Received: 28 February 2023 | Revised: 18 February 2024 | Accepted: 23 February 2024

DOI: 10.1111/iwj.14824

ORIGINAL ARTICLE

IWJ WILEY

How effective is simple mechanical wound debridement in reducing bacterial colonisation? Results of a prospective clinical study

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Abstract

Background and aims: Bacteria in wounds can lead to stagnation of wound healing as well as to local or even systemic wound infections up to potentially lethal sepsis. Consequently, the bacterial load should be reduced as part of wound treatment. Therefore, the efficacy of simple mechanical wound debridement should be investigated in terms of reducing bacterial colonisation. Patients and methods: Patients with acute or chronic wounds were assessed for bacterial colonisation with a fluorescence camera before and after mechanical wound debridement with sterile cotton pads. If bacterial colonisation persisted, a second, targeted wound debridement was performed. Results: A total of 151 patients, 68 (45.0%) men and 83 (55.0%) women were included in this study. The male mean age was 71.0 years and the female 65.1 years. By establishing a new analysis method for the image files, we could document that the bacterial colonised areas were distributed 21.9% on the wound surfaces, 60.5% on the wound edges (up to 0.5 cm) and 17.6% on the wound surroundings (up to 1.5 cm). One mechanical debridement achieved a significant reduction of bacterial colonised areas by an average of 29.6% in the wounds, 18.9% in the wound edges and 11.8% in the wound surroundings and was increased by performing it a second time. Conclusions: It has been shown that even a simple mechanical debridement with cotton pads can significantly reduce bacterial colonisation without relevant side effects. In particular, the wound edges were

the areas that were often most contaminated with bacteria and should be included in the debridement with special attention. Since bacteria remain in wounds after mechanical debridement, it cannot replace antimicrobial therapy strategies, but offer a complementary strategy to improve wound care. Thus, it could be shown that simple mechanical debridement is effective in reducing bacterial load and should be integrated into a therapeutic approach to wounds whenever appropriate.

KEYWORDS

autofluorescence, bacterial wound colonisation, fluorescence camera, wound healing, wounds

Key Messages

- Fluorescence cameras allow immediate visualisation of bacterially colonised wound areas.
- Wound edges are often the most contaminated areas with bacteria.
- Simple mechanical debridement, for example, with cotton pads can be performed quickly and without relevant side effects.
- Simple mechanical debridement already significantly reduces bacterial colonisation of wounds and their surroundings.
- Since bacterial load is reduced but not completely eliminated by mechanical debridement, it should not replace antimicrobial therapy strategies but complement them.

1 | INTRODUCTION

Bacteria have been recognised in recent years as one central important factor in impaired wound healing and other complications. Different stages are distinguished, ranging from harmless contamination to systemic infection.[1] In addition to delayed wound healing, there is a risk of systemic infections, which is often associated with hospitalisation and amputation.[2–4] In addition, morbidity, and mortality increase, as does the risk of systemic spread of infection to potentially life-threatening sepsis.[5,6] For example, the Therapeutic Index for Local Infections (TILI) score is a helpful, validated tool to easily and quickly identify local wound infections. Here, the classic but not specific signs of inflammation, rubor, dolor, calor, tumour and functio laesa, as well as an increase and/or change in the colour or smell of the wound exudate are included as indirect criteria of a local wound infection.[7] Bacteriological testing for pathogens and their resistance to antibiotics is usually performed using semiquantitative swabs or biopsies, as it is possible to see the debris but not which bacteria it contains.[8,9] A disadvantage of these laboratory diagnostics is the waiting time of several days to receive the results and the lack of significance regarding the bacterial distribution pattern on the wound.

A relatively new approach to this problem is offered by fluorescence photography, which makes it possible to visually display bacterial colonisation by means of autofluorescence in wounds and their surroundings in real time on the patient.[10–12] Among other things, this will facilitate clinical wound assessment and the targeted collection of wound swabs, for example, using the Levine technique, as well as support the performance of targeted wound debridement and its success monitoring.[13–15]

Mechanical wound debridement, for example, with cotton pads is a long-established method to support wound healing and reduce the risk of complications.[16,17] Therefore, the purpose of this prospective clinical study was to investigate and visualise the efficacy of simple mechanical wound debridement without antimicrobial agents in reducing bacterial colonisation of wounds and their surroundings using fluorescence photography.

2 | PATIENTS AND METHODS

2.1 | Inclusion and exclusion criteria

Patients with a minimum age of 18 years and with acute or chronic wounds of any aetiology who presented at the certified wound outpatient clinics of the Department of

Dermatology, Venereology and Allergology, the Department of General, Visceral and Transplant Surgery and the Department of Cardiology and Angiology of Essen University Hospital during the period from August 2020 to May 2021 as part of their regular follow-up visits were included in this prospective, noncontrolled intervention study.

Patients with insufficient knowledge of German, pregnant or breastfeeding women and patients with dementia or unable to give consent were excluded.

The wounds were not allowed to exceed a maximum wound extension of 7 cm and must not show any wound undermining, because otherwise an exact analysis by fluorescence photography was not possible.

2.2 | Anamnesis

As part of a standardised anamnesis, patients were asked about previous wound care. Pain was assessed with a numerical rating scale (NRS) ranging from 0 (= no pain) to 10 (= most severe pain imaginable).[18] The dressing change intervals as well as the current wound care were documented and further data such as aetiology, duration of existence of the respective wound and previous course were taken from the existing wound documentation of the corresponding outpatient clinics. In addition, wound sizes were recorded in square centimetres (cm^2) using digital planimetry. The wound-specific quality of life of the patients was assessed using the Wound-Qol ('Quality of Life') questionnaire and is part of the routine of the certified wound outpatient clinics.[19]

2.3 | Practical procedures

The study was conducted during the regular follow-up of wound patients in the respective wound outpatient departments. After verbal and written consent to participate in the study, a standardised medical history was taken from the patients. Subsequently, the patients were positioned according to the wound location, the wound dressing was opened and conventional (Figure 1) as well as fluorescence photographic (Figure 2) photo documentation of the wound using MolecuLight i:X™ (MolecuLight Inc., Toronto, Canada) (Figure 3) followed in a darkened room. Two options for further action emerged from the findings. If bacterial colonisation was detected with the MolecuLight i:X™, a targeted wound swab (MASTASWAB™, MAST GROUP Ltd., UK, Bootle) was taken using the Levine technique from the fluorescent areas for semiquantitative analyses of the pathogen spectrum.

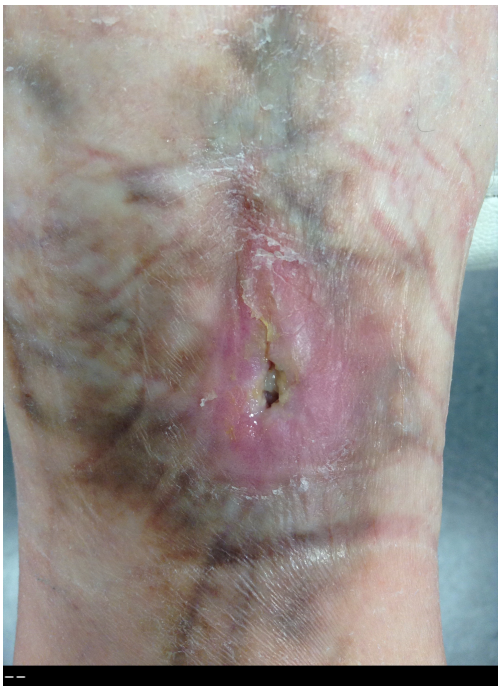

FIGURE 1 Conventional wound photo of an arterial leg ulcer.

To evaluate the effectiveness of mechanical cleansing in reducing bacterial colonisation, an initial mechanical wound debridement was performed using sterile isotonic saline (injection solution sodium chloride 0.9%, Fresenius Kabi Deutschland GmbH, Bad Homburg, Germany) and moistened sterile nonwoven cotton pads (10 × 10 cm, Fuhrmann GmbH, Much, Germany). The moistened pads were used to clean the wounds from the inside to the outside in order to remove wound debris, slough and foreign bodies. This was followed by renewed fluorescence photographic wound documentation. If bacterial fluorescence persisted, a second now more targeted mechanical debridement was performed in the area of bacterial fluorescence and assessed with final wound documentation. If no fluorescence caused by bacterial colonisation was visible in the first wound documentation with the MolecuLight i:X™, no further specific intervention was performed. This procedure was followed by regular wound documentation and treatment by the specialist staff of the respective wound outpatient clinic.

2.4 | Fluorescence photography

Using the MolecuLight i:X™, both conventional and fluorescence photographs were taken before and after each mechanical debridement. By exposing tissue to violet excitation light of wavelength 405 nm in a darkened room, the MolecuLight i:X™ stimulates biological wound

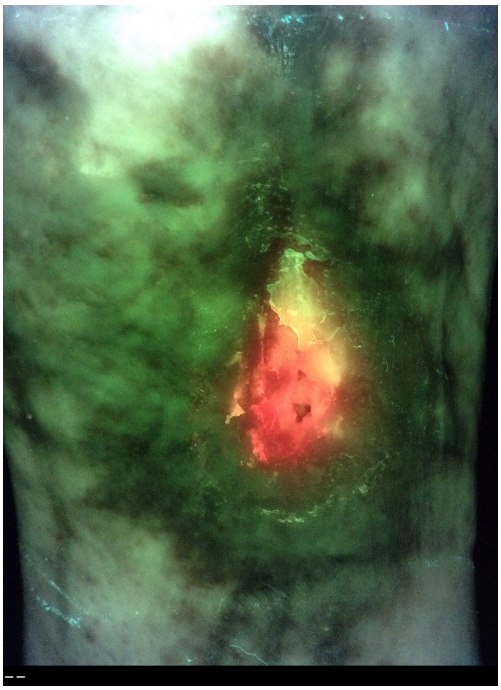

FIGURE 2 Fluorescence photo of an arterial leg ulcer with bacterial fluorescence (red).

components to autofluorescence (Figure 4).[15] The red and blue-green autofluorescence emitted by bacteria can be explained by endogenously produced fluorophores and offers according to the manufacturer's specifications the possibility of real-time visualisation of bacteria on the patient at loads $>10^4$ CFU/g. These include porphyrins, which are a naturally occurring red-fluorescent by-product of heme production by bacteria and are produced by a large proportion of them.[11,20] Blue-green fluorescent fluorophores, termed pyoverdines, are endogenously produced by pseudomonads as part of the iron uptake process and are accordingly specific to this Gram-negative bacterial species.[21] The fluorophores responsible for tissue autofluorescence originate to a large extent from proteins of the extracellular matrix, such as fibrin, collagen, elastin, as well as erythrocytes, and fluoresce in the green and yellow regions of the visible spectrum.[22–24] However, because the MolecuLight i:X™ optically filters out the irrelevant regions of these autofluorescences, tissues on the images appear predominantly in shades of green, which can vary depending on skin colour, providing an anatomical context for comparing areas of possible bacterial colonisation.[15]

2.5 | Data evaluation

To calculate the effectiveness of mechanical debridement using sterile cotton pads in terms of reducing bacterial

FIGURE 3 Conventional wound photo of a venous leg ulcer using MolecuLight i:X™.

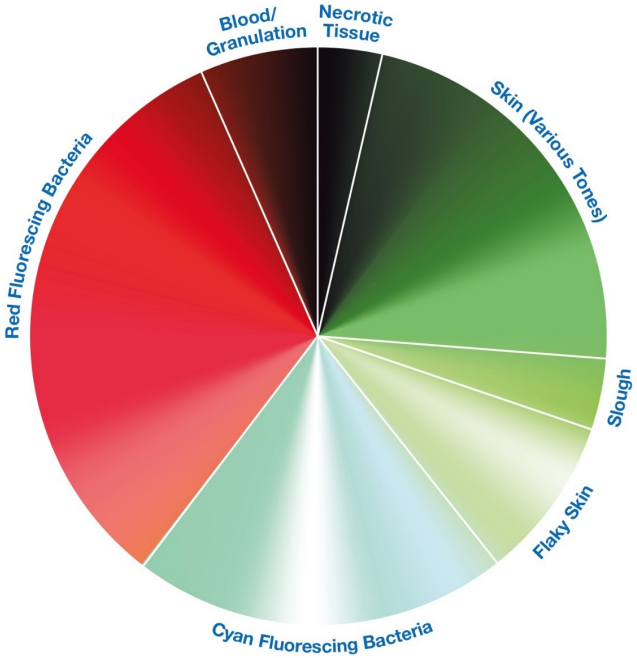

FIGURE 4 Fluorescence spectrum of different tissue and bacterial species.

wound colonisation, the photos taken in the fluorescence mode of the MolecuLight i:X™ had to be evaluated in terms of the reduction of red and/or cyan fluorescence. Since the MolecuLight i:X™ is designed to give the user direct feedback on the remaining fluorescence, there is no tool for quantitative evaluation of the photos. Therefore, we developed an approach to evaluate them separately using Adobe Photoshop® (version 22, Adobe Systems Software Ireland Limited, Dublin, Ireland).

Photographs for which there was clear evidence of reduction were cropped to a uniform size of 1936 × 2592

pixels and the resolution was defined as 620 dpi ('dots per inch'). The definition of this value was necessary to be able to image correct size ratios. All photos taken with the MolecuLight i:X™ were made at a distance of 8–12 cm from the wound. Therefore, this distance can be used to determine a dpi value that correctly reflects the size ratios.

The next step was to define the red and/or cyan areas within the image. For this purpose, these areas were defined individually and manually for each photo using the colour range mode of Adobe Photoshop and saved as a mask. The wound areas were then defined, and this section was also saved as a mask. This mask was duplicated and expanded by either 120 pixels (≈0.5 cm) or 360 pixels (≈1.5 cm) to represent both the wound edge and wound surrounding. Both the dimensions of the wound edge (+0.5 cm around the wound area) and the wound surrounding (+1.5 cm around the wound area) were fixed by us, because there are no uniform definitions for these wound compartments. The masks generated in this way were then combined with each other so that the distribution of bacterial fluorescence over the individual wound compartments could be calculated, as well as the proportion of fluorescence in relation to the area of the wound compartment and the reduction of bacterial fluorescence by a first and, if necessary, second mechanical debridement.

2.6 | Statistical analyses

The SPSS® Statistics 27 analysis software (IBM Corporation, Armonk, NY) was used for statistical analyses. Descriptive data were reported to characterise the patient sample. Binomial logistic regressions were computed to assess the association between the presence of fluorescence-photographically detectable bacterial colonisation as the dependent variable and independent variables including age, sex, presence of a care provider, and odds ratios (OR) were calculated. Changes in bacterial colonisation were assessed for single mechanical debridement using paired *t*-test. If a second mechanical debridement was additionally performed, the change in bacterial colonisation was assessed by Friedman test followed by Dunn-Bonferroni post-hoc test.

3 | RESULTS

3.1 | Patient cohort

A total of 151 patients, 68 (45%) men and 83 (55%) women, from the interdisciplinary wound centre at University Hospital Essen were included in the study. The mean age was 71.0 years for men and 65.1 years for women.

TABLE 1 Wound aetiologies.

Wound aetiology	Frequency	Percentage
Postoperative wounds	69	45.7
Venous leg ulcers	39	25.8
Arterial leg ulcers	11	7.3
Mixed leg ulcers	6	4.0
Others	26	17.2
Total	151	100.0

TABLE 2 Wound locations.

Wound locations	Frequency	Percentage
Lower leg	101	66.9
Head	22	14.6
Foot	12	7.9
Upper arm	5	3.3
Forearm	3	2.0
Thigh	3	2.0
Hand	2	1.3
Back	2	1.3
Chest	1	0.7
Total	151	100.0

3.2 | Wounds

In total, 69 (45.7%) of the 151 patients showed postoperative wounds. In 39 (25.8%) cases, venous leg ulcers were the reason for treatment, in 11 (7.3%) arterial leg ulcers and in six (4.0%) mixed leg ulcers. The remaining 26 (17.2%) patients suffered from less common wound aetiologies, which were grouped under 'other' and included wounds due to pyoderma gangrenosum ($n = 3$), vasculitides ($n = 2$), livedoid vasculopathy ($n = 2$), necrobiosis lipoidica ($n = 1$), Martorell hypertensive ischaemic leg ulcer ($n = 1$) and unspecified wound infections ($n = 17$) (Table 1). The most common wound location was the lower leg with 101 (66.9%) cases. This was followed by the head with 22 (14.6%) and the foot with 12 (7.9%) cases, as well as the upper arm with five (3.3%), forearm three (2.0%), thigh three (2.0%), hand two (1.3%), back two (1.3%) and chest with one (0.7%) patient (Table 2). The duration of existence of the wounds was a median of 20 weeks and an average of 87.4 weeks with

a range of 1 week to 29 years. No duration of persistence could be determined in two patients. In 117 (77.5%) patients, the wound had been present for more than 8 weeks and was therefore classified as chronic, and in 32 (21.2%) patients, the wound was acute. The median wound size was 5.0 cm^2 and the arithmetic mean was 8.4 cm^2 with a range between 0.1 and 44.5 cm^2.

Pain at rest on the NRS scale averaged 1.7 points, with 50.3% reporting a score of 0 points and thus no pain. Severe pain of 8/10 points was reported by 2.0% of patients. For the Wound-Qol, the median score was 14.5 out of a maximum of 68 points.

3.3 | Wound therapy

In 75 (49.7%) of the 151 patients, regular dressing changes were performed by an ambulatory healthcare service, and 76 (50.3%) did it independently. A total of 106 patients primarily used a wound gel, ointment or spray, with the most commonly used representatives being Prontosan Wound Gel® (B. Braun SE, Melsungen, Germany) (39.1%) and Granudacyn Wound Gel® (Mölnlycke Health Care, Düsseldorf, Germany) (23.7%). Five patients used Prontosan Wound Gel® and Iruxol N® ointment (Smith & Nephew, London, United Kingdom) in daily alternation. A total of 45 patients (28.8%) used wound dressings. The most common wound dressing used was ADAPTIC® Fettgaze (Systagenix, Hamburg, Germany) (68.2%), followed by UrgoTül® (URGO, Sulzbach, Germany) (6%) and other wound dressings used much less frequently or only occasionally, such as Biatain Silicone® (Coloplast, Hamburg, Germany), UrgoStart Tül® (URGO, Sulzbach, Germany), UrgoClean AG® (URGO, Sulzbach, Germany) or UrgoTül Silver® (URGO, Sulzbach, Germany). The wound preparations and dressings used were divided into antimicrobial and non-antimicrobial products according to the manufacturer's instructions. Accordingly, 67 (44.3%) of the patients used antimicrobial wound therapy and the remaining 83 (55%) did not. Thereby, binomial logistic regression showed that the use of the wound products advertised as having antimicrobial activity had a significant ($p < 0.001$) impact on the detection of moderate to high bacterial colonisation—with an OR of 0.153 (95% CI [0.057; 0.406]) indicating a significant protective effect in this regard.

3.4 | Bacterial colonisation and fluorescence

In 62 (41.1%) of the examined wounds moderate to high bacterial colonisation, in some cases with several bacterial strains simultaneously, was detected by fluorescence photography and confirmed by wound swabs. Among these, *Staphylococcus aureus* was by far the most frequently detected wound bacterium ($n = 35$; 38.9%), followed by *Pseudomonas aeruginosa* ($n = 9$; 10.0%), *Klebsiella oxytoca* ($n = 7$; 7.8%), *Proteus mirabilis* ($n = 7$; 7.8%) and *Enterococcus faecalis* ($n = 7$; 7.8%). *Escherichia coli* ($n = 6$; 6.7%) and *Streptococcus agalactiae* ($n = 4$; 4.4%) were detected in several patients (Table 3).

TABLE 3 Pathogen spectrum of the wounds examined.

Pathogen spectrum	Frequency	Percentage
Staphylococcus aureus	35	38.9
Pseudomonas aeruginosa	9	10.0
Klebsiella oxytoca	7	7.8
Proteus mirabilis	7	7.8
Enterococcus faecalis	7	7.8
Escherichia coli	6	6.7
Streptococcus agalactiae	4	4.4
Acinetobacter baumanii	2	2.2
Serratia marcescens	2	2.2
Morganella morganii	2	2.2
Streptococcus constellatus	1	1.1
Stenotrophomonas maltophili	1	1.1
Pseudomonas stutzeri	1	1.1
Acinetobacter pittii	1	1.1
Streptococcus dysgalacticae	1	1.1
Klebsiella aerogenes	1	1.1
Enterobacter cloacae complex	1	1.1
Klebsiella pneumoniae	1	1.1
Moraxella catarrhalis	1	1.1
Total	90	100.0

Examination of the distribution of bacterial fluorescence among wound compartments showed that an average of 60.5% (median 67.3%) of bacterial colonisation detected by fluorescence photography was found in the wound edge region. A total of 21.9% (median 1.7%) of the moderate to high bacterial colonisation was in the wound, and the remaining 17.6% (median 10.5%) was in the wound surrounding area (Table 4).

3.5 | Mechanical debridement

From the 62 patients with detectable bacterial colonisation, two (3.2%) refused wound debridement due to severe pain, 21 (33.9%) received only one single debridement and the remaining 39 (62.9%) received two mechanical debridements.

TABLE 4 Distribution of fluorescence among wound compartments.

Distribution of fluorescence to individual wound compartments	Proportion of fluorescence—wound area (%)	Proportion of fluorescence—wound edge (%)	Proportion of fluorescence —wound surrounding (%)
Mean value	21.87	60.51	17.62
Median	1.73	67.30	10.52

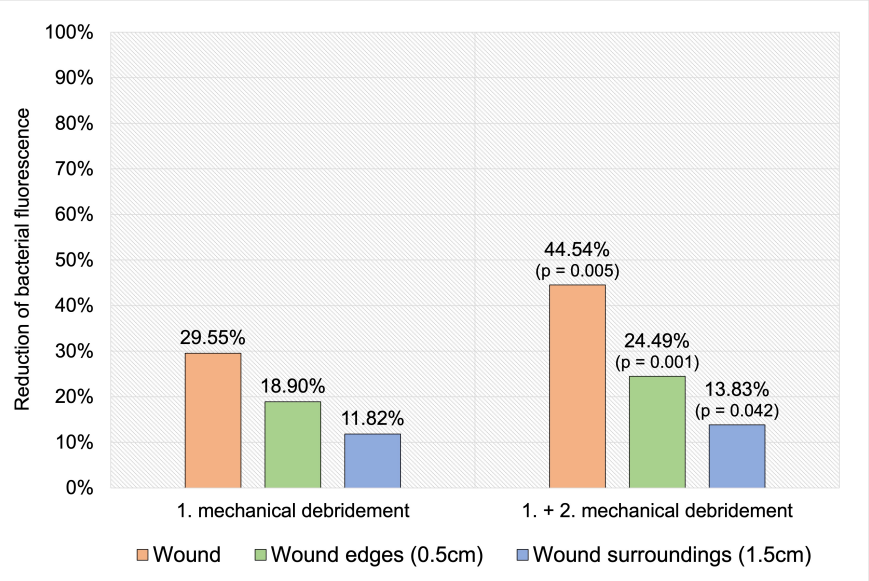

FIGURE 5 Reduction of bacterial fluorescence by single or repeated mechanical debridement.

Because evaluation of the acquired images was not possible in three patients due to poor image quality or incorrect illumination, 57 participants were available for the final evaluation of the first debridement and 37 patients were available for the evaluation of the second debridement.

3.5.1 | Wound area

The fluorescence detected on the wound surfaces averaged 3.86% (SD = 8.10) of the surface area in the 57 patients. This was significantly ($t(56) = 3.30$; $p = 0.002$) reduced to 2.53% (SD = 6.59) by a first mechanical debridement, that is, the relative reduction was 34.46%.

In the 37 patients in whom both the first and second debridement were performed, the mean bacterial colonisation of the wound surface was 2.47% (SD = 5.75), which was reduced to 1.74% (SD = 4.64) by the first debridement (relative reduction 29.55%) and further reduced to 1.37% (SD = 4.07) by the second (relative reduction 21.30%). Accordingly, the bacterial colonisation of the wound surface was significantly ($z = 0.65$; $p = 0.005$) reduced by 44.54% by two mechanical debridements performed (Figure 5).

3.5.2 | Wound edge

Fluorescence detected at the wound edge averaged 10.08% (SD = 12.12) in the 57 patients. This was significantly ($t(56) = 3.1$; $p = 0.003$) reduced to 7.36% (SD = 11.11) by a first mechanical debridement (relative reduction 27.00%).

In the 37 patients in whom both the first and second debridement were performed, the mean bacterial colonisation of the wound edge was 10.74% (SD = 13.25), which could be reduced to 8.71% (SD = 12.57) by the first debridement (relative reduction 18.90%) and to 8.11% (SD = 12.74) the second time (relative reduction 6.89%). The two debridements performed were able to significantly ($z = 0.76$; $p = 0.001$) reduce the bacterial colonisation of the wound edge by a total of 24.49% (Figure 5).

3.5.3 | Wound surrounding

In addition, the fluorescence of the wound surroundings was calculated. This averaged 2.75% (SD = 6.52) in the 57 patients and was significantly ($t(56) = 2.87$; $p = 0.006$) reduced to 2.18% (SD = 6.51) by one mechanical debridement (relative reduction 20.70%). In the 37 patients in

whom both the first and second debridement were performed, the mean bacterial colonisation of the wound surrounding was 3.47% (SD = 7.85), which could be reduced to 3.06% (SD = 7.91) by the first debridement (relative reduction 11.82%) and to 2.99% (SD = 7.92) the second time (relative reduction 2.99%). Overall, the two debridements performed significantly ($z = 0.47$; $p = 0.042$) reduced bacterial colonisation of the wound surrounding by 13.83% (Figure 5).

4 | DISCUSSION

Almost all chronic wounds are at least temporarily colonised with bacteria.[14] These can cause stagnation of wound healing and lead to clinically relevant infections, including potentially lethal sepsis.[25,26] Consequently, bacterial diagnosis in patients with wounds is very important. However, this is often difficult in practice, as standard detection methods require several days before results are available and do not provide any information about the distribution pattern of the bacterial colonisation. With the development of fluorescence imaging in the form of the MolecuLight i:X™, the detection and thus assessment of bacterial colonisation of wounds has now been remarkably simplified. This type of wound assessment now makes it possible for the first time to observe and evaluate the effect of cleansing and debridement measures in real time in clinical practice.

The debridement of wounds is an essential part of modern therapy strategies, which can promote wound healing.[27,28] Different methods are available for this purpose, which have very different advantages and disadvantages in terms of time volume, prior experience and costs, among other things. Mechanical debridement, for example, with cotton pads, is recommended as one good option for mechanical debridement.[17]

In this study, the aim was to evaluate the effectiveness of mechanical debridement using fluorescence photography. However, it already became apparent during the preparatory phase that fewer patients than expected showed fluorescence-photographically detectable bacterial colonisation, so we collected further personal data in order to be able to determine the reasons for this.

4.1 | Wound types

In this study, 32 patients with acute wounds and 117 patients with chronic wounds were included. In the given setting, this distribution was expected because the certified wound outpatient clinics of the University Hospital Essen mainly treat patients with difficult wound healing. Regarding the bacterial colonisation of the wounds, a distinction was only made according to the dichotomous variable acute versus chronic, since an evaluation according to the number of weeks would not have been meaningful in the chosen study design. Accordingly, chronic wounds have a significantly higher risk of moderate to high bacterial colonisation than acute wounds. This can most likely be explained through the factor of time, whose influence on bacterial colonisation has already been observed several times.[14] However, comorbidities such as diabetes mellitus, which are involved in wound chronification and simultaneously increase the risk of bacterial colonisation, may also have an influence.[28]

Although larger wounds have an increased risk of wound infection,[29] we did not find a significant association between wound size and fluorescently detectable bacterial colonisation in our data analysis. However, the significance of our data is limited by the fact that wounds larger than 49 cm^2 could not be included in our study for technical reasons.

4.2 | Bacterial colonisation

The results obtained by wound swabs are in line with previous studies and show *Staphylococcus aureus* (38.9%) and *Pseudomonas aeruginosa* (10%) as the most common pathogens.[30] However, in our cohort, bacterial colonisation was detected in only 41.1% of patients by fluorescence photography. A recent large-scale study from the United States came to a figure twice as high, with 82% of patients,[14] and other smaller studies also showed a significantly higher rate of patients with fluorescence-photographically detectable bacterial colonisation.[31,32] The proportion of colonised wound area was also comparatively low in our population. For example, in the study we published in 2020, bacterial colonisation of the wound area averaged 10.44%, and in the study presented here, it was 2.47% only.[33] The most relevant influence on the significantly lower bacterial colonisation is likely to be the previously applied wound therapy. In our certified wound outpatient clinics, 44.3% of the patients studied were treated with antimicrobial wound therapy, most of which consisted of the use of polyhexanide gel. The efficacy and tolerability of this agent have already been demonstrated in numerous studies, making it the agent of choice for decontamination of bacterially colonised wounds.[34,35] Unfortunately, very few studies have information on the pre-therapy of the wounds studied. Only Moelleken et al. point this out in their article, providing an important clue to the possible cause of the difference observed here. In their pilot study, they

investigated the effects of sharp mechanical debridement on bacterial colonisation using fluorescence imaging in 25 patients with chronic venous leg ulcers. As part of this, wound therapy was switched to a non-antimicrobial therapy at least 1 week before the study to avoid effects on bacterial colonisation. Other relevant factors may be found in the individual environment of the patients. In addition, the care of patients living in Germany cannot be compared one-to-one for example with patients in the United States due to the different healthcare systems.

The evaluation of fluorescence photographic wound images has highlighted that a large proportion of bacterial colonisation $>10^4$ CFU/g is located outside the wound. This observation is consistent with other recent studies and reinforces the importance of increasing focus on the wound edge and wound surrounding, both in microbial detection methods and wound debridement.[14,32,33] A pilot study was the first to demonstrate that the presence of bacteria colonised in the wound edge leads to slower healing, and fluorescence-photography-assisted debridement can reverse the trend.[31] It is still unclear why the wound edges in particular show increased bacterial colonisation. However, fluorescence imaging is an important tool to focus not only on the wound itself, but also on the previously neglected wound surrounding and the wound edge, and to include them in the appropriate therapy.

4.3 | Effectiveness of mechanical debridement

Regarding the effects of mechanical debridement using sterile cotton pads, our results showed that it had a significant effect on the reduction of bacterial colonisation. In the wound bed, this was the greatest with an average relative reduction of 34.46% by one mechanical debridement and decreased in the wound edge and wound surrounding areas with an average relative reduction of 27.0% and 20.7%, respectively. A second, more targeted debridement had an additional positive effect. However, there were interindividual differences in the effects obtained. Thus, in about half of the patients (48.4%), there was almost no effect on visible bacterial fluorescence or, in isolated cases, even an increase. In some patients, there was a complete removal of the bacterial colonisation. A major factor in these very different effects is likely to be the depth of colonisation of the bacteria. Bacteria adhering to the surface can be removed more easily by means of a moistened sterile cotton pad than bacteria that have colonised below the skin surface. In contrast, the increase in fluorescence triggered by bacteria observed in some patients can be explained by the fact that deeper bacteria come closer to the surface due to the removal of upper skin layers and can thus be detected by the MolecuLight i:X™. Similar observations were made by Raizman et al. in their study of 22 patients with DFU, which included the finding that even sharp debridement left moderate to high colonisation of bacteria in 100% of cases.[32] Similarly, Kim et al. found only a slight effect of sharp debridement on bacterial colonisation,[36] and Moelleken et al. also found up to 30% of fluorescence-photographable bacterial colonisation remained, particularly in the wound surrounding area.[33] This study examined the effectiveness of a sharp debridement and demonstrated an average reduction in the wound of 99.4%. This may be partly due to the increased bacterial colonisation adhering to the surface, which may have been caused by the switched non-antimicrobial wound therapy, but more importantly, sharp debridement was performed using ring curettes. This procedure has significantly enhanced the effects achieved by the treatment.

Thus, it can be summarised that purely mechanical debridement with sterile cotton pads can be a first, save and easy to perform step in the reduction of especially superficially adhering bacterial colonisations. However, if the bacteria have already colonised below the upper skin layers, sharp or surgical debridement is a much more effective, but also more invasive, method of elimination.

5 | LIMITATIONS

Some limitations were encountered in the practical use of the MolecuLight i:X™. For example, the maximum wound diameter to be documented was 7 cm. This was due to the fact that the device must always be aligned at a distance of 8–12 cm from the wound. This meant that larger wounds could not be included, so that wound size as an influencing factor on bacterial colonisation could only be considered to a limited extent in this study.

Since the device is hand-held, it was not always possible to photograph the individual wounds at exactly the same distance and angle. These influencing factors can have a partial impact on image evaluation.

Another limitation had to do with the features of the MolecuLight i:X™, which was primarily designed not to perform photo analysis, but to provide the user with direct feedback on the bacterial colonisation. This includes the missing display of bacteria that do not produce porphyrins, such as *Streptococcus*, *Enterococcus* and *Finegoldia*. Furthermore, the maximum penetration depth of the emitted violet light of 1.5 mm makes the identification of deep-seated bacteria impossible. This limitation also applies to the wound swabs. Higher sensitivity could have been achieved with invasive biopsies. However, these were not indicated in all patients.[9]

Overall, these initial results leave sufficient potential for future studies, in particular to investigate the deeper impact of such simple mechanical debridement on wound healing. It would also be desirable to follow up patients in future studies, taking into account the progression of wound healing.

6 | CONCLUSION

In our clinical study, we demonstrated that mechanical debridement with moistened sterile cotton pads is an effective first step in reducing bacterial wound colonisation. This is a simple procedure that can be performed quickly and safely and is quite capable of removing a relevant proportion of the superficial, loosely adhering bacterial colonisation. In daily practice, it is therefore useful to perform fluorescence imaging-assisted debridement as the first step in the treatment of wounds. If there are still fluorescence-photographically displayable bacteria in the area of the wound, these can be treated a second time with targeted mechanical debridement or, better, by means of sharp debridement. Particular attention should be paid to the wound edge, as this often contains most of the bacterial colonisation.

ACKNOWLEDGEMENTS

We thank MolecuLight Inc. (Toronto, Canada) for the free loan of a MolecuLight i:X™ for this study. Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST STATEMENT

Maurice Moelleken has received fees for consulting, lectures and/or studies from the following companies: Adtec Healthcare, Mölnlycke, Rheacell, Urgo. Sebastian Heinrich Krimphove has no conflicts of interests. Frederik Krefting has received fees for consulting, lectures and/or studies from the following companies: Novartis, Lilly, Bristol-Myers Squibb, Janssen, Almirall, Boehringer Ingelheim. Christos Rammos has received fees for consulting, lectures and/or studies from the following companies: Avinger, Biotronik, BD Bard, Cordis, Daichii-Sankyo, Inari, Novartis, Boston Scientific Corp., Veryan, Shockwave, Med Alliance. Anna Ewa Cyrek has received fees for consulting, lectures and/or studies from the following companies: Serag-Wiessner. Sven Benson has received fees for consulting, lectures and/or studies from the following companies: AstraZeneca, Bayer, Celgene, Grünenthal, Janssen-Cilag, Lilly, Novo Nordisk, Sanofi-Aventis, Symbiopharm, Urgo. Joachim Dissemond has received fees for consulting, lectures and/or studies from the following companies: B. Braun, Coloplast, Convatec, Hartmann, Mölnlycke, Lohmann & Rauscher, Smith & Nephew, Urgo.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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How to cite this article: Moelleken M, Krimphove SH, Krefting F, et al. How effective is simple mechanical wound debridement in reducing bacterial colonisation? Results of a prospective clinical study. *Int Wound J*. 2024;21(4): e14824. doi:10.1111/iwj.14824

DOI: 10.1111/iwj.14824
URN: urn:nbn:de:hbz:465-20241230-154845-2