be targeted. Clinicians completed a questionnaire to indicate which wound care procedures were influenced by fluorescence imaging[16]. Detection of bacterial fluorescence led to modified treatment plans in 73% of wounds assessed in the clinical trial, and influenced specific procedures in wounds care including: assessment (74%), sampling location (47%), cleaning (42%), antimicrobial decisions (47%), and change (increase or decrease) in antibiotic usage (36%)[16]. A subsequent, recently completed multi-center clinical trial involving 350 patients and 20 clinicians at 14 clinical sites reproduced these findings and reported a sensitivity in detecting bacterial loads $>10^4$ CFU/g that was 4-fold higher than clinical signs and symptoms assessment alone[31].

4. FLUORESCENCE INFORMATION GUIDES ADVANCED WOUND THERAPIES

Optimal wound care requires frequent monitoring of bioburden as well as of the effectiveness of chosen treatments. Advanced therapies are prevalent in the wound care field, at great benefit to the patient when used appropriately, but most are contraindicated when high bacterial loads are present in the wound. Under the current standard of care, clinicians often guess on when it is appropriate to switch to these advanced and costly therapies. The MolecuLight *i:X* imaging device provides real-time information on bacterial burden in wounds and thus has become an integral part of wound care practice at the Serena Group's clinics. Fluorescence images are now used to provide information on bioburden in wounds both in multiple clinical trials being conducted as well as the daily operations of the clinics' wound care practice. The fluorescence images now inform decision-making on optimal timing of advanced treatment to facilitate wound healing and on the readiness of a wound to successfully accept such treatments when free of clinically significant bacteria.

4.1 Negative Pressure Wound Therapy (NPWT)

NPWT is an advanced wound therapy that involves a sealed wound dressing connected to a vacuum pump to suck up excess exudate, which is thought to promote wound healing[32]. NPWT improves wound closure rates by facilitating formulation of granulation tissue and increasing local blood flow[33-35]. Currently, clinicians rely on manufacturer guidelines, institutional practice standards, and their own experience rather than patient-specific information to inform NPWT treatment decisions (i.e. when to initiate NPWT, timing of dressing changes, and length of total NPWT treatment). This generic approach often results in wounds that have an increase in bioburden after NPWT[36] and increases in costs associated with supplies and clinician time due to the lengthy dressing change time required for these systems. Fluorescence information using the MolecuLight *i:X* imaging device overcomes these challenges by indicating bacterial burden through the sealed NPWT dressing, prior to its removal[30], thereby providing the clinician with an indication of whether the dressing needs to be removed and the wound cleaned, or whether bacteria is absent from the wound, permitting the dressing to remain intact to continue facilitating wound closure. Three cases in which fluorescence imaging information was used to guide the real-time application and monitoring of negative wound pressure therapy (NPWT) are highlighted below.

4.1.1. Clinical Case 1: Fluorescence imaging monitors effectiveness of NPWT used to treat a pressure ulcer

This stage 3 sacral pressure ulcer with delayed healing beyond expectations underwent the MolecuLight *i:X* fluorescence imaging procedure for bacterial presence, location and load (>10^4 CFU/g). Fluorescence images revealed widespread red (bacterial) fluorescent signal throughout the periwound tissues, explaining the healing delay (**Figure 3**). Quantitative cultures from a punch biopsy later confirmed high bacterial loads and an assay indicated elevated bacterial protease activity, also associated with delayed healing. The wound was treated over four weeks with a biofilm disrupting antimicrobial gel (BlastX, NextScience) and negative pressure wound therapy (VAC, 3M). Fluorescence images were captured to monitor treatment effectiveness over a four-week period. At days 14 and 21, images revealed persistence of the bacterial presence (red fluorescence), however an assay to assess protease activity levels (WoundCheck) found that by day 21, the bacterial protease activity had been neutralized in this wound. Over the next week a dramatic reduction in bacterial fluorescence signal was observed and the wound reduced in surface area by 50%. This case highlights the necessary eradication of bacterial loads before substantial wound healing can occur.

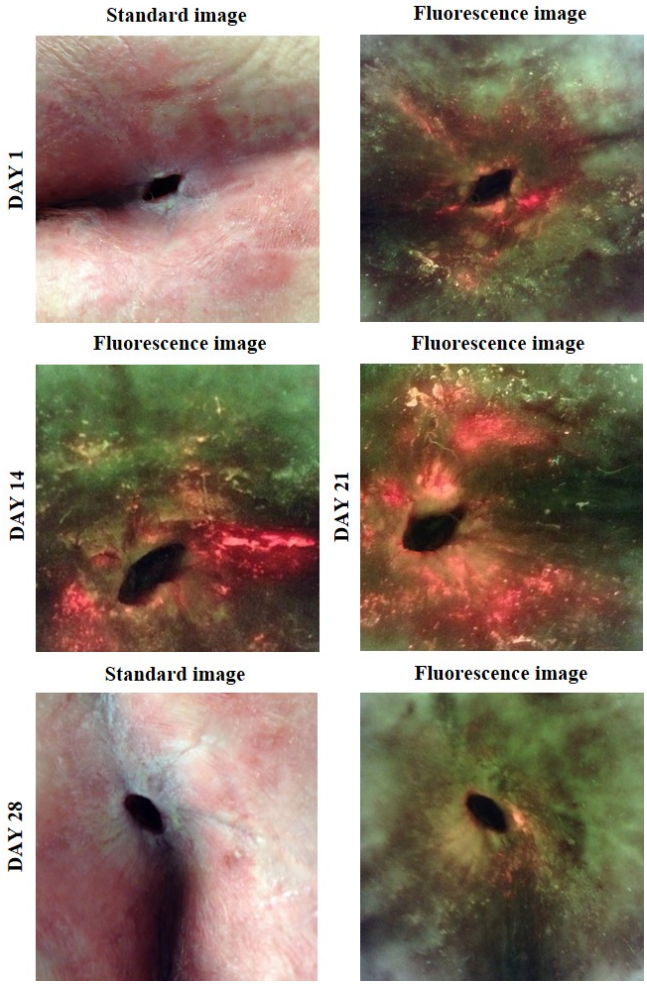

Figure 3**.** Standard and fluorescence images of a stage 3 sacral pressure ulcer undergoing a combination of BlastX and NPWT. Bacterial (red) fluorescence was observed in the periwound region and edge of wound on Day 1, 14 and 21. By day 28, bacterial load in and around the wound was significantly reduced.

4.1.2. Clinical Case 2: Fluorescence imaging guides extent and location of wound cleaning at scheduled NPWT dressing change

NPWT was used to treat an appendectomy abscess in a 58-year old patient. Fluorescence images were used to monitor treatment effectiveness over the weeks of this patient's NPWT care. At the visit shown, fluorescence imaging was acquired through the clear NPWT dressing adhesive layer, prior to its removal. Images revealed red (bacterial) fluorescence under adhesive and on the wound. The red fluorescence was also readily apparently on the wound when imaged after removal of NPWT dressings and persisted after initial wound cleaning. Fluorescence images guided the extent and location of additional cleaning of this wound to maximize removal of the bacterial load before reapplying the NPWT dressing (**Figure 4**).

These cases and prior studies[28, 30, 37] demonstrate the clinical benefits of incorporating fluorescence imaging to monitor effectiveness and to guide timing of NPWT dressing changes. Significant health economic benefits are also observed when fluorescence imaging is used to guide NPWT. With estimates of $89 per each NPWT dressing change[38], NPWT is perceived to be a costly therapy due to the skill and resources involved in applying the dressing and vacuum seal. Real-time monitoring of bioburden in wounds using the MolecuLight *i:X* imaging device enables cost savings and efficient use of resources by *(i*) providing evidence to monitor effectiveness of a selected therapy and (*ii*) enabling acquisition of fluorescence images through the optically transparent adhesive seals of the NPWT, limiting need for unnecessary dressing changes.

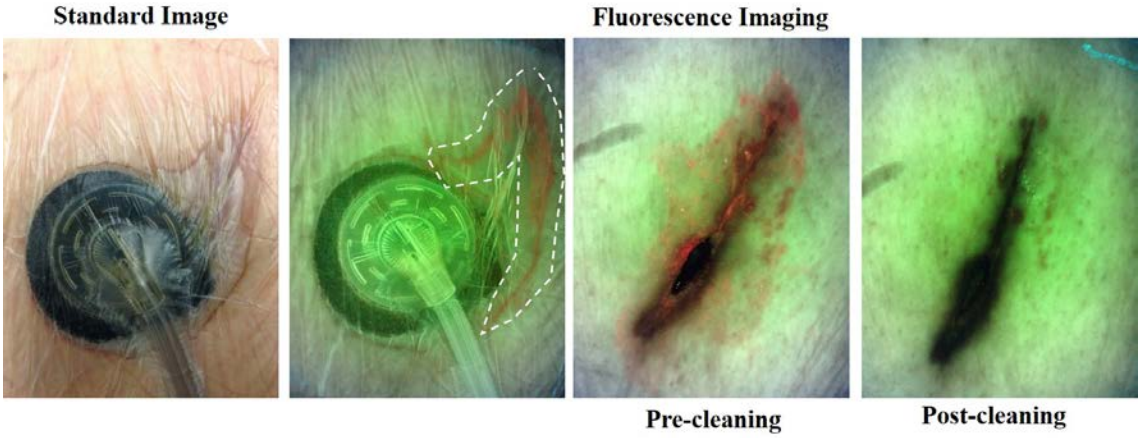

Figure 4: Fluorescence images taken with the *i:X* device during NPWT. Red fluorescence was observed under the adhesive (circled) and on wound. Fluorescence guided location and extent of cleansing at this dressing change. As a result, a significant reduction in bacterial (red) fluorescence was observed post-cleaning.

4.2 Skin Grafts and Skin Substitutes

The clinical and health economic benefits of fluorescence imaging has also been previously reported when guiding application of other advanced wound therapies such as skin substitutes and grafts. Currently, clinical judgement is the primary way of identifying whether wounds are ready for the application of cell-and tissue-based products (CTPs) or skin substitutes, which are used to facilitate healing in large or clinically challenging wounds. In a 5 patient case series by Aung et al., fluorescence imaging when combined with clinical judgement proved to be superior to clinical judgement alone in predicting successful application of CTPs to chronic wounds. In each case, clinical judgement indicated readiness of CTPs, yet fluorescence imaging revealed the presence and location of bacteria burden ($>10^4$ CFU/g) prior to or following application of CTPs. The clinician was blinded to the fluorescence results; substitutes were applied based on clinical judgement and failed in each case. The author noted that if objective information provided by fluorescence imaging had been used to delay application of CTPs until the bacteria was eradicated, this could have resulted in a potential savings of more than $7,660 per patient (associated with failed CTP application over a 4-week period)[39]. If bacterial fluorescence information provided by the MolecuLight *i:X* Imaging device were used to support clinical judgement when deciding on appropriate timing and use of skin substitutes, it could have helped guide extent and location of wound debridement and ensure that wound beds were optimized for use of CTPs.

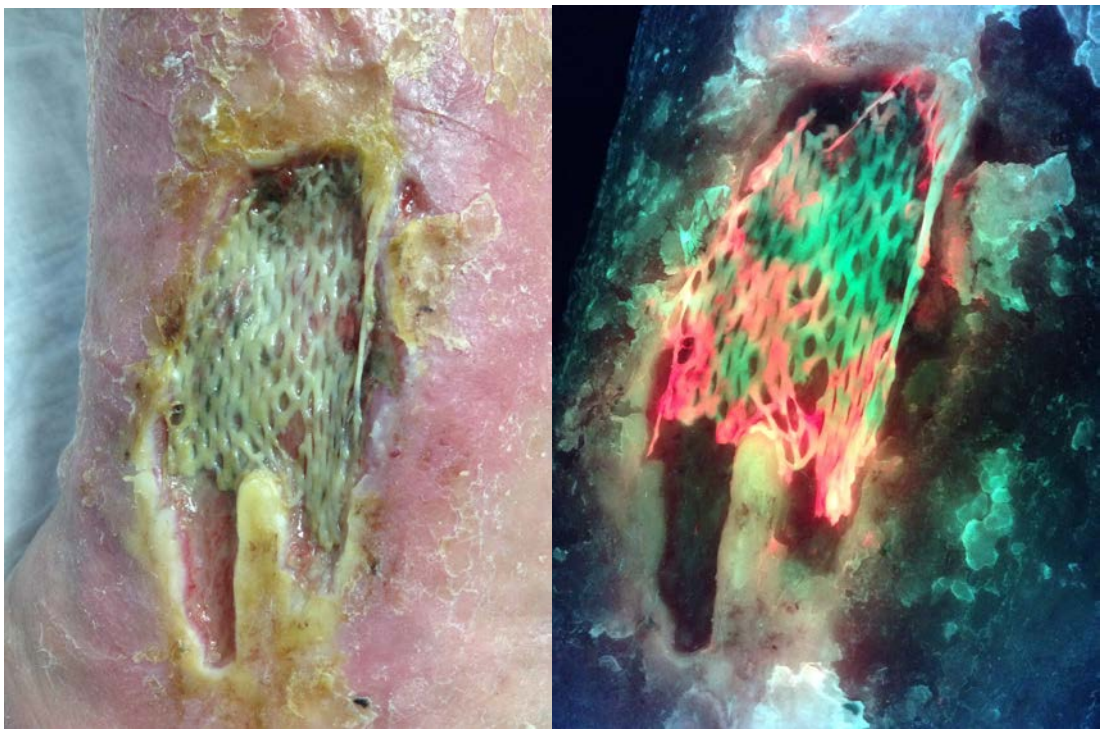

Figure 5. Failing graft on the left. On the fluorescence image on the right the red fluorescence reveals excessive bacterial burden.

5. CONCLUSION

Despite the significant and growing annual costs of wounds to health systems, and the suboptimal results of the clinical standard of care, imaging innovations in the field of wound care have, until recently, been entirely lacking. Reliance on classic signs and symptoms to assess wounds or generic, subjective guidelines to determine appropriate treatment selection provides clinicians with little confidence that their treatment decisions are optimized for wound healing. Fluorescence imaging may be the modality that can revolutionize wound care. The MolecuLight *i:X* is introducing diagnostic imaging to the field of wound care for the first time, by providing objective real-time imaging information on the presence, distribution of bacteria at point-of-care, at loads known to delay wound healing. Fluorescence imaging of bacterial burden provides real-time feedback at the point-of-care on the effectiveness of wound bed preparation and supports informed decision-making on selection of advanced wound care therapeutics.

At the Serena Group's clinics, the routine use of the MolecuLight *i:X* has altered how wounds are assessed and treated and is now a routine and essential clinical tool. Detection of bacterial fluorescence in wounds at the bedside allows for immediate decision-making to prevent spread of infection. Furthermore, fluorescence images provide a source of truth to monitor effectiveness of wound therapies and increases confidence that the treatments selected are optimized to place wounds on a healing trajectory, thereby improving patient outcomes.

REFERENCES

[1] S. R. Nussbaum, M. J. Carter, C. E. Fife *et al.*, "An Economic Evaluation of the Impact, Cost, and Medicare Policy Implications of Chronic Nonhealing Wounds," Value Health, 21(1), 27-32 (2018).

[2] M. Collier, "Recognition and management of wound infections," World Wide Wounds, (2004).

[3] A. R. Siddiqui, and J. M. Bernstein, "Chronic wound infection: facts and controversies," Clin Dermatol, 28(5), 519-26 (2010).

[4] L. Xu, S. V. McLennan, L. Lo *et al.*, "Bacterial load predicts healing rate in neuropathic diabetic foot ulcers," Diabetes Care, 30(2), 378-80 (2007).

[5] R. Wolcott, N. Sanford, R. Gabrilska *et al.*, "Microbiota is a primary cause of pathogenesis of chronic wounds," J Wound Care, 25(Sup10), S33-S43 (2016).
[6] R. Edwards, and K. G. Harding, "Bacteria and wound healing," Curr Opin Infect Dis, 17(2), 91-6 (2004).
[7] C. E. Fife, K. A. Eckert, and M. J. Carter, "Publicly Reported Wound Healing Rates: The Fantasy and the Reality," Adv Wound Care (New Rochelle), 7(3), 77-94 (2018).
[8] IWII, "Wound infection in clinical practice (International Wound Infection Institute)," Wounds International, (2016).
[9] S. E. Gardner, S. L. Hillis, and R. A. Frantz, "Clinical signs of infection in diabetic foot ulcers with high microbial load," Biol Res Nurs, 11(2), 119-28 (2009).
[10] T. E. Serena, J. R. Hanft, and R. Snyder, "The lack of reliability of clinical examination in the diagnosis of wound infection: preliminary communication," Int J Low Extrem Wounds, 7(1), 32-5 (2008).
[11] M. Reddy, S. S. Gill, W. Wu *et al.*, "Does this patient have an infection of a chronic wound?," JAMA, 307(6), 605-11 (2012).
[12] G. Kallstrom, "Are quantitative bacterial wound cultures useful?," J Clin Microbiol, 52(8), 2753-6 (2014).
[13] M. Y. Rennie, L. Lindvere-Teene, K. Tapang *et al.*, "Point-of-care fluorescence imaging predicts the presence of pathogenic bacteria in wounds: a clinical study," J Wound Care, 26(8), 452-460 (2017).
[14] C. Rimington, " Spectral-absorption coefficients of some porphyrins in the Soret-band region," Biochem J, 75(3), 4 (1960).
[15] C. M. Hurley, P. McClusky, R. M. Sugrue *et al.*, "Efficacy of a bacterial fluorescence imaging device in an outpatient wound care clinic: a pilot study," J Wound Care, 28(7), 438-443 (2019).
[16] T. E. Serena, K. Harrell, L. Serena *et al.*, "Real-time bacterial fluorescence imaging accurately identifies wounds with moderate-to-heavy bacterial burden," J Wound Care, 28(6), 346-357 (2019).
[17] M. Y. Rennie, D. Dunham, L. Lindvere-Teene *et al.*, "Understanding Real-Time Fluorescence Signals from Bacteria and Wound Tissues Observed with the MolecuLight i:X(TM)," Diagnostics (Basel), 9(1), (2019).
[18] W. K. Philipp-Dormston, and M. Doss, "Comparison of porphyrin and heme biosynthesis in various heterotrophic bacteria," Enzyme, 16(1), 57-64 (1973).
[19] Y. K. Nitzan, M., "Endogenous Porphyrin Production in Bacteria by δ-Aminolaevulinic Acid and Subsequent Bacterial Photoeradication," Lasers in Medical Science, 14(4), 8 (1999).
[20] L. M. D. Jones, D.; Rennie, M. Y.; Lopez, A. J.; Keim, K. C.; Little, W.; Gomez, A.; Bourke, J.; Ng, H.; DaCosta, R. S. ; Smith, A. C., "In vitro detection of porphyrin-producing wound pathogens with real-time bacterial fluorescence imaging," Future Microbiology, Under review, (2019).
[21] J. M. A. Meyer, M.A., "The Fluorescent Pigment of Pseudomonas fluorescens: Biosynthesis, Purification and Physicochemical Properties," Microbiology, 107, 9 (1978).
[22] M. Monici, "Cell and tissue autofluorescence research and diagnostic applications," Biotechnol Annu Rev, 11, 227-56 (2005).
[23] J. Blomfield, and J. F. Farrar, "The fluorescent properties of maturing arterial elastin," Cardiovasc Res, 3(2), 161-70 (1969).
[24] S. L. Percival, C. Emanuel, K. F. Cutting *et al.*, "Microbiology of the skin and the role of biofilms in infection," Int Wound J, 9(1), 14-32 (2012).
[25] G. A. James, E. Swogger, R. Wolcott *et al.*, "Biofilms in chronic wounds," Wound Repair Regen, 16(1), 37-44 (2008).
[26] C. Attinger, and R. Wolcott, "Clinically Addressing Biofilm in Chronic Wounds," Adv Wound Care (New Rochelle), 1(3), 127-132 (2012).
[27] R. Raizman, D. Dunham, L. Lindvere-Teene *et al.*, "Use of a bacterial fluorescence imaging device: wound measurement, bacterial detection and targeted debridement," J Wound Care, 28(12), 824-834 (2019).
[28] R. Hill, M. Y. Rennie, and J. Douglas, "Using Bacterial Fluorescence Imaging and Antimicrobial Stewardship to Guide Wound Management Practices: A Case Series," Ostomy Wound Manage, 64(8), 18-28 (2018).
[29] E. L. Blackshaw, and S. L. A. Jeffery, "Efficacy of an imaging device at identifying the presence of bacteria in wounds at a plastic surgery outpatients clinic," J Wound Care, 27(1), 20-26 (2018).
[30] R. Raizman, "Fluorescence imaging guided dressing change frequency during negative pressure wound therapy: a case series," J Wound Care, 28(Sup9), S28-S37 (2019).
[31] T. E. H. Serena, K.; Serena, L.; Patel, K.; Sabo, M.; Thibodeaux, K.; Kasper, M.; Le, L.; Halperin, G.; Briggs, P.; Baer, M.; Bullock, N.; DiMarco, D.; Cole, W.; Hamil, R.; Clapp, R. , "Wound assessment paradigm shift: a 350-patient multi-site clinical trial incorporating bacterial fluorescence imaging into standard of care."

[32] J. Webster, Z. Liu, G. Norman *et al.*, "Negative pressure wound therapy for surgical wounds healing by primary closure," Cochrane Database of Systematic Reviews(3), (2019).
[33] A. Wackenfors, J. Sjogren, R. Gustafsson *et al.*, "Effects of vacuum-assisted closure therapy on inguinal wound edge microvascular blood flow," Wound Repair Regen, 12(6), 600-6 (2004).
[34] M. J. Morykwas, L. C. Argenta, E. I. Shelton-Brown *et al.*, "Vacuum-assisted closure: a new method for wound control and treatment: animal studies and basic foundation," Ann Plast Surg, 38(6), 553-62 (1997).
[35] S. Gupta, A. Gabriel, J. Lantis *et al.*, "Clinical recommendations and practical guide for negative pressure wound therapy with instillation," Int Wound J, 13(2), 159-74 (2016).
[36] C. Yang, S. G. Goss, S. Alcantara *et al.*, "Effect of Negative Pressure Wound Therapy With Instillation on Bioburden in Chronically Infected Wounds," Wounds, 29(8), 240-246 (2017).
[37] R. Raizman, "Bacterial fluorescence imaging of wounds undergoing negative pressure wound therapy guides treatment selection and timing of dressing changes: a clinical case series," J Wound Care (Under Review), (2018).
[38] J. J. Kim, M. Franczyk, L. J. Gottlieb *et al.*, "Cost-effective Alternative for Negative-pressure Wound Therapy," Plast Reconstr Surg Glob Open, 5(2), e1211 (2017).
[39] B. Aung, Can Fluorescence Imaging Predict the Success of CTPs for Wound Closure and Save Costs? (2019).