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Prospective clinical study on the efficacy of bacterial removal with mechanical debridement in and around chronic leg ulcers assessed with fluorescence imaging

Maurice Moelleken[1] | **Finja Jockenhöfer**[1] | **Sven Benson**[2] | **Joachim Dissemond**[1]

[1]Department of Dermatology, Venerology and Allergology, University of Essen, Essen, Germany

[2]Institute of Medical Psychology and Behavioral Immunobiology, University of Essen, Essen, Germany

Correspondence
Joachim Dissemond, MD, Department of Dermatology, Venerology and Allergology, University of Essen, Hufelandstraße 55, Essen 45122, Germany.
Email: joachim.dissemond@uk-essen.de

Abstract

Bacterial colonisation in wounds delays healing, mandating regular bacterial removal through cleaning and debridement. Real-time monitoring of the efficacy of mechanical debridement has recently become possible through fluorescence imaging. Red fluorescence, endogenously produced during bacterial metabolism, indicates regions contaminated with live bacteria (>10^4 CFU/g). In this prospective study, conventional and fluorescence photos were taken of 25 venous leg ulcers before and after mechanical debridement, without use of antiseptics. Images were digitally segmented into wound bed and the periwound regions (up to 1.5 cm outside bed) and pixel intensity of red fluorescence evaluated to compute bacterial area. Pre-debridement, bacterial fluorescence comprised 10.4% of wound beds and larger percentages of the periwound area (~25%). Average bacterial reduction observed in the wound bed after a single mechanical debridement was 99.4% ($p<0.001$), yet periwound bacterial reduction was only 64.3%. On average, across bed and periwound, a single mechanical debridement left behind 29% of bacterial fluorescence positive tissue regions. Our results show the substantial effect that safe, inexpensive, mechanical debridement can have on bacterial load of venous ulcers without antiseptic use. Fluorescence imaging can localise bacterial colonised areas and showed persistent periwound bacteria post-debridement. Fluorescence-targeted debridement can be used quickly and easily in daily practice.

KEYWORDS

bacteria, chronic leg ulcers, fluorescence, MolecuLight, wound debridement

1 | INTRODUCTION

Treatment of patients with chronic wounds continues to represent a major interdisciplinary challenge. Wound therapy usually starts with wound cleansing or debridement, irrespective of the underlying genesis.[1] Mechanical debridement, for example with sterile cotton gauze and, if necessary, with curettes, is one of the most common methods. In addition to the removal of devitalised tissue, it is also important to reduce bacterial colonisation, as bacteria can lead to delayed wound healing and further complications such as erysipelas and cellulitis. Within the framework of the ABCDE rule of wound diagnostics, the aim is also to look for bacteria.[2] So far, such examinations have been carried out in routine clinical assessment using bacteriological swabs or biopsies. However, the results are only available after several days as real-time monitoring was not possible. The direct control of the necessity and the sufficient execution on debridement was, up to now, exclusively carried out by the visual assessment by the therapists, who have varied experience and no assistive devices.

Another important and often neglected aspect is the bacterially contaminated periwound area. Especially in patients with venous leg ulcers, irritations, and eczema can often be found in the periwound, for example due to stasis dermatitis or contact allergies. There is a risk of bacterial recontamination and an increase in the size of the wounds.[3,4] Whether a wound or the periwound region is bacterially colonised can currently only be assumed clinically. A new fluorescence imaging device offers the possibility of real-time visualisation of bacteria on the patient at loads $>10^4$ CFU/g.[5] Thus, monitoring the success of the bacterial removal can be carried out directly and, if necessary, a new, targeted debridement can be carried out. However, a quantification of bacterial area per wound on the displayed images has not been carried out.

The aim of this study was to investigate whether real-time monitoring and quantification of the reduction of bacterial colonised area resulting from a single mechanical debridement are possible. A new technique for quantifying the area of bacterial fluorescence pixels was developed. We report that wound debridement efficacy differs between the wound bed and the periwound area, and therefore should be examined and evaluated separately.

Key Messages

- periwound area is often more contaminated with bacteria than the wound bed
- periwound area is often not, or only insufficiently, included in wound debridement and wound cleansing. This can lead to re-colonisation of the wound bed
- a single mechanical debridement with sterile cotton gauze and ring curettes (if required) can reduce most of the bacterial load very efficiently. This process is relatively simple, inexpensive, and safe
- fluorescence imaging enables immediate localisation of bacterial contaminated areas (loads $>10^4$ CFU/g) and can be used easily and quickly for monitoring of debridement efficacy

2 | PATIENTS AND METHODS

2.1 | Inclusion and exclusion criteria

This prospective clinical pilot trial was designed to include patients with chronic venous leg ulcers whose wounds had existed for at least 8 weeks and whose wound size was 1 to 65 cm^2.

Exclusion criteria were defined as patients under 18 years of age, non-debridable wounds due to severe pain, necrotic tissue, patients during pregnancy or lactation, cognitively impaired patients, and wounds treated locally or systemically with antibiotics within 1 week prior to the start of the study.

2.2 | Treatment process

At least 1 week before the examination, the patient's therapy was switched to non-antimicrobial wound care in order to avoid any influence of this therapy on bacterial colonisation. On the day of the examination, the wound dressing was opened, a conventional photo was taken, and in a completely darkened room, a fluorescence photo of the wound including the periwound area was taken. Subsequently, a mechanical debridement of the wound and the periwound region up to 1.5 cm outside the wound bed was performed using physiological saline solution, sterile cotton gauze, and, if necessary, a 4 mm ring curette. After completion of the debridement, a conventional and a fluorescence photo were taken again, and the wound was bandaged. Thus, one conventional and one fluorescence photo were taken per wound both before and after mechanical debridement. Fluorescence photos were taken of (a) the wound without periwound region, (b) wound and 0.5 cm of periwound, (c) wound including 1.0 cm of periwound, and (d) wound including 1.5 cm of periwound. A maximum distance of 1.5 cm was chosen to keep the wound including the

periwound area of 1.5 cm to all sides on a single photo to minimise errors in the calculation.

2.3 | Fluorescence imaging of wounds

The MolecuLight *i:X* (MolecuLight Inc., Toronto, Canada), developed for medical wound imaging and documentation, is capable of immediately visualising fluorescence using safe violet light (wavelength of 405 nm) and specialised optical filters to capture relevant fluorescence signals from wound tissues and bacteria (Figure 1). In a sufficiently dark room with an optimal distance of 8 to 12 cm between the camera lens and the wound surface, the device excites tissue and bacteria with its violet light LEDs. In response, tissue fluoresces various shades of green due to fluorescence of extracellular matrix components while most bacteria fluoresce red due to endogenous porphyrin production. *Pseudomonas* uniquely fluoresces cyan. Fluorescence photos and videos can be captured to visualise and locate bacteria at loads $>10^4$ CFU/g. In addition, a two-dimensional wound measurement can be generated using the wound measurement app within the device (Figure 2).[5]

2.4 | Image analysis

2.4.1 | Image segmentation

With the image processing program GNU Image Manipulation Program version 2.10.12 (GIMP Team), the fluorescence photo and the conventional photo were processed,

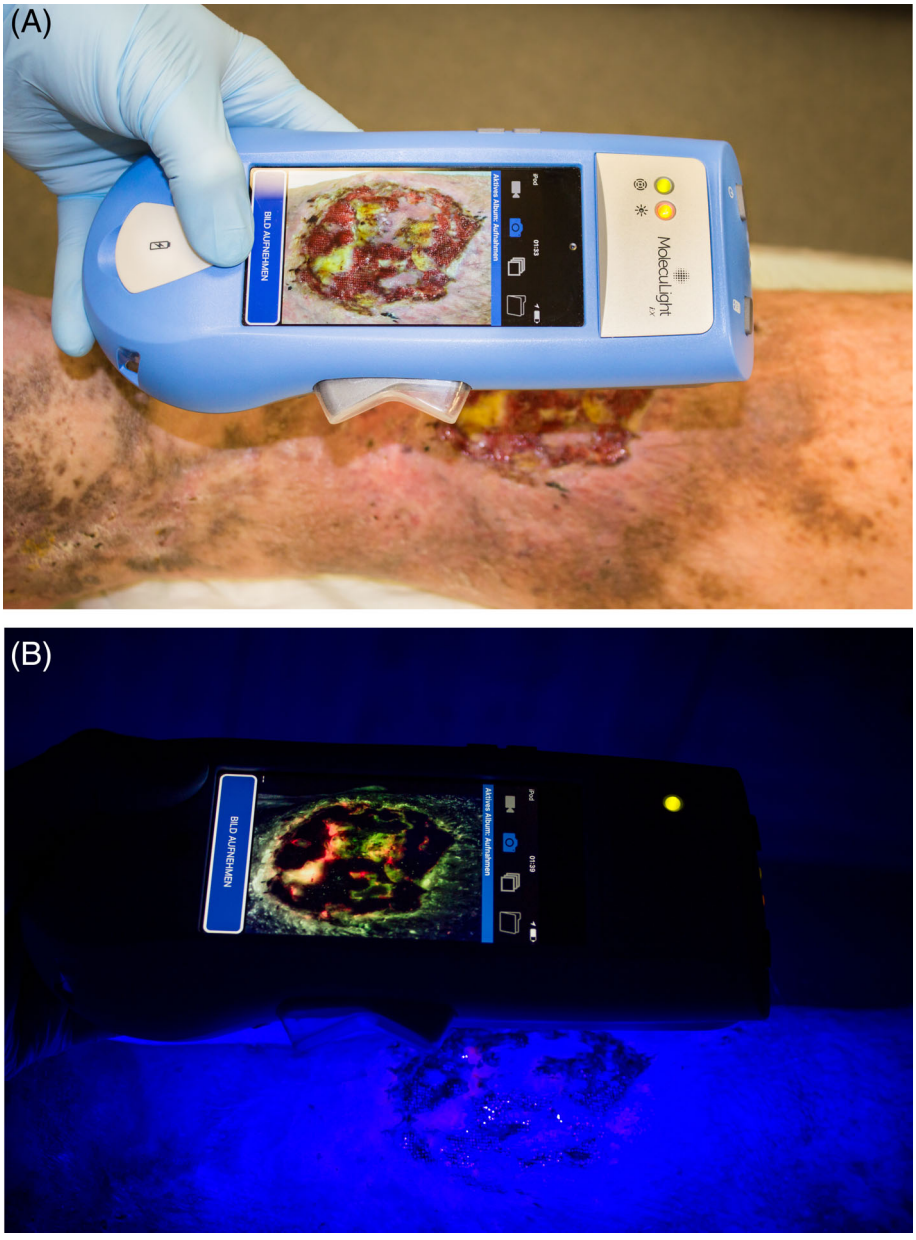

FIGURE 1 (a) Application example of a conventional image using the MolecuLight *i:X* imaging device. (b) Application example of a fluorescence image using the MolecuLight *i:X* imaging device

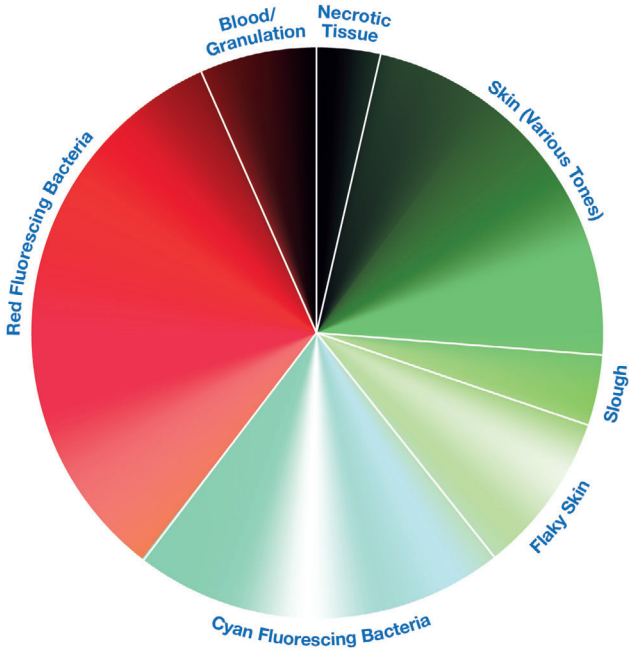

FIGURE 2 Colour scale for the interpretation of MolecuLight autofluorescence photography

respectively. The wound bed within the images was defined and a digital mask applied such that areas outside the regions of interest were assigned a black background with a colour value of 0, to exclude colour influences of the wound surrounding during evaluation. Three additional images were generated for analysis of the periwound tissue measured at three distances: 0.5, 1.0, and 1.5 cm from the wound edge. These three photos were also masked with a black background to exclude colour influences of outside regions. The same procedure was performed for all fluorescence photos after mechanical debridement (Figure 3).[6]

2.4.2 | Image analysis

The analysis program ImageJ (Wayne Rasband, National Institutes of Health, Bethesda, MD) further evaluated the colour spectra of each pixel. The majority of bacterial strains produce red fluorescence. In the RGB colour space, which indicates colour values for the red component, green component, and blue component of 0 to 255, depending on their intensity, a red component of R170 was defined as the threshold value. Anything larger than a red component of R170 represents a red fluorescence visible to the eye. The four fluorescence photos before and after mechanical debridement were evaluated so that a comparison could be made between these two recording times.

By subtracting all black pixels (R0, G0, B0) from the total number of all pixels of a photo (wound + wound edge + black background), the number of pixels could be reduced to those of the wound and the wound surrounding. This method made it possible to count all red pixels with a red component of ≥R170 and to relate them to the entire wound area or the defined wound surrounding. The proportion of red pixels in all coloured pixels was calculated. This value corresponds to the percentage of wound bed or wound surrounding area colonised by bacteria at loads $>10^4$ CFU/g.[7]

2.5 | Statistical analysis

Statistical analyses were conducted with SPSS statistics and analysis software (IBM, Armonk, NY). For descriptive data presentation, mean values (and range) or median (and percentiles) were reported. For comparison of wounds and wound surroundings before and after one-time mechanical debridement, nonparametric Wilcoxon tests were computed. Nonparametric tests were used because Kolmogorov-Smirnov tests indicated that data were non-normally distributed. The alpha level was set at .05 for all analyses.

3 | RESULTS

This prospective clinical trial included a total of 25 patients, including 10 women and 15 men, with chronic venous leg ulcers. The average age was 71.3 years (range: 50-85 years).

3.1 | Bacterial status pre-debridement

Before mechanical debridement, the wounds had a bacterial fluorescent-positive (red) area averaging 10.44% of total wound area. Up to 0.5 cm of the periwound region, red fluorescent pixels were 23.69% of total wound area. This increased to 26.01% with up to 1.0 cm of periwound region, and was 23.62% when up to 1.5 cm of periwound region was included (Figure 4). When the wound and 0.5 cm of periwound area were combined, the percentage of the total wound area with red fluorescence present was 15.44%. This value increased up to 18.96% when combining wound and up to 1.0 cm of periwound region, and was 18.75% of total wound area when combining wound centre with up to 1.5 cm of surrounding periwound area (Table 1).

3.2 | Bacterial status post-debridement

With a single mechanical debridement, the red fluorescence of the wound beds was reduced by 99.4%, from

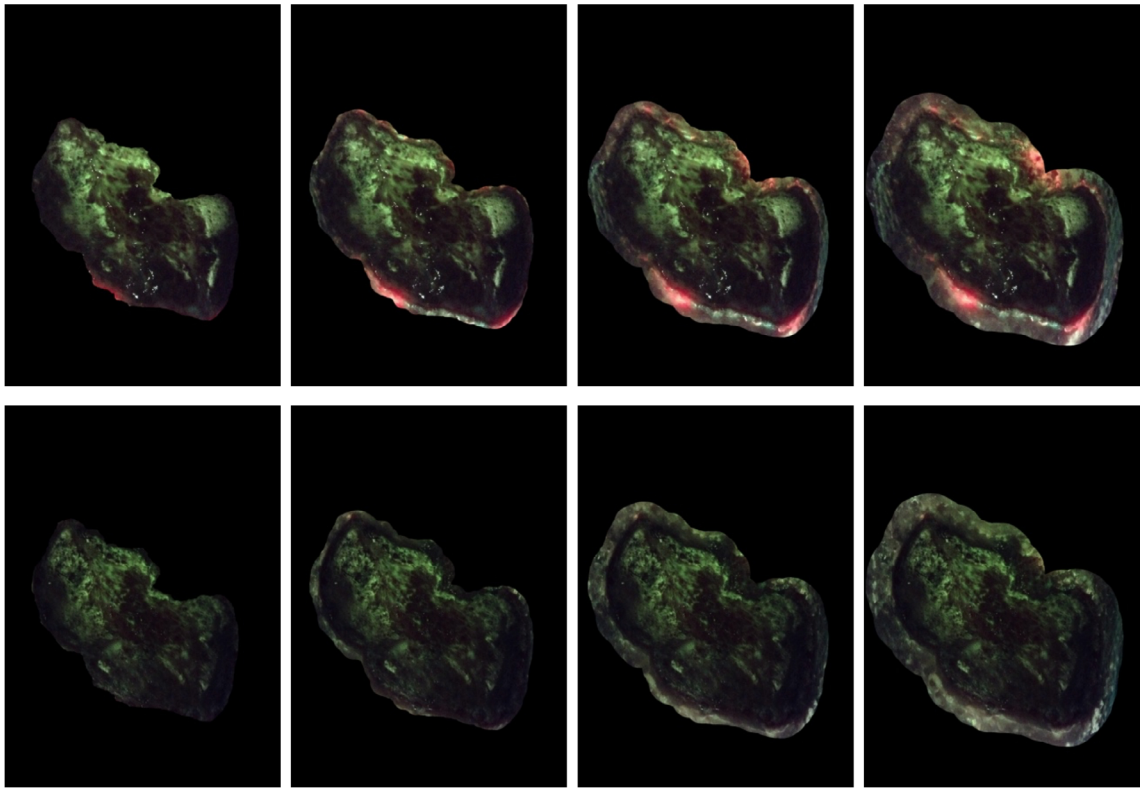

FIGURE 3 Photo documentation of a wound. Top: fluorescence photos before debridement: Wound, wound + 0.5 cm periwound area, wound + 1.0 cm periwound area, wound + 1.5 cm periwound area. Bottom: fluorescence photos after debridement: wound, wound + 0.5 cm periwound area, wound + 1.0 cm periwound area, wound + 1.5 cm periwound area

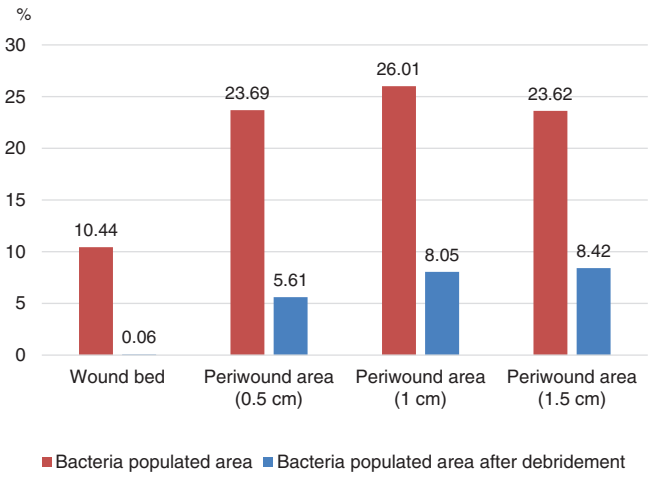

FIGURE 4 Comparison of areas populated with bacteria before and after debridement. x-axis with percentage of bacterial colonisation. y-axis with localisation information: wound bed & periwound area of 0.5 to 1.5 cm

10.44% of total wound area to 0.06% ($P < .001$). Debridement also resulted in a reduction of bacterial load in the periwound area, though not to the same extent. Debridement decreased periwound red fluorescence by 76.32% (from 23.69% to 5.61%; $P < .001$) when up to 0.5 cm of the periwound was included in the analysis. When the periwound region was analysed up to 1.0 cm, a reduction of 69.05% occurred (from 26.01% to 8.05%; $P < .001$); and up to 1.5 cm of 64.34% (from 23.62% to 8.42%; $Z = -3.528$, $P < .001$). When comparing the results of the wound in total, including the periwound up to 0.5 cm, the percentage red pixels are reduced by 87.99% (15.44% vs 1.85%), up to 1.0 cm by 79.27% (18.96% vs 3.93%), and up to 1.5 cm by 73.26% (18.75% vs 5.02%) (Figure 4, Tables 1 and 2).

4 | DISCUSSION

It is well established that bacteria at clinically significant loads in chronic wounds can lead to a delayed healing, deterioration of wound conditions, and contribute to progression of wound size.[8] In addition, there is a danger that bacterial infections resulting in sepsis can occur. Regular wound cleansing is therefore an essential component of modern wound care.[9] In addition to the bacterial colonisation of the wound bed, bacteria are also present in the periwound area, the region surrounding the wound. This study, in addition to other recent works,

TABLE 1 Results of the areas colonised with bacteria in % before and after debridement, as well as the reduction of areas colonised with bacteria after debridement in %

Localisations	Areas colonised by bacteria		Reduction
	Before debridement	After debridement	
Wound bed	10.44%	0.06%	99.38%
Periwound area up to 0.5 cm	23.69%	5.61%	76.32%
Periwound area up to 1.0 cm	26.01%	8.05%	69.05%
Periwound area up to 1.5 cm	23.62%	8.42%	64.34%
Wound bed + periwound area up to 0.5 cm	15.44%	1.85%	87.99%
Wound bed + periwound area up to 1.0 cm	18.96%	3.93%	79.27%
Wound bed + periwound area up to 1.5 cm	18.75%	5.02%	73.26%

TABLE 2 Summary of the results

	Baseline	Post-debridement	*Z*-score	*P*-value[a]
Wound bed	0.12 (0.02/6.15)	0 (0/0.05)	**$Z = -3.805$**	**<.001**
0.5 cm periwound area	11.49 (0.15/33.83)	0.07 (0/0.72)	**$Z = -3.805$**	**<.001**
1.0 cm periwound area	10.90 (0.43/43.99)	0.30 (0.01/7.62)	**$Z = -3.815$**	**<.001**
1.5 cm periwound area	7.76 (0.88/46.04)	0.34 (0.03/5.37)	**$Z = -3.528$**	**<.001**

[a]Results for comparisons of the bacterial colonisation of baseline before mechanical debridement and after mechanical debridement using Wilcoxon tests. Significant differences are printed in bold. The values are given as median (25th/75th percentile).

reports prevalent colonisation of periwound tissue that persists post-debridement, which puts the wound bed at risk of recontamination and potentially re-colonisation. This finding should be communicated to wound clinicians and counteracted in wounds through evidence-based practices. Guidelines state that wounds should be subjected to regular wound cleansing using low cytotoxic antiseptics and, if necessary, mechanical debridement for example using a sterile gauze, ring curette, sharp spoon, or scalpel to remove vital tissue, necrosis, foreign bodies, and wound coverings. Surgical debridement with appropriate analgesia should be considered in the case of pronounced findings.[1,9] However, the region to be debrided relies on clinician judgement. Swabs and semi-quantitative microbiological analysis to assess bacterial load in the wound area can be performed, but swabs taken for microbiological testing usually take several days before a result is available. As such, clinicians typically rely on visual interpretation and subjective experience to determine if bacterial elimination following debridement was successful.[4,10,11]

A novel fluorescence imaging device now enables real-time detection of bacteria in wounds. This fluorescence information can be used to assess efficacy of wound debridement. Our results showed almost complete eradication of bacterial fluorescence in the wound bed after a single mechanical debridement with physiological saline solution, sterile gauze, and, if necessary, ring curettes without the use of any antiseptics. In addition to removing dead tissue, fibrin, and contamination, mechanical wound cleaning and debridement removes a large proportion of bacteria from the wound area and wound environment. Cleaning the periwound region should also reduce the risk of recontamination.[10,12] We could not find a correlation between the size of the wound and the extent of bacterial colonisation. It was striking that in the larger wounds, the most pronounced bacterial colonisation was found in the area of the wound edges. Recolonisation by the more contaminated periwound area could play a relevant role here. Yet, this study of chronic venous leg ulcers observed that standard debridement left behind 36% of periwound bacterial fluorescence signal, indicating either that regions or high bacterial load were missed or that bacteria reside deeper within the periwound tissue, remaining after superficial debridement. A recent study of diabetic foot ulcer debridement reported that periwound bacterial fluorescence persisted after aggressive debridement in 100% of debrided wounds.

4.1 | Fluorescence imaging for microbial detection

In a clinical trial, the positive predictive value (PPV) of red fluorescence on MolecuLight *i:X* fluorescence images

was investigated in relation to the visualisation of bacteria in wounds. In 60 wounds, including 47 diabetic foot ulcers, 12 chronic venous leg ulcers, and 1 amputation wound, red fluorescence was found to indicate bacterial loads $>10^4$ CFU/g with a PPV of 100%. Altogether 30 of the fluorescence positive wounds were analysed by biopsy with subsequent real-time quantitative polymerase-chain-reaction (qPCR) and the remaining 30 wounds by curettage and semi-quantitative bacterial cultures. Biopsy and microbiological analysis (either qPCR or semi-quantitative analysis) confirmed bacterial colonisation in all 60 wounds with red fluorescence. It can therefore be assumed that red fluorescence in wounds corresponds to the reliable detection of the presence of bacterial colonisation. Similar PPV values have been reported in other studies: Hurley et al (2019, n = 50 swabs) reported a PPV of 95% for red fluorescence and 100% for cyan fluorescence confirmed using swabs and semi-quantitative cultures; Serena et al (2019, n = 19 biopsies) reported a PPV of MolecuLight images of 100% confirmed using DNA pyrosequencing. These results, consistent across studies and sampling and analysis methods, support the basic assumption that bacteria are represented by red fluorescence in images taken using the MolecuLight *i:X* imaging device, and reflect an important foundation of the current study.[13]

Serena et al focused on the problem that the identification of wounds moderately to severely colonised wit bacteria, identification which is often ambiguous or misinterpreted.[14] Microbiological swabs require at least 24 hours until a result is available. Classic signs and symptoms scores (CSSs) such as NERDS (non-healing, exudate, red and bleeding surface or granulation tissue, debris, smell, or unpleasant odour) or STONEES (size is bigger, temperature is increased, osteomyelitis probe to or exposed bone, new or satellite areas of breakdown, exudate, erythema/edema, and smell), or the International Wound Infection Institute guidelines for assessing CSS, are therefore used at the point-of-care to identify infection and high bacterial loads in wounds. The scores are highly subjective and can therefore lead to misinterpretation. Serena et al (2019) found that of the 19 study wounds, 18 had moderate to severe bacterial colonisation according to qPCR yet in only four of the 19 wounds were these bacterial loads detected by CSS scores. By combining CSS scores with fluorescence imaging, accuracy was increased from 26.3% to 73.7% and sensitivity from 22.2% to 72.2%. The authors described that use of real-time fluorescence imaging allowed for immediate detection of bacteria and led to immediate actions, such as mechanical debridement, to reduce bacteria. Similarly, in a study of paediatric burns, contactless, painless, and immediate bacterial detection was possible using this fluorescence imaging device. In this patient population, rapid and effective wound disinfection is important to prevent septic progression.[15] These user-friendly aspects can also be confirmed by our clinical applications. The results of the present study are in line with these previous findings as we show that fluorescence imaging enabled quick, reliable, non-contact visualisation of wound regions colonised by bacteria. The images generated can also be used for patient and therapist education. The visualisation makes the patient or therapist more aware of the colonisation.

Similar to our approaches and results, Blumenthal et al used fluorescence imaging for evaluating debridement efficacy in military and trauma wounds. Fluorescence was used to control the success of mechanical debridement performed under fluorescence guidance. After mechanical debridement, a clear decrease in red fluorescence and microbiologically proven reduction of bacterially colonised areas was observed. These investigations have been carried out on other wound types, but the results can be easily compared with our results.[16] However, the application of our method for determining the surface bacterial colonisation in this study could have contributed to a better objectification of the results presented.[17]

In the future, the combination of fluorescence imaging for immediate assessment and localisation of bacterial colonisation and an additional bacteriological smear, especially to exclude multi-resistant bacteria, could also be a useful combination for clinical routine.[14,16,18-20]

4.2 | Limitations of the study

From a bacterial density of 10^4 CFU/g, a detectable red fluorescence colour is observed. If the bacterial density is below this limit, no reliable detection of bacteria is achieved using fluorescence. Furthermore, the violet 405 nm light has a maximum penetration depth of 1.5 mm. Deeper contaminations with bacteria cannot be detected, for example in fistulae or under a pressure callus, or the colour changes to a yellowish hue due to the combination of red fluorescence with bright green from the overlying callus. Fibrin coatings, crusts, and wound fluids can have an influence on the measured fluorescence.[5,21]

The vast majority of bacteria produce porphyrins as a metabolic by-product of haem metabolism. These are excited at the 405 nm wavelength used, resulting in red fluorescence. *Pseudomonas aeruginosa* uniquely produces endogenous *siderophores* or pyoverdines in addition to porphyrins; these pyoverdines produce a light cyan-blue fluorescent colour. Therefore, cyan-blue fluorescence should also be considered in the image evaluation in addition to red fluorescence in future studies.

A limitation in the application of PC-controlled evaluation are very bright reflections in moist wounds, as these appear whitish and contain a variety of colours in high

intensity. In the examined wounds, only very small, punctiform reflections were found. If larger reflections occur, this leads to a falsified increase in the red detection on segmentation analysis – despite red fluorescence not being visible to the eye on the fluorescence image – and congruently to a false high bacteria detection. Similarly, this problem with false high values can also occur with very scaly skin. These false values are specific to the image analysis being performed, not to visual inspection of the image with the naked eye. However, if wounds bleed, the haemoglobin in the blood absorbs the violet light, dampening any red fluorescence signal. The results can then be false negative; therefore, it is advised that any blood be wiped away prior to fluorescence imaging.[21,22]

5 | CONCLUSION

The results of our study show that in patients with chronic venous leg ulcers, the periwound area is usually more contaminated with bacteria than the wound bed, possibly due to clinician focus on this region when cleaning and debriding. A single mechanical debridement can already reduce a large part of the bacterial load without the use of antiseptics. This procedure is simple, inexpensive, and safe. The new fluorescence imaging procedure is a helpful aid in monitoring debridement effectiveness through immediate, contactless imaging of regions with high bacterial loads. Fluorescence imaging can be used easily and reliably for wound evaluation, targeted debridement, and monitoring of treatment effectiveness.

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ORCID

Joachim Dissemond https://orcid.org/0000-0003-3893-328X

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