Use of a bacterial fluorescence imaging system to target wound debridement and accelerate healing: a pilot study

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Objective: Optimal wound-bed preparation consists of regular debridement to remove devitalised tissues, reduce bacterial load, and to establish an environment that promotes healing. However, lack of diagnostic information at point-of-care limits effectiveness of debridement.

Method: This observational case series investigated use of point-of-care fluorescence imaging to detect bacteria (loads >10⁴CFU/g) and guide wound bed preparation. Lower extremity hard-to-heal wounds were imaged over a 12-week period for bacterial fluorescence and wound area.

Results: A total of 11 wounds were included in the study. Bacterial fluorescence was present in 10 wounds and persisted, on average, for 3.7 weeks over the course of the study. The presence of red or cyan

fluorescent signatures from bacteria correlated with an average increase in wound area of 6.5% per week, indicating stalled or delayed wound healing. Fluorescence imaging information assisted in determining the location and extent of wound debridement, and the selection of dressings and/or antimicrobials. Elimination of bacterial fluorescence signature with targeted debridement and other treatments correlated with an average reduction in wound area of 27.7% per week (p<0.05), indicative of a healing trajectory.

Conclusion: These results demonstrate that use of fluorescence imaging as part of routine wound care enhances assessment and treatment selection, thus facilitating improved wound healing.

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bacterial load • debridement • fluorescence imaging • point-of-care systems • wound healing

he economic burden of wounds in the US costs Medicare an estimated \$28.1 billion to \$96.8 billion annually. 1-3 The associated costs of care and risk for severe complications (such as infection or amputation) increases the longer a wound remains open.^{4,5} A stall in wound closure may be due to a variety of systemic and local factors, among them high bacterial burden.⁶ At bacterial loads of 10⁴ colony-forming units (CFU) per gram of tissue, delayed wound healing is observed, and wound status worsens for each additional log increase in bacterial load.7 Wounds with bacterial loads that exceed 106CFU/g are considered clinically infected.8 The presence of devitalised tissue further impairs wound healing by preventing new tissue from forming, thus creating a nidus for bacterial growth.9

Frequent and thorough debridement is used to remove these barriers to the healing process¹⁰ and establish a balanced healing environment to control infection.¹¹ Debridement, in line with standard clinical practice, aims to remove necrotic and fibrous tissue and reduce bacterial burden in the wound. However, thorough removal of bacterial burden via debridement is challenging as bacteria are invisible to the naked eye and many infected wounds do not show clinical signs and symptoms of infection.¹² As a result, bacteria at loads

that hinder healing may persist in the wound, further prolonging wound chronicity. Although swabs or wound biopsies can be used to confirm presence of bacterial burden in wounds, use of these procedures varies widely and results take days to obtain. The lack of objective information at point-of-care to guide appropriate debridement contributes to delays in wound healing.

Diagnostic procedures to identify bacterial burden and monitor wound closure at the bedside could help to improve wound healing by providing objective information on the location and extent of bacterial colonisation in wounds. Fluorescence imaging of bacteria provides immediate information on the presence and location of moderate to heavy bacterial loads (>10⁴CFU/g)¹³ in wounds and surrounding tissues that are otherwise invisible to the naked eye. The handheld diagnostic imaging device used in this case series emits a safe violet (405nm) light that excites porphyrinproducing bacteria to produce a unique red fluorescent signature. Fluorescence imaging detects 87% of the most common wound pathogens,14 including Pseudomonas aeruginosa, which uniquely produces a cyan fluorescent signature due to endogenous pyoverdine production; a small number of bacterial genera (Streptococcus and Enterococcus) do not emit detectable fluorescence signals. 15 Multiple clinical studies report positive predictive values over 87% when using fluorescence imaging to detect bacteria at loads greater than $10^4 \text{CFU/g}^{\,13,16,17}$ on and beneath the surface of wounds, up to 1.5mm depth.¹⁸ The device also contains digital wound area measurement software that automatically detects the wound border

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The aim of this 12-week case series was to evaluate the utility of fluorescence imaging to detect bacterial burden in excess of 10⁴CFU/g in wounds, monitor changes in wound area, and inform treatment selection of lower extremity hard-to-heal wounds that were negative for clinical signs and symptoms of infection. Fluorescence (FL) imaging provided information on bacterial presence that was used to guide debridement, dressing selection and/or decision to use antimicrobials or antibiotics.

Methods

Study design and participants

All patients >18 years of age with an open wound of the lower extremity were eligible for this prospective observational case series. Wounds were included regardless of mechanism of injury, wound size or wound duration. Patients signed a photography-release consent form and were not compensated for participation. Data collection and follow-up were conducted over a 12-week period, or until the wound healed or the patient was lost to follow-up. Information on type of wound, pre-study wound duration (in weeks), wound location and any comorbidities, previous treatments and/or medications was collected from each participant. At each weekly visit, wounds were cleansed with saline and underwent assessment for clinical signs and symptoms of infection using the checklist from the International Wound Infection Institute (IWII).5 This was followed by standard and fluorescence imaging, wound measurement and, when indicated, targeted debridement guided by fluorescence information.

Wound area imaging and measurement

Wound images were captured using a non-contact, handheld fluorescence imaging device (MolecuLight *i:X*, MolecuLight Inc.,Canada). This fluorescence imaging device emits a safe violet light (405nm) and uses specialised optical filters to capture relevant fluorescence from tissue and bacteria. ¹⁸ Presence of bacteria is indicated immediately by appearance of red fluorescence (indicative of porphyrin-producing bacteria, for example, *Staphylococcus aureus* and most wound pathogens, Grampositive and negative, or cyan fluorescence (specific to pyoverdine-producing *Pseudomonas aeruginosa*) ^{13,14,18} on the device screen. In contrast, background tissue appears as various shades of green due to collagen. ¹⁸ Wounds exhibiting red or cyan fluorescence are considered to have moderate to heavy (>10⁴CFU/g) levels of bacteria. ^{13,20-22}

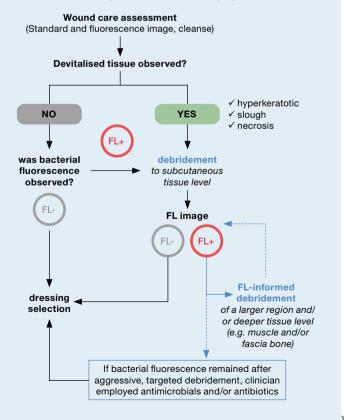
The wound care clinician captured standard and fluorescence images after training on device use and image interpretation. ^{18,23} A range finder on the device was used to ensure that all images were taken at the optimal imaging distance (8–12cm). On either side of the wound, two yellow wound measurement calibration

stickers were placed. A standard image was acquired under normal room light conditions. The clinician used the manual wound measurement function to outline the wound borders on the device's screen. The measurement software then calculated wound area (cm²), as well as maximum length and width of the wound (cm). Stickers were removed and the room was made dark in preparation for fluorescence imaging. Light sensors on the device indicated when the room was dark enough for fluorescence images to be acquired. Fluorescence images were then captured and used to determine presence and location of bacterial loads of 10^4CFU/g or more. Tollowing fluorescence imaging, a decision of fluorescence-positive (red or cyan, 'FL+') or negative ('FL-') was made for each wound.

Debridement decision tree

Fluorescence images were used to guide debridement, dressing selection and/or decision to use antimicrobials or antibiotics, creating an evidence-based clinical decision tree (Fig 1). Clinician workflow using the fluorescence

Fig 1. Debridement decision tree. Images positive for bacterial fluorescence were used to guide debridement. Persistence of bacterial fluorescence provided evidence that a larger region and/or deeper tissue level was warranted. This loop of imaging and fluorescence-informed debridement was repeated until bacterial fluorescence was eliminated. If debridement was not able to eliminate bacterial fluorescence, antimicrobials and/or antibiotics were employed. 'FL+' indicates presence of bacterial fluorescence while 'FL-' indicates absence of bacterial fluorescence detected using the fluorescence imaging device



practice

imaging device was as follows: the clinician first determined whether devitalised tissue (characterised by hyperkeratotic tissue, presence of slough and necrosis) was present in the wound. Presence of devitalised tissue in the wound, regardless of fluorescence signature, prompted debridement to the subcutaneous level. If there was no indication of devitalised tissue and fluorescence images were negative for red or cyan fluorescence, standard evaluation and monitoring was completed. If red or cyan (bacterial) fluorescence was observed, debridement was carried out to the subcutaneous level. Debridement was performed as per standard of care using a surgical blade or curette. After debridement, standard and fluorescence images were captured once more to reassess for presence of moderate to heavy bacterial burden. If red or cyan fluorescence signal persisted in fluorescence images, fluorescence-informed debridement was carried out on a potentially larger region and/or to a deeper level (muscle and/or fascia). This feedback loop of fluorescence imaging and fluorescence-informed debridement was repeated to deeper tissue levels (as far as bone) as needed, until bacterial fluorescence was no longer evident. Persistence of red or cyan fluorescence, despite multiple rounds of debridement, was indicative of deeper bacterial burden and prompted clinicians to employ antimicrobials or antibiotics. After debridement was completed, all wounds were measured using the measurement application.

Statistical analysis

Wound area was reported as individual data points, collected weekly. Slopes of weekly change in wound area (expressed as a percentage, %) were calculated using linear regression modelling. Weekly change in

wound area for each patient was then categorised based on presence or absence of bacterial fluorescence at time of wound area measurement, and reported as average \pm standard error of the mean (SEM). An unpaired t-test was used to compare average slope of wound area changes when bacterial fluorescence was present compared with periods where bacterial fluorescence was absent. A significance level of p<0.05 was chosen for statistical tests.

Results

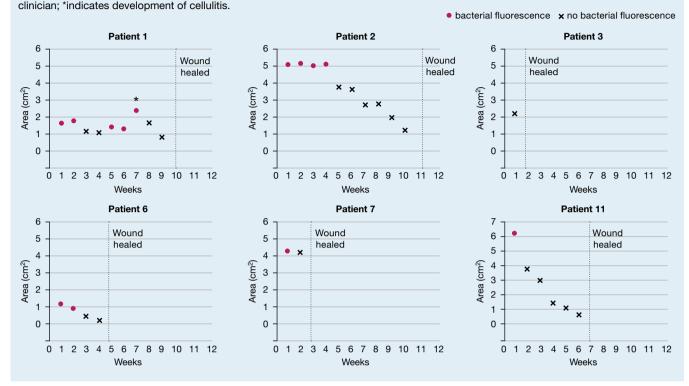
A total of 11 patients (six women, five men) were assessed over a 12-week period. Of these, six patients completed the 12-week assessment; three patients were lost to follow-up and two patients entered another research study. Median patient age was 74.9 years (range: 60–99 years). All patients included in the study had ankle brachial pressure index (ABPI) results within the normal limits. Wound types included: trauma wounds (n=3), venous leg ulcers (VLU, n=5), and diabetic foot ulcers (DFU, n=3). Mean duration of wounds was 16.5 weeks (range: 4-32 weeks) before observation period. At initial assessment, average wound area was 8.1cm² (range: 1.1–35.0cm²). Wound depth was not measured by the clinician, except for patient two. Wounds were located on the right lower leg (n=5), left lower leg (n=3), left heel (n=1), right heel (n=1) and left distal foot/post-trans metatarsal amputation (n=1); most wounds had exposed tissue to the subcutaneous layer (n=9), but partial thickness (n=1) and fascia/tendon (n=1) were also observed.

At initial assessment, no wounds were suspected of infection, based on clinical signs and symptoms (Table 1) but all wounds were considered to have

Table 1. Baseline characteristics of wounds in case series.

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Patient ID	Wound type	CSS assessment*	Region of bacterial fluorescence observed during predebridement assessment [†]	Wound area (cm²)	Wound duration (before study)		
1	Trauma	Negative	Bed + periwound	1.6	16 weeks		
2	Trauma	Negative	Bed + periwound	5.1	13 weeks		
3	VLU	Negative	Negative fluorescence	2.2	4 weeks		
4	Trauma	Negative	Bed	4.8	28 weeks		
5	DFU	Negative	Bed + periwound	22.4	12 weeks		
6	DFU	Negative	Periwound	1.1	14 weeks		
7	VLU	Negative	Periwound	4.3	4 weeks		
8	DFU	Negative	Periwound	4.3	16 weeks		
9	VLU	Negative	Bed + periwound	35.0	32 weeks		
10	VLU	Negative	Bed + periwound	1.9	26 weeks		
11	VLU	Negative	Bed + periwound	6.3	7 weeks		

Wounds exhibiting three or more clinical signs and symptoms (CSS) per category in the International Wound Infection Institute checklist⁵ would have been considered positive for infection or high bacterial loads; †Wounds where red or cyan fluorescence was present were considered positive for bacterial fluorescence indicative of moderate to heavy bacterial loads; DFU—diabetic foot ulcer; VLU—venous leg ulcer



delayed healing (>12-week duration) and fluorescence signals from bacteria (red or cyan) were observed in 10/11 wounds. The presence or absence of bacterial fluorescence in wounds predicted their wound healing. Six out of the 11 (55%) wounds observed in this study healed over the 12-week follow up period, with an average time to heal of 6.3 weeks from initial study visit. In these wounds, removal of bacterial fluorescent signature corresponded with trend for decreased wound area, indicating a reduction in wound size and sustained healing trajectory (Fig 2). Elimination of bacterial fluorescence through targeted debridement or other treatments was associated with an average reduction in wound area of 27.7±10.1% per week (Fig 4). In contrast, the appearance of red or cyan (bacterial) fluorescence was associated with an average increase in wound area of 6.5±10.8% per week (from initial wound size), indicating a non-healing trajectory (Fig 4). Wounds did not heal when fluorescent signature from bacteria persisted, and no trend in wound area was observed (Fig 3). Together, these results suggest that presence of bacteria at loads sufficient to produce detectable fluorescence (>10⁴CFU/g)¹³ hinders healing, as indicated by stagnant or increasing wound area, and eradication of fluorescent signatures from bacteria is associated with a healing trajectory.

Fluorescence imaging following initial CSS-guided debridement revealed persistence of red or cyan (bacterial) fluorescence that warranted an additional

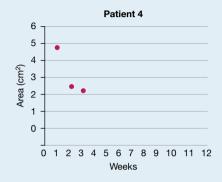
round of debridement in 12.5% (7/56) of assessments. In five of these instances, persistence of red or cyan fluorescence prompted a second round of debridement to a deeper and/or larger area (Table 2). In one instance, fluorescence information resulted in debridement to a superficial/open wound level where initial CSS assessment indicated evaluation of the wound only (that is, CSS indicated no debridement was necessary). In three other instances, fluorescence images revealed a deeper level of bacteria warranting a switch from either superficial/wound level of debridement to the subcutaneous level (n=2), or a switch from muscle/ tendon level of debridement to bone (n=1). In another instance, fluorescence imaging information also enabled visualisation of bacterial burden in regions missed by initial CSS-guided debridement, resulting in a second

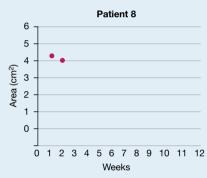
Table 2. Fluorescence information guided a second round of debridement to a deeper level or larger area.

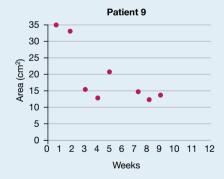
Debridement based on CSS	Debridement guided by fluorescence imaging	Number of instances			
None	Superficial/open wound	1			
Superficial/open wound	Subcutaneous	2			
Muscle/tendon	Bone	1			
Subcutaneous	Subcutaneous (larger area)	1			
CSS—clinical signs and symptoms assessment					

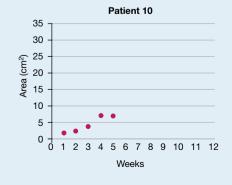
Fig 3. Wounds where bacterial (red or cyan) fluorescence persisted remained on a non-healing trajectory over the duration of the study. Wound area was measured at each weekly visit. Presence of bacterial fluorescence in wounds is denoted by red symbols. Patients 4 and 8 were lost to follow up at weeks four and three respectively; patients 9 and 10 entered another research study at weeks 10 and six, respectively

• bacterial fluorescence x no bacterial fluorescence









round of debridement over an additional 20cm² area of the wound.

Patient 1

A 67-year-old female presented with a trauma wound on the left lower leg as a result of an automobile accident. At initial presentation, the wound duration was 16 weeks and wound area was 1.6cm². Clinical assessment using the IWII checklist⁵ suggested absence of infection in the wound. Fluorescence imaging at initial assessment clearly demonstrated positive bacterial fluorescence in the wound and periwound region. Debridement to the subcutaneous level was performed at the initial visit, but red (bacterial) fluorescence persisted (Fig 5a, d). The patient was treated with an enzymatic debriding agent and hydrophobic bacterial-binding nonadherent contact layer. At visit five (Fig 5b, e), the wound was deemed negative for infection based on CSS assessment, but fluorescence imaging revealed an alarming red fluorescence signature in the periwound region and the wound area increased to 1.4cm², indicating early signs of cellulitis. The patient was then placed on doxycycline hyclate (100mg twice a day for 10 days). On visit six, the wound size had decreased to 1.3cm² and fluorescence imaging revealed absence of red fluorescence from bacteria in the wound bed and scant red fluorescence in the periwound region (Fig 5c, f). At the visit seven, the wound had increased in size to 2.4cm2 and red fluorescence was still observed in the periwound tissues. The patient was then placed on another seven-day course of doxycycline hyclate (100mg). By visit eight, the wound had reduced in size to 1.7cm² and no red fluorescence was detected in and around the wound. The wound continued to decrease in size to 0.8cm² at visit nine, and by visit 10, the wound was completely healed.

Patient 2

A 63-year-old male presented with a trauma wound to his right lower leg after falling off a ladder. At initial presentation, the duration of the wound was 13 weeks and measured 5.1cm² in area and 0.7cm in depth (Fig 6a). The wound did not appear to be overtly infected but had a moderate amount of devitalised tissue present. Fluorescence imaging at initial assessment indicated evidence of red and cyan fluorescence in and around the wound, indicating presence of bacteria (Fig 6e). Post debridement, the cyan fluorescence was reduced, but red fluorescence increased (Fig 6f). The patient was provided with a daily enzymatic debridement agent for at-home treatment and a hydrophobic bacterial-binding nonadherent contact layer was used. At subsequent visits, debridement to the subcutaneous layer was performed on the wound based on bacterial fluorescence on images. After undergoing debridement at visit five, the wound was negative for bacterial fluorescence (6h) and wound size had decreased to 3.8cm². Fluorescence remained negative at subsequent visits, and by visit 11, the wound had completely healed.

Discussion

Results from this observational study demonstrate that early detection and ongoing monitoring of bacterial burden in wounds can facilitate improved wound healing. Fluorescence imaging of bacteria using the handheld imaging device enabled visualisation of the load (>104CFU/g) and location of bacteria in wounds at the bedside. Incorporating this diagnostic imaging procedure into standard wound assessment provided information at point of care on bacterial burden in wounds. This information helped clinicians to determine the location and extent of debridement as well as selection of appropriate antimicrobials. An overall positive impact on wound management was observed as six of these previously hard-to-heal wounds (averaging 16.5 weeks in duration before study participation) healed in an average of 6.3 weeks, over the course of the study.

In 10 of the 11 wounds assessed, bacterial fluorescence was detected (at loads >104CFU/g) despite absence of CSS. These findings are in line with recent studies, ^{24–26} and confirm the ability of fluorescence imaging to detect presence of bacteria (at loads >10⁴CFU/g), in wounds asymptomatic for CSS. It is plausible that at 10⁴CFU/g, symptoms of infection may not manifest; but even at bacterial loads (>106CFU/g) known to indicate infection,8 fluorescence imaging has been shown to detect 50% more wounds than CSS assessment. 13,16 Together, these findings suggest that fluorescence imaging has the potential to reliably alert the practitioner to clinically significant levels of bacteria, even in the absence of overt clinical signs and symptoms of infection. This is the 'early detection' so critical to prevention of disease progression that has been emphasised in other fields such as cancer, but to date has been absent from wound care infection diagnosis.

The wounds included in this case series had an average duration of 16.5 weeks before participation in

Fig 4. Average per week percentage area change. Bars represent average change in wound area when bacterial fluorescence was present (red) compared with absence of fluorescence (open bar). Error bars denote standard error of the mean. *p<0.05 measured by an unpaired t-test

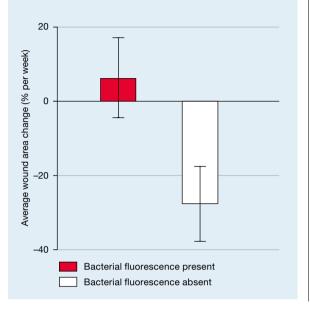


Fig 5. Patient 1, a 67-year-old female with a trauma wound on left lower leg as a result of an automobile accident. Initial wound assessment revealed a wound area of 1.8cm² (a). Red fluorescence (white arrows) was detected at visit one (d). At visit five, wound area increased (b) and the periwound area exhibited red fluorescence (e). By visit six (c), red fluorescence signal had resolved (f)



Fig 6. Patient 2, a 63-year-old male with a trauma wound to his right lower leg after falling off a ladder. At initial visit, the wound had a moderate amount of devitalised tissue, and a wound area of 5.1 cm² (a) and moderate to heavy bacterial load (cyan and red fluorescence, denoted by white arrows) in and around the wound (e). Debridement at visit one (b) resulted in a decrease in cyan but an increase in red fluorescence (f). At visit five, wound size decreased to 3.8cm² (c) and a marked decrease in red fluorescence was observed (g). Following debridement (d), bacterial fluorescence was resolved (h)



Fig 7. Patient 6, a 71-year old female with a 14-week history of a diabetic foot ulcer of the right heel. At initial visit, the wound measured 1.1cm² (a) and showed evidence of moderate to heavy bacterial load (cyan and red fluorescence, white arrows) in and around the wound pre-debridement (e). After initial debridement (b), a decrease in cyan fluorescence was observed but there was an increase in red fluorescence (f). By visit four, wound size decreased to 0.2cm² (c) but bacterial fluorescence was still observed (g). Following debridement (d), the wound was negative for bacterial fluorescence, and the periwound showed slight bacterial fluorescence (h)



this study. The duration of these wounds suggests that they may be stalled in the wound healing pathway, preventing progress towards the proliferation phase of healing in which wound edges begin to contract and wound area is reduced.²⁷ Many factors may contribute to stalled healing, but previous reports,^{7,28–31} and evidence from this study, suggest that presence of bacteria is a critical factor contributing to delayed

healing. A 25% decrease in wound area over four weeks is considered the threshold for a healing trajectory.³² Here, we observed that elimination of bacterial fluorescence from wounds was associated with a pivot from a nonhealing to a healing trajectory, associated with an average decrease in wound area of 27.7% per week. This shift towards healing with eradication of bacterial fluorescence is highlighted in patient 2. Before participation in this observational case series, this trauma wound had a duration of 13 weeks. Bacterial fluorescence persisted in this wound for the first four weeks of visits and was associated with an average decrease in wound area of 0.3%, which is predictive of a non-healing trajectory. Elimination of bacteria through fluorescence-targeted debridement and other treatments, resulted in a 53% reduction in wound area over four weeks, predictive of a healing trajectory.

The periwound region is not typically a region of focus in wound bed preparation as this area is typically intact skin and can appear healthy on visual inspection. Interestingly, in this series of lower extremity wounds, fluorescence imaging revealed bacterial fluorescence (at loads >104CFU/g) in the periwound region in 81% (9/11) of wounds. This is not the first study to report bacterial fluorescence from the periwound region. In a clinical trial of 50 hard-to-heal (chronic) wounds, Raizman et al. reported fluorescence indicative of bacteria in the periwound of 89% of wounds²⁰ and Farhan et al. detected bacteria in the peripheries of 88% of wounds.²⁵ However, we show here for the first time that the presence of periwound bacterial loads is associated with delayed healing and fluorescenceguided debridement of these regions to eliminate fluorescent signature from bacteria was associated with a shift of wounds onto a healing trajectory. Larger studies are warranted to better understand the potential detriment of bioburden harboured in periwound tissues, but these findings suggest that fluorescence imaging of hard-to-heal wounds can be used to enhance detection and targeted removal of bioburden in this region of the wound that is typically overlooked during routine assessment. The increased focus on periwound tissue health, and not just on the management of bioburden in the wound bed, has the potential to improve healing rates and reduce reulceration.

Standard debridement practices, which entail visual assessment of the wound, had little effect on the reduction of clinically significant bacterial loads and emphasised that the current standard of care for wound bed preparation does not maximise removal of bacterial burden. This has previously been shown by others, based on comparisons of pre- and post-debridement

microbiological analysis. 33,34 Similarly, in a study of 22 DFUs, 20 fluorescence imaging revealed that bacterial fluorescence (loads >10 4 CFU/g) was left behind in 100% of study wounds after standard of care debridement. The information on bacterial load and location provided at point of care by fluorescence imaging information supported the medical necessity for additional debridement. Through fluorescence-targeted debridement of these wounds, the bacterial fluorescence was reduced. 20

Limitations

There are limitations to this study that should be noted. This was a prospective, observational study of only 11 wounds, some of which were lost to follow-up throughout the 12-week study duration. Larger, controlled studies are required to definitively establish a relationship between fluorescence-guided wound care and wound healing rates. In addition, as with any diagnostic tool, the device itself has limitations. Bacteria cannot be visualised when located >1.5mm from the surface, due to current limitations of optical imaging. Therefore, the device does not replace the need for standard assessment for clinical signs and symptoms of deeper infection. Visualisation of red or cyan fluorescence signals indicates bacteria at loads known to delay healing⁷ but does not necessarily mean that infection is present. No information is provided on specific bacterial species or antibiotic sensitivities; should a clinician require that information the wound would need to be sampled.

Conclusion

Wound care clinicians are often challenged with having to make multiple decisions when managing a wound (selection of dressing, timing of dressing change etc.), while simultaneously being limited by a lack of objective, reliable information to guide these decisions.^{35–37} In this report, we demonstrate that fluorescence imaging provided objective evidence that improved treatment decision-making, including informing the extent of debridement, application of antimicrobial therapies, and selection of appropriate secondary dressings. Fluorescence-guided treatment facilitated a switch to a healing trajectory in those wounds when a bacterial fluorescence signature was no longer present. The ability to visualise bacteria in wounds at point of care enables a more proactive wound management strategy that may help to accelerate healing of hard-to-heal wounds. JWC

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Reflective questions

- When debriding a wound, what information do you use to guide the extent and location of debridement?
- How can point-of-care information on bacterial burden in wounds influence your treatment planning?
- Fluorescence imaging and digital wound measurement provided documented evidence of wound healing progress. What information do you currently use to determine whether a wound is on a healing trajectory?

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