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Treatment of infected wounds with cold atmospheric plasma: a case series

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Abstract: Due to its inactivating effect on bacteria, cold atmospheric plasma (CAP) has been shown to be successful in the treatment of hard-to-heal (chronic) and infected wounds. In this case series, 15 patients with a total of 20 wounds were treated with a mobile CAP device and the bacterial load of the wound simultaneously observed using a MolecuLight i:X (MolecuLight Inc., US) camera. In 60% of cases, the bacterial load could be brought under control despite minimal CAP application. This procedure offers the advantage of being able to directly visualise wound bacterial load and, therefore, the inactivation of bacteria is also directly visible. The use of the two devices complemented each other; supporting wound management and analysis of its effectiveness.

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bacterial load • chronic wound • cold atmospheric plasma • hard-to-heal • infected wound • non-invasive physical plasma • wound • wound bed preparation • wound care • wound dressing • wound healing

Controlling the bacterial load of a wound and the resulting delay in wound healing are among the greatest challenges in vascular medicine. In recent years, many new therapeutic methods have been developed as potential solutions.[1–3] Negative pressure wound therapy (NPWT) is one of the most established and most evaluated methods, and hyperbaric oxygen therapy for the treatment of diabetic foot wounds has also been an integral part of modern wound care for many years. Additionally, treatment methods, such as magnetic resonance therapy, ultrasound and laser technology, are also becoming increasingly popular and have shown good empirical results.[1–6]

Another promising treatment approach is cold atmospheric plasma therapy (CAP). Its advantage lies in that it can be successfully used in all phases of wound healing, due to its antimicrobial and cell-regenerating properties.[7–14] It has also been recognised by the European Wound Management Association (EWMA) as a promising treatment option for hard-to-heal (chronic) wounds.[4]

Hard-to-heal wounds, in particular, are highly susceptible to bacterial contamination.[5,6] They often persist for long periods and wound secretions can cause maceration, creating an ideal environment for bacteria. A wide variety of pathogens can typically be detected; *Staphylococcus aureus* and *Pseudomonas aeruginosa* are frequently found in infected, inflamed wounds.[7,8]

To adapt wound care specifically to the type of bacterial colonisation, it is important to first determine the infection status. This can be achieved by bacterial fluorescence imaging, using devices such as the MolecuLight i:X (MolecuLight Inc., US) wound imaging device, or by screening methods, such as those described by Cutting and Harding.[14,15] These methods can also help guide targeted CAP application and assess the clinical success of the therapy.

In this case series, a mobile CAP device was used. The device generates cold plasma directly from the ambient air and transfers it to the wound bed.

Plasma is a partially ionised gas. Just as ice first turns into liquid water when heated and then into steam, gases can be turned into plasma by adding energy.

CAP, generated at atmospheric pressure and temperatures <40°C, can be used in medicine to treat acute and hard-to-heal wounds.[10–12] It has two main effects. First, it promotes human cell activity, supporting cell division and even angiogenesis.[13,14] Second, it has a bactericidal effect, eliminating bacteria and fungi—including antibiotic-resistant strains.[16–22] CAP has already shown promising results in treating hard-to-heal wounds.[11,23–25] Its effectiveness is attributed to various mechanisms, including the normalisation of wound pH.[10,26]

This case series aimed to determine the effects of CAP therapy on the bacterial load of hard-to-heal wounds over three weeks, focusing on wounds with simple or critical colonisation, as defined by the World Union of Wound Healing Societies' classification.[16]

Methods

Patients with wounds of different aetiologies, from three clinics in Italy, were included in the study, which took place between 2021 and 2022. Inclusion criteria were: hard-to-heal wounds of all aetiologies of >2 months' duration. Children and patients who were pregnant and/or breastfeeding were excluded.

Infections were evaluated using the Cutting and Harding criteria[27] and using an autofluorescence-based

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system to visualise bacterial load (MolecuLight i:X fluorescence imaging).

Ethical approval and patient consent

Ethical approval was not required for this study as this was a clinical, post-marketing study with a CE-certified medical device (plasma care, terraplasma medical GmbH, Germany), used according to the manufacturer's specifications. Before the patient underwent treatment, they provided written informed consent to participate in the study, and for the publication of anonymised data and photographs.

Bacterial fluorescence:

The MolecuLight i:X is a handheld fluorescence imaging system that enables rapid, non-invasive visualisation of bacterial colonisation in wounds. The device uses violet light (405nm) to excite fluorescent molecules produced by bacteria, detecting two specific fluorescence signals:

- Red fluorescence: indicates the presence of porphyrin-producing bacteria (e.g., *Staphylococcus aureus*, many Gram-positive and Gram-negative pathogens)
- Cyan (blue-green) fluorescence: specific to pyoverdine-producing bacteria, such as *Pseudomonas aeruginosa*.

A fluorescence signal suggests a bacterial load of $>10^4$ colony-forming units per gram (CFU/g), which is considered clinically relevant for critical colonisation or infection.[24] The advantage of the method is the real-time diagnostics by direct visualisation of bacterial load without time-consuming laboratory tests.

In combination with CAP, the use of the fluorescence imaging system enables precise application of CAP to fluorescent areas for optimised local bioburden reduction and quantitative follow-up by comparing fluorescence intensity at different timepoints (as in this case series: day (D)0, D7 and D21).

Clinical signs of infection

Clinical criteria for the identification of wound infection are commonly based on a list created by Cutting and Harding.[27] This list is based on empirical data generated in a large, multidisciplinary clinical practice over decades. The classic signs and symptoms of wound infection include: inflammation; new or increasing pain; local heat; swelling; advancing redness and purulence.[16] However, these indicators are less commonly observed in hard-to-heal wounds and, therefore, clinicians must be familiar with the secondary signs of infection, including pain, increase in wound size, friable granulation tissue, increased exudate levels and delayed healing.[17–19]

Wound bed preparation score and wound size

Wound bed preparation (WBP) is an established concept in the treatment of hard-to-heal wounds. The basic method of WBP was published in 2000 by Falanga.[28] It is a systematic approach to wound management by identifying and removing barriers to healing. The concept was originally developed in plastic surgery, and includes: wound assessment; debridement; moisture balance; bacterial balance; and wound cleaning.

The WBP score is based on work by Falanga et al.[29] which establishes a new classification system that evaluates the following parameters: healing margins (wound edge effect); presence of eschar; wound depth and granulation tissue; amount of exudate; presence of oedema; dermatitis in the wound edge area; callus and/or fibrosis; and a pink/red wound bed. Each parameter is assigned a score from 0 (worst score) to 2 (best score) and all parameter scores are added together to give a total score. The best possible score a wound can achieve is, therefore, 16, and the worst possible score is 0.[29]

The wound area was measured using the device WoundViewer (Omnidermal, Italy), an automatic system based on artificial intelligence.[30]

Treatment protocol

All patients had previously been treated with antiseptics (such as polyhexanide) for at least two weeks. Since no improvement was achieved with standard of care (SoC), CAP treatment was started as an add-on to SoC. 'Improvement' was defined according to the EWMA in its consensus document: *Hard-to-heal wounds: a holistic approach*.[31] The guidelines state: 'If no signs of healing tendency are observed after four weeks of standard therapy, the treatment strategy should be reconsidered.' In addition, according to the *Consensus guidelines for the identification and treatment of biofilms in chronic nonhealing wounds*, effective therapies typically show measurable progress (e.g., granulation tissue) within this period.[32]

The SoC was not modified to ensure the outcome was not affected by any change. Baseline visit was at D0, with follow-up visits carried out at D7 and D21.

Treatment included CAP application with the plasma care device at DO and D7 for two minutes each time to assess indicators of persistent infection. Progress of wound healing was assessed at D21.

Results

Patient characteristics

A total of 15 patients (11 female, four male) with 20 wounds were included in this case series. The patients ranged in age from 43–90 years, with a median age of approximately 75 years.

The underlying aetiologies of the wounds were diverse, reflecting a broad spectrum of hard-to-heal wound conditions commonly observed in vascular medicine and dermatology. The most frequent wound causes included: arterial ulcers (three patients); vasculitis (three patients); psoriatic arthritis-related ulcers (two patients); as well as individual cases of osteomyelitis, calciphylaxis, scleroderma-associated vasculitis and arterial disease, diabetic foot ulcers, venous ulcers, and pressure ulcers; and one patient had a wound of non-defined dermatological disease.

The duration of the wounds varied significantly, ranging from 2–180 months (15 years), with several

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wounds persisting for >5 years, particularly in patients with osteomyelitis, vasculitis or arterial disease. This variation highlights the hard-to-heal and treatment-resistant nature of the wounds included in the study.

Evaluation with bacterial fluorescence imaging

The wounds were examined for bacterial colonisation at D0, D7 and D21 using the bacterial fluorescence imaging device. Fig 1 and Fig 2 illustrate the difference in fluorescence between wounds infected with *Pseudomonas aeruginosa* (pyoverdine-producing) and wounds infected with other bacteria, such as *Staphylococcus aureus* (porphyrin-producing).

Fig 1 shows images of a patient's infected leg ulcer with signs of critical colonisation by *Pseudomonas aeruginosa*. This is illustrated by the bright green (cyan) glow due to pyoverdine in the middle of the wound (Fig 1a, white arrow). No evidence of pyoverdine fluorescence after seven days (Fig 1b, 1c). There was no evidence of infection with other porphyrin-producing bacteria in this wound.

There are clear indications for colonisation with *Pseudomonas aeruginosa* and other bacteria in the leg ulcer of the patient shown in Fig 2. Fig 2a shows the bright green (cyan) glow due to pyoverdine distributed in the wound edges and periwound area, particularly at the lower wound edge (which is an indicator for *Pseudomonas aeruginosa)*. The red fluorescence (an indicator for other bacteria), especially visible in the centre of the large wound area (white arrows) progressively decreased.

The colonisation of all wounds with *Pseudomonas aeruginosa* (cyan fluorescence) and mixed colonisation (cyan + red fluorescence) was quantified by area on a scale from 0 (no fluorescent area) to 3 (heavily colonised wound).

The bacterial load was significantly reduced within 21 days. Between the first (D0) and second therapies (D7), the number of *Pseudomonas aeruginosa* colonies was reduced from 30 to 12 by D7 and to six by D21 (Fig 3). Other bacteria were also reduced from 28 colonies on D0 to 16 colonies by D7 and to eight by D21. The reduction of *Pseudomonas aeruginosa* by more than half after just one treatment is particularly notable. Fig 3 shows the reduction of bacteria in all wounds over 21 days. *Pseudomonas aeruginosa* was eliminated in 13 wounds, and porphyrin-producing bacteria were eliminated in 11 wounds.

Analysis of the Cutting and Harding rating

The Cutting and Harding rating was performed according to the classic signs of infection and additional parameters, such as wound odour, exudate, pain, wound edge condition, etc.[27]

The total infection scores assessed by the Cutting and Harding method are shown in Fig 4. The reduction in bacterial colonisation was 82.3%

Clinically, at D7, three wounds were free of infection, 15 wounds had improved and two remained unchanged. By D21, infection had been cleared in six cases and 14 cases showed improvement; no wounds remained unchanged or had worsened (Fig 5).

Wound bed preparation

The sum of the WBP score values for all wounds was 107 at D0; 143 at D7; and 242 at D21, representing an increase of 126.2%.

Fig 6 shows the increase in the WBP score over the 21-day observation period. On average, the wounds improved from a median of 5.6 to a median of 11.3.

Wound size

Analysing the individual wound area, the following was observed:

- Healed=0
- Improved (reduced in size)=15
- Worsened (increased in size)=5

Note that while the 20 wounds had a combined total area of 202.8cm² (mean: 10.1cm²; median: 4.29cm²) at D0, the five wounds that worsened accounted for almost one third of the area at baseline (66.2cm² on D0) and nearly half of the total area at D21 (91.33cm² of 195.57cm²) (Table 7).

Of the five wounds that worsened, three showed contamination with *Staphylococci* at the D21 follow-up. The responding wounds reached an overall area reduction of 22.0% within 21 days with only two CAP treatments. The number of worsened cases was too small to identify a common pattern.

Discussion

Hard-to-heal wounds remain a major therapeutic challenge in vascular medicine, particularly due to persistent bacterial colonisation and its impact on wound healing progression. Despite advancements in wound care, the need for effective, non-invasive and well-tolerated methods that address both microbial burden and tissue regeneration remains unmet in many clinical scenarios. In this context, CAP has emerged as a promising adjunct therapy due to its dual bactericidal and cell-stimulating effects.[10,24,33,34]

The treatment regimen used in this study, with only two CAP applications at one-week intervals, was intentionally minimal to test the efficacy of the system under reduced treatment intensity, corresponding to the lower limit of current clinical recommendations. Despite this, the results are compelling, with 15 wounds showing clinical improvement, and an average wound size reduction of 22% observed among the responders. In contrast, five wounds worsened, with three of those testing positive for contaminating *Staphylococcus aureus* at follow-up, highlighting the well-known virulence and resistance mechanisms of this common wound pathogen.[21,22]

Microbiological assessment through two complementary diagnostic approaches, such as fluorescence imaging and the Cutting and Harding

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Fig 1. Fluorescence of *Pseudomonas aeruginosa* in bright cyan (white arrow) on day (D)0 **(a)**; D7 **(b)** and D21 **(c)**. No visual evidence of *Pseudomonas aeruginosa* from D7

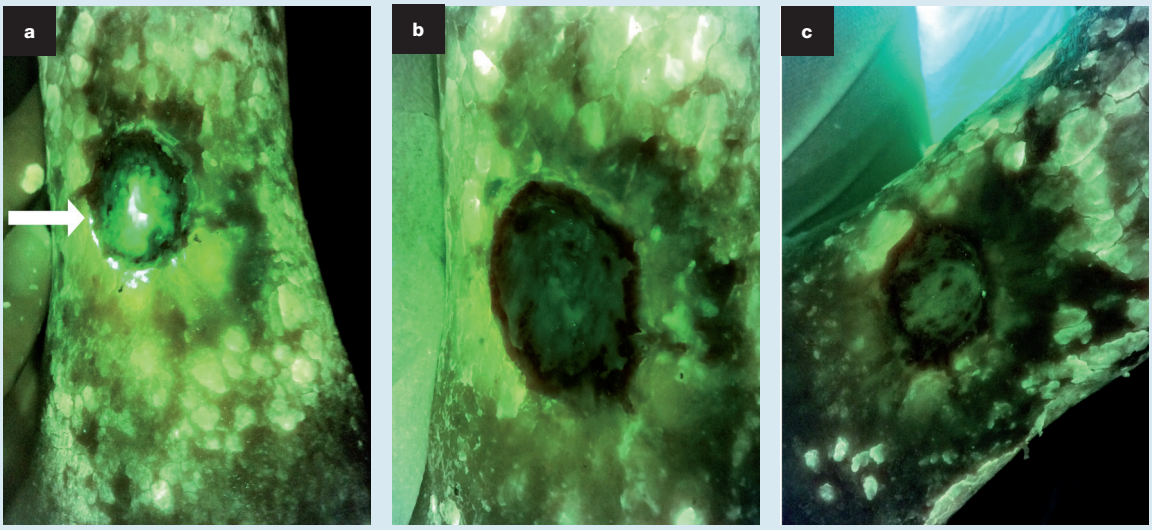
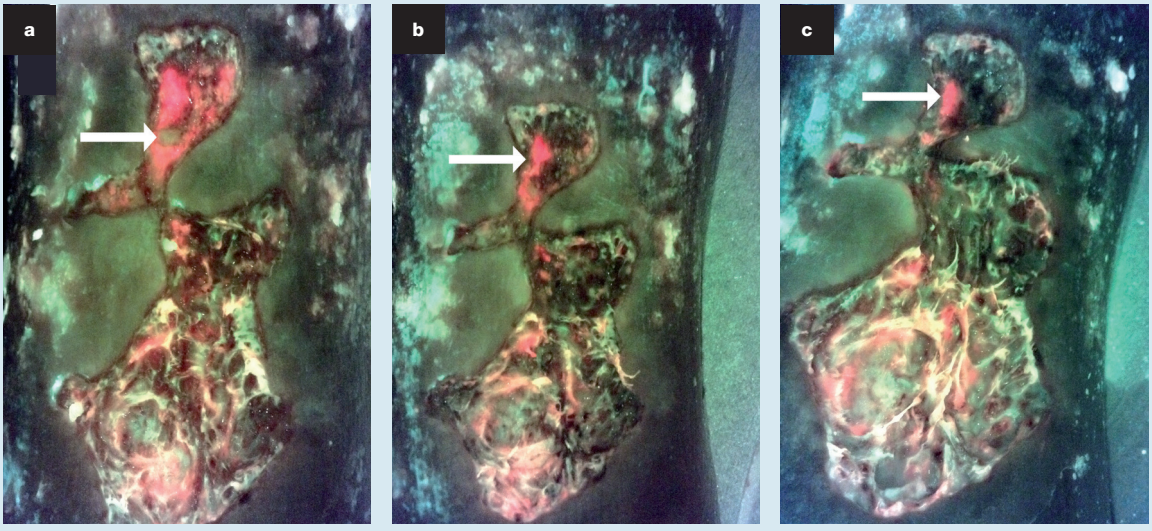


Fig 2. Fluorescence of a wound with mixed colonisation on day (D)0 **(a)**; D7 **(b)**; and D21 **(c)**. *Pseudomonas aeruginosa* appears in cyan. The fluorescence of other bacteria appears in red due to the porphyrin (white arrow)



infection criteria,[14] provided further insights. Complete bacterial load control was achieved in 60% of cases using fluorescence assessment, while a reduction in bacterial burden was observed in 85% of cases according to the Cutting and Harding method.[14] These findings reinforce the bactericidal efficacy of CAP, even under minimal exposure, and highlight its potential as a valuable tool in wound management.

The combination of CAP therapy and bacterial fluorescence imaging represents an innovative approach to treating hard-to-heal infected wounds. The MolecuLight i:X used in this study enables real-time visualisation of bacterial load, providing a rapid, non-invasive alternative to conventional microbiological techniques.

In this study, the fluorescence imaging device was used to evaluate the efficacy of CAP therapy. The results demonstrated that just two applications within three weeks led to a significant reduction in fluorescence signals: in 60% of wounds, bacterial load was no longer detectable, and *Pseudomonas aeruginosa* colonies were eliminated in 13 out of 20 cases. These findings highlight the potential of CAP as an anti-infective therapy, particularly for critically colonised wounds.

However, discrepancies between fluorescence diagnostics and clinical scoring systems were also observed. While the Cutting and Harding criteria indicated improvement in 85% of cases, this did not always correlate with fluorescence patterns. This underscores the limitations of older infection scoring

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Fig 3. Bacterial reduction from day (D)0 to D7 and to D21. Left: *Pseudomonas aeruginosa*, right: porphyrin-producing bacteria, such as *Staphylococcus aureus*. *Infection/bacterial colonisation was rated on a scale of 0-3, and then total calculated for all wounds combined

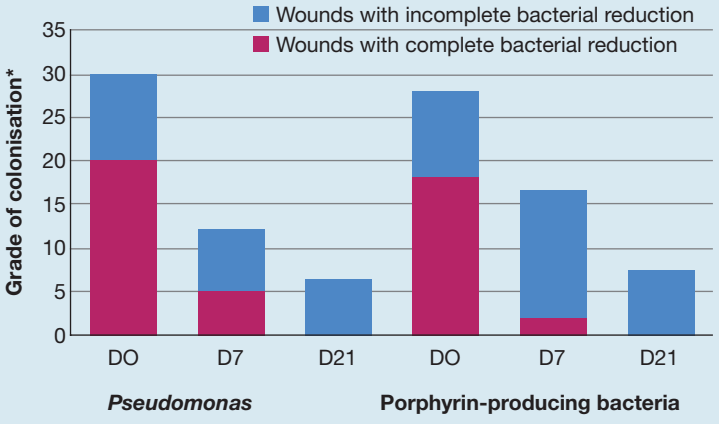
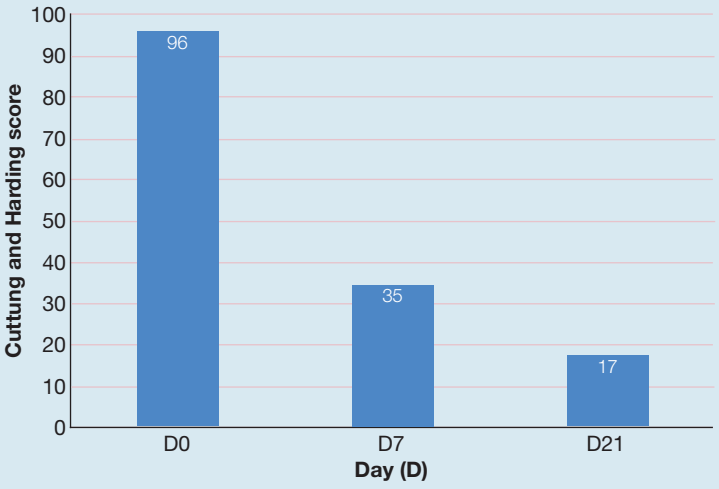


Fig 4. Evaluation of the wounds using the Cutting and Harding score[14]



systems and emphasises the need for future studies to incorporate more current standards. Additionally, integrating supplementary methods, such as biofilm detection or the CAse REport guidelines[23] further enhance diagnostic accuracy.

In summary, this study demonstrates that the combination of fluorescence-based diagnostics and CAP therapy holds promise for accelerating wound healing in hard-to-heal infected wounds. However, broader clinical implementation will require further studies with standardised protocols and modern infection criteria.

At the same time, the discrepancy between the two diagnostic methods underscores an important point: achieving consistent, objective infection diagnostics in vascular wound care remains difficult, even when validated tools are used. This calls for further refinement in diagnostic standardisation and combined assessment approaches in future studies.

Patient safety and tolerability are critical in any novel therapy. In this present study, no side-effects, pain or discomfort were reported during or after CAP application, confirming the tolerability of the plasma care device and supporting its suitability for use in-patient populations.

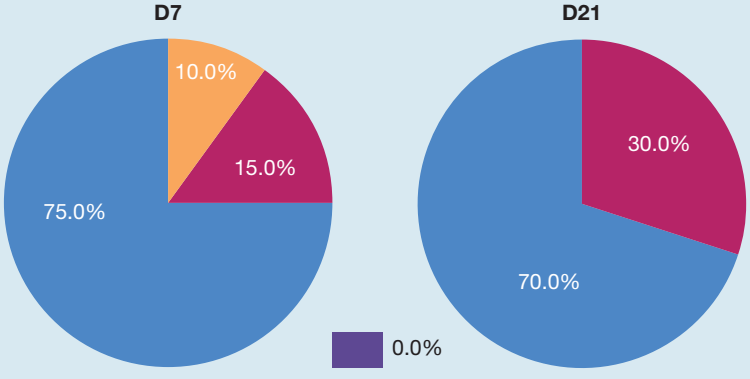
Taken together, these results demonstrate that CAP therapy with the device used in this study is not only effective in reducing microbial burden and improving wound status, but also well-tolerated and applicable in routine care. The study design, with minimal application frequency, suggests that even short CAP interventions can yield measurable clinical benefits. However, the persistence of bacterial contamination in a subset of patients, particularly involving *Staphylococcus aureus*, indicates that more frequent or sustained applications may be required in certain cases to achieve optimal outcomes.

Limitations

This study has limitations, including its observational nature, small sample size, and absence of a control group. Further randomised controlled trials are needed to confirm these findings, determine the ideal application frequency, and define which wound types and microbial profiles respond best to CAP therapy.

Fig 5. Evaluation of infection signs

Wounds	D7		D21	
Cleared	3	15.0%	6	30.0%
Improved	15	75.0%	14	70.0%
Unchanged	2	10.0%	0	0.0%
Worsened	0	0.0%	0	0.0%
Total	20		20	



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Conclusion

This observational study demonstrates that CAP therapy using the plasma care device is a safe and effective method for reducing bacterial load and supporting wound healing, even when applied under minimal treatment conditions. The results are particularly encouraging in terms of infection control, with most wounds showing clear improvement after just two applications over three weeks.

However, the optimal treatment frequency and application protocol remain to be determined. While the current study highlights the effectiveness of limited CAP use, it is likely that more frequent or prolonged applications could further enhance outcomes, particularly in larger wounds or wounds with critical bacterial colonisation. Some wounds that did not respond in this study may have benefited from a more intensive CAP regimen.

Further clinical studies with larger patient cohorts and varied application schedules are warranted to explore these possibilities. Such research will help define evidence-based guidelines for CAP use and better tailor treatment to individual wound characteristics. JWC

Fig 6. Wound bed preparation (WBP) score (0–16, worst to best score) averaged over all treated wounds

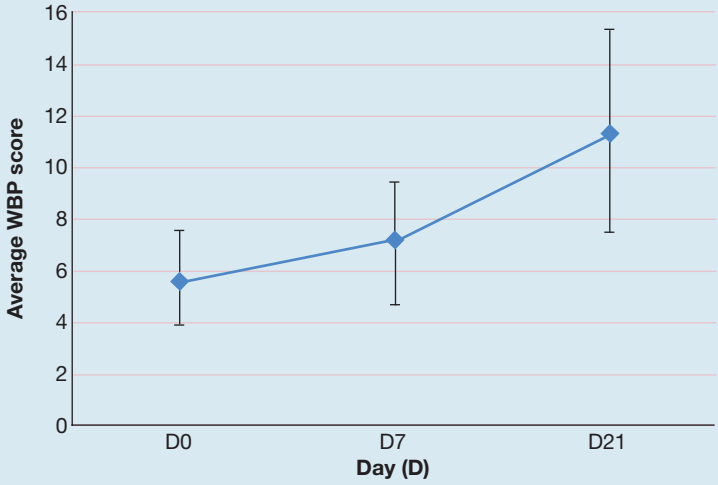
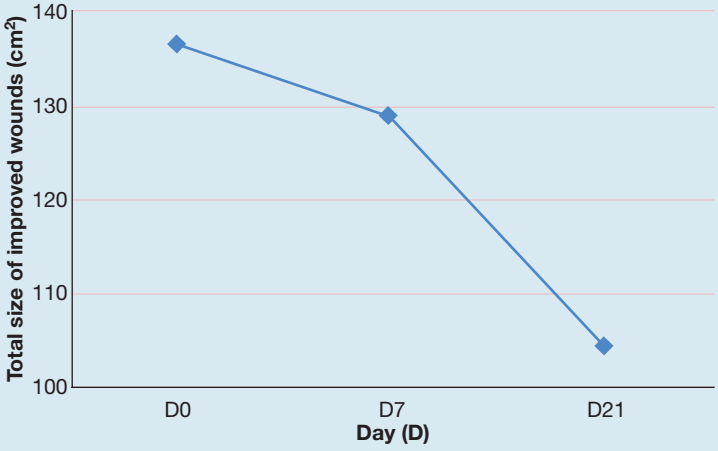


Fig 7. Total wound size of all 15 improving wounds



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Reflective questions

- What is the most effective way of visualising the effect of cold atmospheric plasma (CAP)?
- How might CAP be used in wound bed preparation?
- How effective is CAP with limited applications on reducing bacteria and in wound bed preparation?

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