

Comparative study of two antimicrobial dressings in infected leg ulcers: a pilot study

• **Objective:** The aim of the study was to compare the efficacy of a microorganism-binding (MB) dressing with a silver-containing hydrofiber (SCH) dressing in controlling the bacterial loads of heavily colonised or locally infected chronic venous leg ulcers, before surgical management with homologous skin grafts.

• **Method:** A randomised comparative single centre study recruited patients presenting with hard-to-heal critically colonised or locally infected leg ulcers, who could be treated with skin grafting. Inclusion criteria included; ulcers of vascular aetiology, over 18 years old, a wound duration ≥ 6 months and ankle brachial index (ABPI) > 0.6 . Patients were randomly assigned to treatment with SCH dressings (Aquacel Ag) or MB dressing (Cutimed Sorbact). Dressings were changed daily over a four-day observation period, after which they were taken for a skin grafting procedure. Swab samples from ulcer beds were taken in order to quantify the bacterial load at inclusion (D0) and at the end of the observation period day 4 (D4). No antibiotics were administered before or during the evaluation period.

• **Results:** Both groups ($n=20$ SCH, $n=20$ MB) were similar in gender, age, pathophysiology (both had 15 patients with venous leg ulcers and 5 with arterial leg ulcers), ulcer surface, ulcer duration, treatment-related pain and initial bacterial load. Analysing bacterial load variation showed a significant reduction of bacterial burden at D4 in both groups. In the SCH group, we found an average bacterial load reduction of 41.6%, with an average reduction of 73.1% in the MB group ($p < 0.00001$). No serious adverse events were reported.

• **Conclusion:** Our evaluation confirmed that MB and SCH dressings are effective in reducing the bacterial burden in critically colonised or locally infected chronic leg ulcers, without inducing adverse events, with MB dressings significantly more effective.

• **Declaration of interest:** There were no external sources of funding for this study. The authors have no conflicts of interest to declare.

leg ulcers; infection; bacterial load; antimicrobial dressing; efficacy; skin allograft

Skin grafting failure due to infection was proposed in 1951 by Jackson.¹ In 1967 Krizek et al. published data showing that on average 94% of grafts survived when $\leq 10^5$ CFU/g were present in the tissue biopsies, whereas 19% survived when count exceeded 10^5 CFU/g.² Another study³ demonstrated the presence of *Pseudomonas aeruginosa* and *Staphylococcus aureus* results in a significant probability of the skin graft failing to take. These findings were supported by Hogsberg et al.,⁴ who concluded that a successful skin graft 'take' is less likely to occur with wounds containing more than 10^5 viable bacteria per gram of tissue.

Bacteria can secrete a large number of enzymes such as hyaluronidase, fibrinolysins, and proteases. In the case of skin grafting, these may damage the growth of capillaries through the fibrin layer between the granulation tissue and the graft.

Critical colonisation is used to describe the level of bacteria that inhibits wound healing but does not display classical signs of infections.⁵ The term, which has been part of the wound care vocabulary for a

long time, is frequently challenged⁶ but not yet disproved. Synonyms for critical colonisation include: silent infection, covert infection, occult infection, refractory wound, subclinical infection, indolent wound, stunned wound, subacute infection and recalcitrant wound.⁵ This means that clinical criteria are required to diagnose concealed infection.

Robson et al.⁷ defined infection as a level of $>10^5$ microorganisms/g of tissue, and using quantitative bacteriology, they found that wounds undergoing delayed closure with <10 CFU/g healed successfully, while those with $>10^5$ CFU/g did not.

For ulcers with high bacterial loads, the correct choice of a dressing to reduce bioburden is important. Adequate delivery of bactericidal agents to an infected ulcer can be very difficult; the dressing must be able to effectively decrease the microorganism population (planktonic and biofilms), with a broad spectrum of action. The dressing must not be toxic or induce resistance. It is widely accepted that topical antibiotics should be avoided owing to the risk of increasing bacterial resistance and contact dermatitis.⁸

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Silver-containing dressings are used worldwide for the local management of colonised or infected leg ulcers. We routinely use a silver-containing Hydrofibre dressing. (SCH: Aquacel Ag, ConvaTec, NJ, US). The dressing releases silver ions on the wound bed or inside the dressing, these need to come into contact with and get inside bacteria to exert their bactericidal action. Bacterial destruction may result in the release of substances capable of prolonging the inflammatory response. Silver ion release has to be slow in order to provide a long lasting antimicrobial effect.

Systemic uptake of silver ions with deposition in organs like liver and kidney has been demonstrated.⁹ Even if silver's systemic toxicity seems very low, there is no clear evidence about the effects of long-term exposure to high levels.⁹ Another concern when using silver dressings is that silver at higher concentrations may exert a local cytotoxic effect binding fibroblasts and keratinocytes resulting in delayed healing.¹⁰ Finally, the most important concern, is the onset of bacterial resistance to silver, which has been reported for *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Citrobacter freundii*.¹¹ Silver resistance determinants are often located on mobile genetic elements, facilitating their spread.¹² Even if the risk of widespread resistance to silver in wound care seems low, it has to be carefully monitored.¹³

As concerns persist about silver's potential toxicity, and the risk of bacterial resistance to silver,⁹ we wanted to explore the clinical efficacy of a microorganism-binding (MB) dressing (Cutimed Sorbact, BSN Medical; Hamburg Germany), available locally for the treatment of critically colonised or infected wounds. MB dressings have antimicrobial capabilities. The dressing, which is designed to be in contact with the wound bed, is coated with dialkylcarbamoyl chloride (DACC) making the dressing hydrophobic. Wound bacteria are largely hydrophobic in nature and when in proximity to the hydrophobic dressing become bound to the dressing and are removed from the wound bed with dressing change. The result is a reduced wound bacterial load.¹⁴ The antimicrobial properties of MB dressings are based on a physical effect; as a result, no bacterial resistance is expected or has been demonstrated.¹⁵

Objective

The aim of our study was to evaluate the efficacy of MB versus SCH dressings, before surgical management with skin grafting, in controlling the bacterial load of heavily colonised or locally infected chronic leg ulcers.

Materials and methods

This was a comparative, randomised, single centre pilot study. Patients with vascular leg ulcers (venous and arterial) and considered suitable for

wound management with skin grafting were recruited for the study.

Signed informed consent was obtained from patients. The study complied with the Helsinki Declaration and the rules of the local ethical committee.

Inclusion criteria

Patients older than 18 years, of both genders, with critically colonised (multiplying bacteria causing delayed healing without sign of infection) or locally infected (multiplying bacteria with sign of local tissue damage) ulcers of vascular aetiology, duration ≥ 6 months and ankle brachial pressure index (ABPI) > 0.6 .

Exclusion criteria

Patients were excluded if they were younger than 18 years, had ulcers without signs of critical colonisation or infection, had ulcers of immunological or diabetic origin, were receiving cortisone or immunosuppressive treatment, had a ulcer duration < 6 months, or had an ABPI < 0.6 .

Treatment protocol

Following inclusion, patients were randomly, using List Randomizer, assigned to treatment with SCH (20 patients) or MB dressings (20 patients). After an observation period of 4 days, during which time dressings were changed daily, patients were taken to the operating room for a planned skin grafting procedure. In cases of an incomplete wound bed preparation, with some areas of ulcer bed still covered by slough or necrotic tissue, sharp debridement was performed before skin grafting.

For the purpose of the present comparative study, the type of dressing was the only modification introduced to the management protocol. All products had the CE mark and were used according to the manufacturers' instructions.

Inelastic compression was used on all patients throughout the treatment period before and after the skin grafting. The level of compression was adapted individually depending on the ulcer aetiology and the peripheral vascular conditions. Patients with venous leg ulcers had compression up to 40mmHg,¹⁶ while patients with arterial leg ulcers had lower levels of compression. In no case did the compression level exceed 40mmHg.¹⁶

The primary outcome was the ulcer bacterial load. Secondary outcomes were:

- Ease of dressing application and removal
- Treatment related pain variation
- Adverse events.

Primary outcome bacterial quantification

At inclusion (D0) and upon conclusion of the observation period (D4) swab samples from ulcer beds were taken in order to quantify bacterial load. After cleansing of the ulcer bed with Ringer's solution,

samples were taken from clinically chosen 1cm² areas by pressing and rotating the swab tip uniformly. In some cases, marks were made on the periwound skin in order to identify the same area for further swabbing procedures. Swabs were transferred to the laboratory and cultured for aerobic bacteria on Agar plates. The results were checked after 5 days. No antibiotics were administered to any patients before or during the evaluation period.

Secondary outcomes

Ulcer-related pain was evaluated using a visual analogue scale (VAS) where 0 represented absence of pain and 10 represented agonising pain.

Two nurses and one doctor provided their opinion about the features of the dressing, its conformability and ease of use.

Statistical analysis

Given the exploratory nature of the study we did not establish or test any hypothesis. Data were analysed using descriptive statistics and comparative tests including Student's t-tests to analyse differences between groups regarding demographic data, wound size, ulcer duration time, pain scores, bacterial loads at D0 and D4. ANOVA tests were used to analyse bioburden variation between D0 and D4 within and between groups, with p values <0.05 considered statistically significant.

Results

There were 20 patients allocated to each group with similar demographics in each, gender (16 male, 24 female) and age (69.5 ± 13.5 years). The aetiology of the lesions was also similar—in each group, 15 patients presented with venous leg

ulcers and five with arterial leg ulcers (Tables I and II).

All patients completed the study. Surgical sharp debridement was not required in any case.

The statistical analysis found no significant difference between groups regarding wound size (p=0.48), ulcer duration time (p=0.47) or bacterial load at D0 (p=0.21; Tables III, IV and Fig 1).

Staphylococcus aureus, methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella*, *Enterobacter cloacae*, and *Proteus mirabilis* were most frequently found on the ulcer beds. In general we found a polymicrobial burden with bacterial species equally distributed in the two groups. The recorded data does not allow us to make any further comparison between bacteria species.

Primary outcome bacterial quantification

The average bacterial load was similar in both groups at D0, that is, 9.1 × 10⁵CFU/cm² and 8.5 × 10⁵CFU/cm² in the SCH and MB groups, respectively. After analysing bacterial load within each group, the results showed a significant reduction of bacterial burden at D4 in both groups. In the SCH group, the average bacterial load reduction was 41.6%, with a reduction of 73.1% in the MB group. When comparing bacterial load between groups at D4, the reduction was significantly higher in the MB group (p< 0.0001; Fig 1).

Secondary outcomes

Dressing application and removal was found to be atraumatic and simple for both dressing types. Average ulcer-related pain scores were 4.65 and 4.75 at D0 in the SCH and MB groups, respectively. Pain scores decreased in both groups, –35% in the SCH group and –38% in the MB group. The statistical analysis found no significant difference between groups at D0 (p=0.41) or at D4 (p=0.89; Fig 2).

Of the 40 patients, 20 (10 SCH and 10 MB) required analgesics before treatment. At D4, only 4 patients in the SCH group and 3 in the MB group still required analgesics. Only four patients in the SCH group and five in the MB group needed more than one piece of the dressing at each dressing change. Using more than one piece of the dressing did not have any effect on bacterial load reduction.

Two patients in the SCH group reported intense burning following the application of the dressing. The burning sensation lasted for a few hours, then disappeared without further problems and without the need for analgesics.

No serious adverse events related to the dressings were seen during the present study.

Discussion

Clinical practice has demonstrated that the majority of leg ulcers heal within 4–6 months when correctly

Table I. Ulcer aetiology and gender distribution

Group	Aetiology (n)	Male (n)	Female (n)	Total (n)
MB	Venous	5	10	15
	Arterial	4	1	5
SCH	Venous	5	10	15
	Arterial	2	3	5
TOTAL		16	24	40

MB - microorganism-binding dressing; SCH - silver-containing hydrofiber dressing

Table II. Age by group and gender

Group	Male (years)	Female (years)	Total (years)
MB	67.2 ± 9.9	71.2 ± 14	69.4 ± 12.2

managed through the use of well-established protocols including appropriate wound management, dressings and compression.

For the proportion of ulcers that do not respond to standard care, a multidisciplinary team approach, which must include a vascular surgeon and a plastic surgeon, is required.¹⁷ Other therapeutic alternatives need to be considered to increase the probability of healing. Skin grafting is one of these alternatives.

Using the technique proposed by Levine et al.,¹⁸ Bill et al.¹⁹ quantified bacterial loads in 38 non-healing wounds without classical signs of infection. Tissue biopsy showed $>10^5$ bacteria/g in 28 of the biopsied wounds. Of those identified, the quantitative swab technique detected 79% of the infected wounds. The quantitative information allowed modification of the management plan, resulting in wound healing.

Our findings of the bacteria present are similar to those reported by Gjødtsbøl et al.²⁰ who found that chronic wounds are colonised by multiple bacterial species (aerobic and anaerobic), and that once bacteria are established many of them persist within the wound.

A problem with quantitative bacterial cultures (biopsy or swab) is that it may take up to 48 hours to obtain a result, after the decision to graft is typically made. As a result, this methodology is most commonly applied to research. The clinical reality is that surgeons must trust their knowledge and frequently take a more aggressive approach to make sure that the wound bed is clinically 'clean' before grafting.

Another problem with methods in quantitative bacteriology is the difficulty to detect or the underestimation of hard-to-cultivate bacteria. Traditional quantitative methods are limited when determining a threshold value for bacterial bioburden, mainly because *in vitro* growth is needed, and they can only

Table III. Average surface by group and ulcer type

Group	Mean surface (cm ²)		
	Venous	Arterial	Both
MB	42.6	72.0	50.0
SCH	42.9	74.0	50.7

MB - microorganism-binding dressing; SCH - silver-containing hydrofiber dressing

Table IV. Average duration by group and ulcer type

Group	Mean duration (months)		
	Venous	Arterial	Both
MB	34.4	26.4	32.4
SCH	33.5	26.4	31.7

MB - microorganism-binding dressing; SCH - silver-containing hydrofiber dressing

detect viable and cultivable bacteria. A potential solution to this problem may be the panbacterial real-time polymerase chain reaction (RT-PCR), which can quickly determine bacterial loads and provides more precise data on bacteria species.

However, RT-PCR requires specific reagents for each bacterial species. However, the development of a 'Universal' reagent based on the 16S-rRNA gene (a prokaryotic rRNA found in all bacteria), which has a stable structure that changes little over time, allows a quick determination of total bacterial burden with high sensitivity and the detection of both aerobic and anaerobic bacteria.

Gentili et al.²¹ published the results of a 4-week clinical evaluation in 19 patients (20 wounds) presenting hard-to-heal vascular leg ulcers, treated with Sorbact dressings. The results showed that the dress-

Fig 1. Comparison of bacterial loads at day 0 and day 4

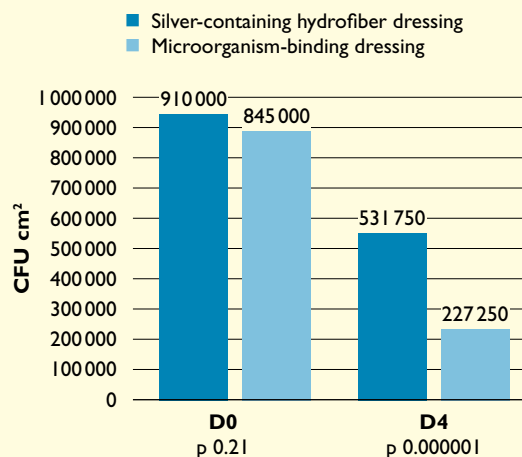
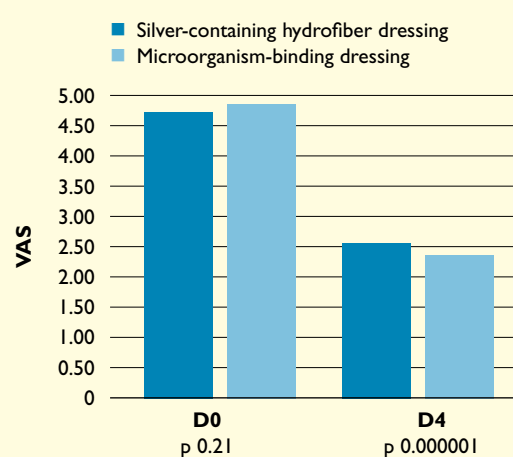


Fig 2. Comparison of pain scores at day 0 and day 4



ing promoted healing in 7 patients and improvement in another 8. They used quantitative 16S RT-PCR to assess bacterial loads. The initial bacterial load was considerably different in the samples ranging from 4.38×10^3 – 2.44×10^8 bacterial genomes/mg of tissue. Nevertheless, the average of the total bacterial load before the treatment was 4.41×10^7 /mg of tissue, which decreased to 1.73×10^5 /mg of tissue, corresponding to a significant 254-fold decrease in the total bacterial load in the healing wounds, whereas in the non-healing wounds they found only a non-significant 5.3-fold decrease of the total bacterial load. The results allowed them to confirm the suitability of 16S RT-PCR quantification of total bacterial load as a quick and sensitive parameter of wound evolution when performed on tissue biopsies.

When designing our pilot study protocol, we took into consideration the available experimental data about the technology on which the MB dressing is based.^{15,22–25} The dressings mechanism of action constitutes a paradox: microorganisms are trapped not destroyed, and eliminated from the wound at dressing change. The mesh structure allows conformability and ease of application. Because of their mechanism of action, it is unlikely that MB dressings will cause bacterial resistance or have systemic absorption and local or systemic toxicity. As bacteria are removed intact, the release of bacterial endotoxins is prevented and the local inflammatory response is reduced. However, a change in the current assumptions about antimicrobial dressings is required to accept that local antimicrobial activity is achieved without using more conventional antimicrobial substances.

In general, the frequency of dressing change depends on the quantity of wound exudate, the wound status and bioburden. For all our patients with these types of wounds, included or not in this study, we change the dressings every day. This is based on the following reasons:

- To assess the wound on a daily basis
- Our belief that a daily change could have a better impact on the preoperative preparation of the wound bed as it could provide a more intense antimicrobial effect
- The fact that the preparation period is short
- MB dressings are indicated to be changed daily.

It is important to highlight the observed pain reduction, which was probably due to a reduction of bacterial load and inflammation as a direct result of the dressings and compression. The presence of silver could be seen as a cause for stronger pain in the SCH group; however, the results didn't show any significant difference between the two groups.

Limitations and future studies

By definition, pilot studies are size-limited and our trial is not an exception. New technologies are not

always easy to assess, and in the absence of reliable evidence, pilot studies are a good way to obtain baseline data to assist the designing of further research. A further larger trial is necessary to confirm our data.

Blinding of treatment does not apply to this study. Devices used during a comparative trial are expected to perform similar actions, but as both dressings are physically different, blinding is not possible. What could have been blinded here were the initial assessment of the wound and the assessment of outcomes by different expert clinicians. However, implementing this type of blinding during the present trial was logistically difficult because of the short observation period. A complete analysis about blinding in wound research can be found in a document published by the EWMA's Patients Outcome Group.²⁶

This study may also be limited by the swabbing of the wound for bacteria. Although qualitative biopsies are more reliable, they are also more invasive. Hence, we chose to swab the wound area carefully in the same place with the same method.

The observation period was limited to 4 days as a direct result of our protocol of care, which we have adapted for this type of patients. The four-day period is intended to prepare the wound for surgery. Owing to the short duration of the study, we did not record data on ulcer development and healing rates. It is highly likely that by increasing the chance of the graft taking we are improving healing outcomes. However, we focused this pilot study on bacterial loads to investigate the dressings' antimicrobial efficacy.

There are limitations around the identification of the bacterial species, which are due to the traditional culture methods we used. As RT-PCR becomes increasingly accessible and widely used, more clinical evidence will be available, and it is highly likely that the bacteriological criteria for wound infection that we apply today will be challenged and modified in the future.

Conclusions

Our evaluation seems to confirm that, independently from their mechanisms of action, MB dressings as well as SCH dressings are both effective in reducing bacterial burden in critically colonised or locally infected chronic venous leg ulcers without inducing adverse events.

In this pilot trial, MB dressings were significantly more effective in reducing bacterial numbers than SCH dressings. However, the size of the population, represents a challenge regarding comparative efficacy. A trial including a larger population, a longer follow up and the use of PCR techniques for quantitative bacteriology are required to confirm these results. ■

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