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 ≤10^5CFU/g were present in the tissue biopsies, whereas 19% survived when count exceeded 10^5CFU/g.[2] Another study[3] demonstrated the presence of *Pseudomona aeruginosa* and *Staphylococcus aureus* results in a significant probability of the skin graft failing to take. These finding 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 challenged[6] 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 >10^5CFU/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 Hydrofiber 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*, *Pseudomona 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 1cm^2 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), *Pseudomona 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 x 10^{5}CFU/cm^2 and 8.5 x 10^{5}CFU/cm^2 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
SCH	64.3 ±10.9	72.5 ± 16.7	69.7 ± 15.2
TOTAL	65.9 ± 10.1	71.9 ± 15.2	**69.5 ± 13.6**

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

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 biospied 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 Gjødsbøl 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 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-

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

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

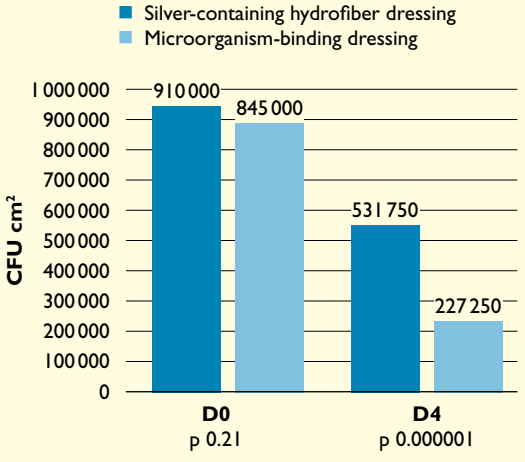
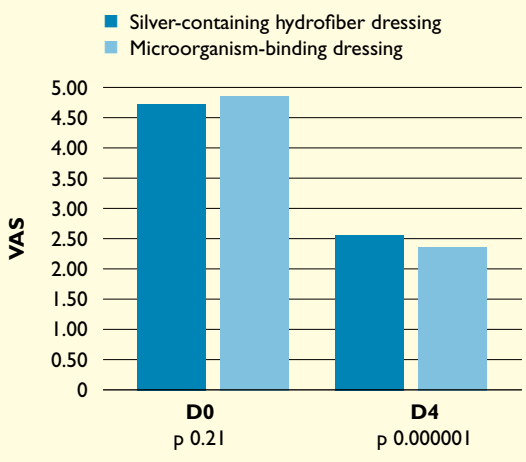


Fig 2. Comparison of pain scores at day 0 and day 4



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 x 10^3–2.44 x 10^8 bacterial genomes/mg of tissue. Nevertheless, the average of the total bacterial load before the treatment was 4.41 x 10^7/mg of tissue, which decreased to 1.73 x 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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SURGICAL INFECTIONS
Volume 17, Number 4, 2016
Mary Ann Liebert, Inc.
DOI: 10.1089/sur.2015.223

Original Articles

Randomized Controlled Trial Evaluating Dialkylcarbamoyl Chloride Impregnated Dressings for the Prevention of Surgical Site Infections in Adult Women Undergoing Cesarean Section

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Abstract

Background: Surgical site infections (SSI) occur in 1.8%–9.2% of women undergoing cesarean section (CS) and lead to greater morbidity rates and increased treatment costs. The aim of the study was to evaluate the efficacy and cost-effectiveness of dialkylcarbamoyl chloride (DACC) impregnated dressings to prevent SSI in women subject to CS.
Methods: Randomized, controlled trial was conducted at the Mazovian Bródno Hospital, a tertiary care center performing approximately 1300 deliveries per year, between June 2014 and April 2015. Patients were randomly allocated to receive either DACC impregnated dressing or standard surgical dressing (SSD) following skin closure. In order to analyze cost-effectiveness of the selected dressings in the group of patients who developed SSI, the costs of ambulatory visits, additional hospitalization, nursing care, and systemic antibiotic therapy were assessed. Independent risk factors for SSI were determined by multivariable logistic regression.
Results: Five hundred and forty-three women undergoing elective or emergency CS were enrolled. The SSI rates in the DACC and SSD groups were 1.8% and 5.2%, respectively ($p = 0.04$). The total cost of SSI prophylaxis and treatment was greater in the control group as compared with the study group (5775 EUR vs. 1065 EUR, respectively). Independent risk factors for SSI included higher pre-pregnancy body mass index (adjusted odds ratio [aOR] = 1.08; [95% confidence interval [CI]: 1.0–1.2]; $p < 0.05$), smoking in pregnancy (aOR = 5.34; [95% CI: 1.6–15.4]; $p < 0.01$), and SSD application (aOR = 2.94; [95% CI: 1.1–9.3]; $p < 0.05$).
Conclusion: The study confirmed the efficacy and cost-effectiveness of DACC impregnated dressings in SSI prevention among women undergoing CS.

Cesarean section (CS) remains to be one of the most common surgical procedures performed worldwide and available data indicate that surgical interventions constitute approximately 0.4%–40.5% of all deliveries [1]. Depending on the definition and the observational period, surgical site infection (SSI) occurs in about 1.8%–9.8% of all CS patients and leads to greater morbidity rates, prolonged hospitalization, and increased number of hospital readmissions [2–9]. Post-cesarean SSI has been estimated to extend the period of hospitalization by 4 d, and at the same time generating an additional cost of 3716 EUR per patient [9].

A recently published pilot study revealed a downward trend in SSI rates after CS if dialkylcarbamoyl chloride (DACC) impregnated dressings were used as method of post-operative SSI prevention [10]. The characteristic feature of the dressing, whose fibers were covered with a hydrophobic derivate of fatty acids, is its solely physical mechanism of action. It uses the interaction between hydrophobic molecules in the presence of aqueous medium, as well as the fact that the majority of pathogens responsible for the development of SSIs demonstrate moderate to high cell surface hydrophobicity (CSH) [11]. High CSH allows microorganism

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to adhere to cells and to initiate infection, at the same time causing their aggregation on the surface of the impregnated dressing, decreasing both their number in the wound bed and proliferation [12–16]. Described mechanism of action is not associated with the release of additional antimicrobial substances, thus eliminating the risk of cytotoxicity and sensitization, which is particularly important during the periods of puerperium and lactation [16]. To date, the efficacy of DACC-impregnated dressings has been proven in the treatment of venous, arterial and pressure ulcers, burns, diabetic foot, and hard-to-heal post-traumatic and post-operative surgical incisions [17–20].

Taking into consideration the steadily growing CS rates, as well as factors that are responsible for impaired wound healing in women of reproductive age, such as obesity, diabetes mellitus, or smoking, it is vital to search for new, efficient, and safe strategies of preventing obstetric SSI. Also, from the point of view of healthcare system economics, it is important to avoid additional costs, which would allow for a widespread application rather than in high-risk patients only. Therefore, the aim of the study was to evaluate the efficacy and cost-effectiveness of DACC impregnated dressings in the prevention of post-operative SSI among CS women.

Patients and Methods

Setting and study population

The single-blinded, randomized, controlled clinical study was conducted between June 2014 and April 2015 at the Mazovian Bródno Hospital, a tertiary referral center and a clinical hospital of the Medical University of Warsaw. Local Ethics Committee approved of the study (reference no. KB/127/2014 received on June 10, 2014) and written informed consent was obtained from all participants. The trial was registered with ClinicalTrials.gov (reference no. NCT02168023).

The inclusion criteria were: Patient age >18 y, emergency or elective CS, singleton or multiple pregnancy, mental and physical capacity to consent to participation in a clinical trial, CS performed by transverse skin incision followed by a transverse uterine incision in its lower segment, antibiotic prophylaxis administered zero to 30 min before the surgery, and wound irrigation with octenidine solution before subcutaneous tissue closure.

The patients were randomly assigned to two groups, depending on the applied dressing. Patients with DACC impregnated dressing (Sorbact Surgical Dressing®, ABIGO Medical AB, Sweden) constituted the study group, whereas women with standard surgical dressing (SSD) (Tegaderm + Pad®, 3M Health Care, St. Paul, MN) were recruited as control groups. Simple randomization with the 1:1 allocation ratio, conducted by an operating room (OR) nurse, was used to alternate the patients; even number: DACC dressing, and odd number: SSD. For masking purposes, all dressings were placed in white envelopes and sealed. The surgical team was blinded to the type of dressing until skin closure.

Data on patient demographics, peri- and post-operative course were collected from hospital medical records. Demographic parameters included: Age, race, pre-pregnancy weight, weight gain during pregnancy, height, pre-pregnancy body mass index (BMI), parity, gestational age; presence of diabetes mellitus (pre-gestational or gestational diabetes), hypertension (chronic hypertension or pregnancy-induced hypertension); smoking in pregnancy, history of previous CS, and presence of singleton/multiple pregnancy.

Peri- and post-operative parameters included: Type of dressing; mode of CS (emergency, elective); duration of the surgery; surgeon experience (resident, assistant specialist, consultant); type of anesthesia (spinal, general); presence of meconium stained amniotic fluid (MSAF); hemoglobin concentration 24 h before and 24 h after the surgery; receipt of blood transfusion, and length of post-operative hospital stay.

SSI-related parameters included: Presence of superficial or deep SSI during the first 14 d after the surgery, wound dehiscence; onset of the first symptoms of SSI; the need for systemic antibiotic therapy, hospital re-admission and/or re-operation; the number of ambulatory visits; length of additional hospitalization, and identification of the pathogen responsible for SSI.

The technique of a transverse skin incision (Pfannenstiel) followed by a transverse uterine incision in its lower segment was used in all women, as described previously [10]. For subcutaneous tissue and skin incision closure, single monofilament absorbable suture (Monosyn 2/0, B. Braun Melsungen AG, Germany) and subcuticular continuous monofilament non-absorbable suture (Prolene 2-0, Ethicon, Somerville, NJ), were used respectively. All patients received antibiotic prophylaxis (1g of cefazolin) administered zero to 30 min before the surgery according to Polish National Consultants of General Surgery and Clinical Microbiology recommendations and wound irrigation with octenidine solution (Octenisept®, Schülke & Mayr GmbH, Germany) proceeded subcutaneous tissue closure [21]. Octenidine is a cationic, surface active, topical antimicrobial agent with high bactericidal and fungicidal activity, and cytotoxicity similar to that of other common antiseptics such as chlorhexidine [22].

The study plan resembled the one described in the pilot study [10]. Briefly, the dressing was left in situ for the first 48 h post-operatively in all participants, unless there were reasons for replacement, e.g., wound hemorrhage or detachment of the dressing. After that time, the dressing was removed and the first clinical assessment of wound healing was performed. The patients were discharged home on post-operative day three, unless contraindicated, and recommended to revisit on day seven for skin suture removal and second wound evaluation. Third and final wound assessment was scheduled for post-operative day 14. Patients who failed to report for follow-up visits were excluded from the final analysis. Each wound assessment during patient hospitalization, the follow-up visits, or in case of patient's self-referral to an ambulatory center, was performed by one of two authors (PS, MB), blinded to the type of the dressing used.

Study outcome definitions

The symptoms of superficial or deep SSI were analyzed according to the U.S. Centers for Disease Control and Prevention (CDC) criteria [23]. Wound dehiscence was defined as separation of the skin, subcutaneous tissue, or fascia, resulting from infection. Time of primary hospitalization was defined as period from the day of surgery (day zero) to discharge from the hospital. Time of additional hospitalization

was defined as period from the first SSI symptoms to treatment completion and discharge from the hospital (in cases when SSI developed during primary hospitalization and was the main reason for prolonged stay in the hospital), or from day one of re-admission because of SSI until treatment completion and discharge from the hospital (in cases when primary hospitalization was finished). Emergency CS was defined as procedure performed within 30 min of the decision. Surgeon experience was determined on the bases of specialization in obstetrics and years of experience: Resident—physician in specialist training, assistant specialist—specialization in obstetrics for ≤5, consultant—specialization in obstetrics for >5 y.

Cost data analysis and definitions

In case of SSI occurrence, the following costs were analyzed: Systemic antibiotic therapy, ambulatory visits, additional hospitalization, and additional nursing care. The costs were calculated in Polish zloty (PLN) and then converted to Euro (EUR), based on the Polish National Bank exchange rate from June 1, 2015 (1 EUR = 4.1 PLN).

The cost of antibiotic therapy, defined as the cost of therapy from day one to the last day of SSI treatment, was calculated on the basis of antibiotic prices from the central hospital pharmacy and according to the manufacturer's specifications. The cost of ambulatory visit was calculated on the basis of classifying the patients into one of the Diagnosis Related Groups of Polish National Health Fund (DRG: W40), based on the diagnosis from the International Statistical Classification of Diseases and Related Health Problems v.10 (ICD-10-CM; o86.0—infection of obstetric surgical wound; o90.0—disruption of cesarean delivery wound), and the performed procedure, in accordance with the International Classification System for Surgical, Diagnostic and Therapeutic Procedures v.5.22 (ICD-9-CM; 86.28—non-excisional debridement of wound, infection or burn; 93.57—application of another wound dressing). Using the abovementioned data, the cost of a single ambulatory visit was estimated at 54 PLN (13 EUR). The costs of additional hospitalization in SSI patients who required prolonged primary hospitalization or readmission, together with the costs of additional nursing care obtained from the hospital financial office, amounted to 316 PLN (77 EUR) and 173 PLN (42 EUR) per day, respectively.

In order to determine the total costs of prophylaxis and treatment of SSI in both groups, the costs mentioned above were supplemented with the costs of dressings based on mean retail prices: DACC 11.5 PLN (2.8 EUR) and SSD 20 PLN (4.9 EUR).

Statistical analysis

Statistical analysis was performed using the R package v. 3.0.1 (The R Foundation for Statistical Computing, Vienna, Austria). Continuous variables were compared using the Mann-Whitney *U* test. For categorical variables, the χ^2 test or the Fisher exact test were applied. The p-value of <0.05 was considered as statistically significant.

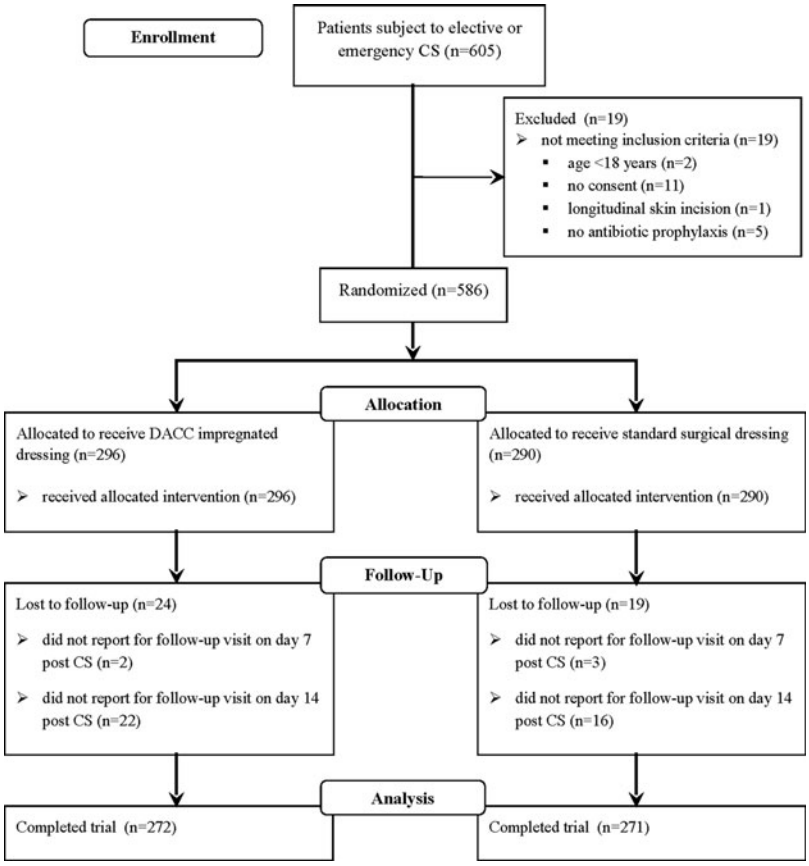

FIG. 1. CONSORT 2010 flow diagram of the recruitment process and randomization. CS, cesarean section; DACC, dialkylcarbamoyl chloride

Table 1. Characteristics of Patients Who Underwent Cesarean Section during the Study Period from June 2014 to April 2015

	Study group (n = *272*)	*Control group* (n = *271*)	*p*
Age (y)	31.2 ± 4.8 (18 – 43)	30.6 ± 4.8 (18 – 44)	.08
≤20	5 (1.8%)	4 (1.5%)	.63
21–30	114 (42.0%)	126 (46.5%)	
31–40	148 (54.4%)	134 (49.4%)	
>40	5 (1.8%)	7 (2.6%)	
Race			
Caucasian	269 (98.9%)	268 (98.9%)	>.999
Non-Caucasian	3 (1.1%)	3 (1.1%)	
Pre-pregnancy weight (kg)	65.8 ± 13.2 (40 –138)	66.4 ± 14.6 (39 –129)	.69
Weight gain during pregnancy (kg)	14.3 ± 6.0 (0 – 40)	14.4 ± 5.6 (0 – 38)	.64
Height (m)	1.66 ± 0.06 (1.48– 1.81)	1.65 ± 0.06 (1.4 – 1.84)	.55
Pre-pregnancy BMI (kg/m^2)	23.9 ± 4.5 (16.3 – 47.7)	24.2 ± 4.9 (14.5 – 43.6)	.57
BMI <25	193 (70.9%)	176 (64.9%)	.31
BMI ≥25 and <30	53 (19.5%)	62 (22.9%)	
BMI ≥30	26 (9.6%)	33 (12.2%)	
Parity			
Primiparous	131 (48.2%)	150 (55.4%)	.11
Multiparous	141 (51.8%)	121 (44.6%)	
Gestational age (wks)	38.1 ± 2.4 (24 – 41)	38 ± 2.5 (24 – 41)	.92
< 37 wks	32 (11.8%)	46 (17.0%)	.11
Diabetes mellitus	26 (9.6%)	35 (12.9%)	.28
PGDM	9 (3.3%)	8 (2.9%)	
GDM	17 (6.3%)	27 (10.0%)	
Hypertension	24 (8.8%)	34 (12.5%)	.08
Pre-pregnancy HTN	11 (4.0%)	8 (2.9%)	
PIH	13 (4.8%)	26 (9.6%)	
Smoking during pregnancy	20 (7.3%)	20 (7.4%)	>.999
Mode of CS			
Elective	214 (78.7%)	211 (77.9%)	.90
Emergency	58 (21.3%)	60 (22.1%)	
Previous CS	96 (35.3%)	87 (32.1%)	.49
Multiple pregnancy	5 (1.8%)	7 (2.6%)	.76
Duration of surgery (min.)	36.3 ± 9.0 (17 – 82)	36.7 ± 11.4 (17 –125)	.94
≤25	35 (12.9%)	34 (12.5%)	.86
25–50	218 (80.1%)	221 (81.5%)	
>50	19 (7.0%)	16 (6.0%)	
Surgeon experience			
Resident	106 (39.0%)	113 (41.7%)	.56
Assistant specialist	73 (26.8%)	77 (28.4%)	
Consultant	93 (34.2%)	81 (29.9%)	
Type of anesthesia			
Spinal	225 (82.7%)	221 (81.6%)	.81
General	47 (17.3%)	50 (18.4%)	
MSAF	20 (7.3%)	21 (7.7%)	.99
Pre-operative Hgb (g/dL)	12.2 ± 1.0 (8.3 – 14.9)	12.3 ± 1.1 (8.7 – 16.1)	.77
Post-operative Hgb (g/dL)	10.9 ± 1.0 (7.1 – 14.2)	11 ± 1.2 (6.5 – 14.6)	.39
Δ Hgb (g/dL)	1.3 ± 0.7 (0.1 – 3.5)	1.3 ± 0.9 (0.1 – 6.5)	.17
Blood transfusion	6 (2.2%)	4 (1.5%)	.75
Length of post-operative hospital stay (d)	4.3 ± 1.9 (3 – 14)	4.6 ± 2.1 (3 – 15)	.22

Data are expressed as mean ± SD/ (range) or as frequency (%).
BMI = body mass index; PGDM = pre-gestational diabetes mellitus; GDM = gestational diabetes mellitus; HTN = hypertension; PIH = pregnancy induced hypertension; CS = cesarean section; MSAF = meconium stained amniotic fluid; Hgb = hemoglobin concentration; Δ Hgb = change in hemoglobin concentration.

In order to identify the factors responsible for post-operative SSI in women after CS, univariate logistic regression, followed by multivariable logistic regression with backward selection based on the Akaike Information Criterion, were performed.

On the basis of the pilot study results, power analysis indicated that a sample size of 248 for each of the two groups was required to detect a difference in SSI proportion, with a power of 90% and $\alpha = 0.05$.

Results

During the study period, between June 2014 and April 2015, there were 1144 deliveries, including 605 cesarean

Table 2. Primary and Secondary Study Outcomes

	Study group (n = 272)	*Control group* (n = 271)	*p*
No. of patients with SSI (%)	5 (1.8)	14 (5.2)	.04
No. of patients with SSI and wound dehiscence (%)	1 (0.4)	2 (0.7)	>.99
No. of patients with SSI who required systemic antibiotic treatment (%)	0	4 (1.5)	.13
No. of patients with SSI who required hospital readmission (%)	0	3 (1.1)	.24
No. of patients with SSI who required surgical intervention (%)	0	0	-
	Study group (n = 5)	*Control group* (n = 14)	
Time of SSI occurrence (d)	7.4 ± 1.14 (6-9)	9.1 ± 3.6 (3–14)	.26
No. of ambulatory visits	4.6 ± 1.67 (2-6)	2.9 ± 1.1 (1– 4)	.02
Length of additional hospitalization (d)	0	8.2 ± 3.2 (5–11)	.22

Data are expressed as mean ± SD/ (range) or as frequency (%)
SSI = surgical site infection.

sections (52.9%), at the Department of Obstetrics, Gynecology and Oncology (Fig. 1). Among the women undergoing CS, 19 failed to meet the inclusion criteria: Two were <18 y of age, 11 were not in the capacity to or failed to consent to participation in the study, one patient had CS performed by a longitudinal skin incision, and five patients did not receive antibiotic prophylaxis. Out of the 586 patients who were deemed eligible for the study and who were randomly assigned into either the DACC group (study group, n = 296) or the SSD group (control group, n = 290), 43 (7.3%) failed to report for follow-up visits and were excluded from further analysis. In the final stage, the study and control groups consisted of 272 and 271 patients, respectively.

Table 3. Microorganisms Isolated from Surgical Site Infections during the Study Period from June 2014 to April 2015

Microorganisms	*Study group* No. (%)	*Control group* No. (%)
Enterobacteriaceae	1 (9.1)	9 (56.25)
Klebsiella pneumoniae	0	3
Proteus mirabilis	0	1
Enterobacter cloacae	0	2
Escherichia coli	1	3
Coagulase-positive Staphylococci	2 (18.2)	1 (6.25)
MSSA	2	1
Coagulase-negative Staphylococci	1 (9.1)	1 (6.25)
MSSE	1	0
Staphylococcus hominis	0	1
Anaerobes	2 (18.2)	1 (6.25)
Bacteroides fragilis	1	0
Prevotella bivia	0	1
Peptoniphilus asaccharolyticus	1	0
Enterococcaceae	2 (18.2)	1 (6.25)
Enterococcus faecalis	2	1
Streptococcus sp.	2 (18.2)	0 (0)
Other	1 (9.1)	3 (18.75)
Total	11 (100)	16 (100)

MSSA = methicillin susceptible *Staphylococcus aureus*; MSSE, methicillin susceptible *Staphylococcus epidermidis*

Patient characteristics are presented in Table 1. There were no substantial differences between the DACC and the SSD groups with regard to patient demographics and perioperative course. Surgical site infections were observed substantially more often in the SSD group (Table 2). Incisional SSIs occurred during the first 14 post-operative days in 5.2% of patients from the control group as compared with 1.8% of women from the study group ($p = 0.04$). No statistically significant differences were found as far as the presence of post-operative wound dehiscence, receipt of systemic antibiotic therapy, or re-admission rates were concerned. Regardless of the fact that women who received the DACC dressing did not require systemic antibiotic therapy and additional hospitalization, the number of ambulatory visits was substantially higher in the study group as compared with the control groups, 4.6 vs. 2.9, respectively ($p = 0.02$) (Table 2). Mean time of additional hospitalization in the SSD group was 8.2 d. In both groups there were no cases of SSIs in patients with diabetes mellitus, both pre-existing before pregnancy and gestational, and with chronic arterial hypertension. All study participants were HIV-negative.

Enterobacteriaceae, coagulase-positive and negative *staphylococci*, anaerobes, *Enterococcaceae*, and *Streptococcus* sp. were the pathogens responsible for most SSI cases in both groups (Table 3). Microbiological analysis revealed strains of *Enterobacteriaceae* as the dominant group of pathogens isolated in patients from the SSD group, accounting for more than half of the identified microorganisms (56.25%). A similar correspondence was not observed in the DACC group, where *Enterobacteriaceae* constituted 9.1% of the isolated strains, with no dominant group of pathogens.

The univariate analysis revealed pre-pregnancy BMI of $\geq 30\,kg/m^2$ (odds ratio [OR] = 4.5; [95% CI: 1.3–14.8]; $p = 0.009$), pregnancy induced hypertension (OR = 5.1; [95% CI: 1.4–16.2]; $p = 0.008$), and smoking in pregnancy (OR = 5.0; [95% CI: 1.3–15.7]; $p = 0.009$) to be the factors that substantially increase the risk for SSI, and

Table 4. Univariate Analysis of Risk Factors for Surgical Site Infection in Females after Cesarean Section

	No. (%) of patients			
	SSI (n = *19*)	*No SSI* (n = *524*)	*OR (95% CI)*	*p*
Age (y)				
≤30	10 (52.6)	239 (45.6)	1.0	-
31–40	8 (42.1)	274 (52.3)	0.7 (0.23– 2.0)	.48
>40	1 (5.3)	11 (2.1)	2.2 (0.4 –17.9)	.41
Race				
Caucasian	18 (94.7)	519 (99.0)	1.0	-
Non-Caucasian	1 (5.3)	5 (1.0)	5.7 (0.1 –55.2)	.19
Weight gain during pregnancy (kg)				
≤10	7 (37.0)	132 (25.0)	1.0	-
>10	12 (63.0)	392 (75.0)	0.6 (0.2 – 1.8)	.28
Pre-pregnancy BMI (kg/m^2)				
BMI <25	9 (47.4)	360 (68.7)	1.0	-
BMI ≥25 and <30	4 (21.0)	111 (21.2)	0.99 (0.23– 3.2)	>.999
BMI ≥30	6 (31.6)	53 (10.1)	4.5 (1.3 –14.8)	.009
Parity				
Primiparous	14 (73.7)	267 (51.0)	2.7 (0.9 – 9.7)	.06
Gestational age				
< 37 wks	5 (26.3)	73 (13.9)	2.2 (0.6 – 6.7)	.17
Hypertension				
PIH	5 (26.3)	34 (6.5)	5.1 (1.4 –16.2)	.008
Smoking during pregnancy	5 (26.3)	35 (6.7)	5.0 (1.3 –15.7)	.009
Mode of CS				
Elective	13 (68.4)	412 (78.6)	1.0	-
Emergency	6 (31.6)	112 (21.4)	1.7 (0.5 – 4.9)	.27
Previous CS	3 (15.6)	180 (34.4)	0.4 (0.7 – 1.28)	.14
Multiple pregnancy	2 (10.5)	10 (1.9)	6.0 (0.6 –31.6)	.06
Duration of surgery (min.)				
≤25	4 (21.0)	65 (12.4)	1.0	-
>25	15 (79.0)	459 (87.6)	0.53 (0.16– 2.27)	.28
Surgeon experience				
Resident	8 (42.1)	211 (40.3)	1.0	-
Assistant specialist	5 (26.3)	145 (27.7)	0.9 (0.2 – 3.2)	>.999
Consultant	6 (31.6)	168 (32.0)	0.9 (0.3 – 3.2)	>.999
Type of anesthesia				
Spinal	14 (73.7)	432 (82.4)	1.0	-
General	5 (26.3)	92 (17.6)	1.7 (0.5 – 5.1)	.36
MSAF	3 (15.8)	38 (7.2)	2.4 (0.4 – 8.9)	.17
Pre-operative Hgb				
≤12 g/dL	6 (31.6)	191 (36.4)	0.8 (0.2 – 2.3)	.81
Post-operative Hgb				
≤10 g/dL	3 (15.8)	97 (18.5)	0.8 (0.15– 3.0)	>.999
Δ Hgb				
≥3g/dL	1 (5.3)	9 (1.7)	3.2 (0.07–25.1)	.30
Length of post-operative hospital stay (d)				
≤5	13 (68.4)	393 (75.0)	1.0	-
6–10	5 (26.3)	123 (23.5)	1.2 (0.3 – 3.8)	.78
>10	1 (5.3)	8 (1.5)	3.7 (0.08–31.9)	.27
Dressing type				
SSD	14 (73.7)	257 (49.1)	1.0	-
DACC	5 (26.3)	267 (50.9)	0.3 (0.09– 1.03)	.04

SSI = surgical site infection; BMI = body mass index; PIH = pregnancy induced hypertension; CS = cesarean section; MSAF = meconium stained amniotic fluid; Hgb = hemoglobin concentration; Δ Hgb = change in hemoglobin concentration; SSD = standard surgical dressing; DACC = dialkylcarbamoyl chloride-impregnated dressing; CI = confidence interval; OR = odds ratio.

application of the DACC impregnated dressing as the factor that lowers the risk (OR =0.3; [95% CI: 0.09–1.03]; p = 0.04) (Table 4).

In order to identify independent risk factors for SSI, a separate multivariable logistic regression with backward selection was performed. The following parameters were found to influence the risk for SSI: Pre-pregnancy BMI (aOR = 1.08; [95% CI: 1.0–1.2]; $p < 0.05$), smoking in pregnancy (aOR = 5.34; [95% CI: 1.6–15.4]; $p < 0.01$) and SSD application (aOR = 2.94; [95% CI: 1.1–9.3]; $p < 0.05$).

Total estimated cost of SSI prophylaxis and treatment was greater in the control group as compared with the study

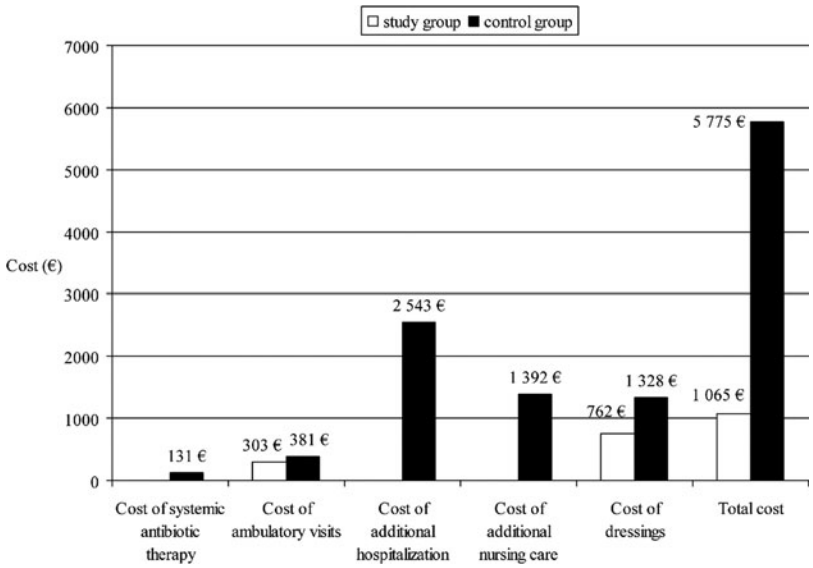

FIG. 2. Cost of treatment attributable to surgical site infection after cesarean section by dressing type.

group, and amounted to 5775 EUR vs. 1065 EUR, respectively (Fig. 2). In the study group it comprised only the cost of ambulatory visits, whereas in the control group total cost encompassed additional expenses because of prolonged hospitalization and additional nursing care. Similarly, systemic antibiotic treatment with metronidazole, cefuroxime, ceftriaxone, amoxicillin, ciprofloxacin, or gentamicin used alone or in combination was necessary only in patients in the control group.

Discussion

The presented study was a single-center, randomized controlled trial, aiming to evaluate the efficacy of DACC impregnated dressings to prevent SSI in women after CS. To the best of our knowledge, it has been the first prospective study on the use of DACC dressings in a large cohort of pregnant women.

Our results confirmed effectiveness of the DACC dressings in SSI prevention after CS. Application of the hydrophobic dressing resulted in a decreased rate of SSI and its considerably milder course, with no need for systemic antibiotic therapy and hospital readmissions. As a consequence, the total cost of SSI treatment was lower in the DACC group and was a result of ambulatory visits only. Despite the fact that the total number of the ambulatory visits was substantially higher in the study group, the dominant element in the total treatment cost was the length of additional hospitalization, with mean duration of 8 d in the group with SSD.

Multivariable logistic regression analysis revealed obesity, smoking, and the use of a standard occlusive dressing as the three independent factors that increase the risk for incisional SSI after CS. The adverse effects of the first two factors have been well-documented in the literature [2,3,5,7,8]. In case of obesity, excessive thickness of subcutaneous tissue is believed to cause tissue hypoperfusion and hypoxygenation, impeding the healing process and antibiotic penetration [24]. The risk of SSI is often additionally increased by the presence of hyperglycemia, prolonged surgery time because of technical difficulties, the need for a longer skin incision, and more blood loss. Also, proper wound hygiene and care may be hampered by the location of the incision between skin folds, what may predispose to the development of infection. As far as smoking is concerned, the components of tobacco smoke cause tissue hypoxia, impair the function of inflammatory cells, and limit fibroblast proliferation and migration, thus delaying wound healing [25–27].

The type of dressing used in prevention of SSI after obstetric operations is of the utmost importance from the point of view of the study goals. Similarly to subcutaneous drains or surgical staples used for skin closure, the type of the applied dressing may affect the risk of SSI [2,5]. Obtained results revealed an almost three-fold increase of SSI risk in patients who received the SSD.

Microorganisms responsible for SSI were similar in both groups, with the exception of more numerous *Enterobacteriacae* strains found among the control groups, which may be explained by the fact that approximately 25% of *Enterobacter* spp. strains isolated from surgical incisions are characterized by high CSH, whereas hydrophobic properties are found in 88% of the *Enterobacter cloacae* strains alone [28]. In case of *Klebsiella pneumoniae*, CSH is affected by the presence of O-antigen lipopolysaccharide or polysaccharide capsule, making bacteria more hydrophilic and, as a result, less susceptible to adhesion to the hydrophobic surface of the dressing [29]. Neither the abovementioned properties of bacterial strains nor the effect of the remaining factors on the CSH of the isolated pathogens were the subject of the investigation. It has been proven that the use of octenidine solution, just as bacterial culture in carbon dioxide atmosphere in the presence of serum, resembling wound conditions under an occlusive dressing, increase CSH, contrary to antibiotics used in pre-operative prophylaxis [11,16,30].

Our study is subject to several limitations, including the fact that the effectiveness of the impregnated dressings was analyzed in a group of women undergoing CS, which, unlike most surgical patients, constitute young population with few comorbidities. Also, the observed SSI incidence after CS

most probably does not reflect the total SSI rate because of the fact that analysis included only superficial and deep SSI, as well as shorter than recommended by the CDC period of observation. Exclusion of organ/space SSI from the analysis was imposed by the fact that the effect of the dressing on the incidence of such infections after CS is limited and the shortened time of the observation, from the recommended 30 d to 14 d, was conditioned by lack of the possibility of effective medical supervision and low patient compliance after that time, as described by Wilson et al. [4]. At the same time, the literature reports indicate that superficial and deep SSIs account for 93%–100% of all cases of SSI following CS, with 78%–100% occurring within 14 d of the surgery [2–4,6,7]. As the subject of the study included only superficial and deep SSI such risk factors as number of vaginal examinations, duration of labor or pre-term rupture of membranes were not included in the analysis, taking into account their correlation with organ/space infections.

To conclude, the use of a DACC-coated dressing decreased the SSI rates among patients after CS and proved its cost-efficacy. Weight reduction before conception, abstaining from smoking in pregnancy, and application of dressings that are effective in SSI prophylaxis, are the key factors which might prevent SSIs after CS.

Acknowledgments

Statistical analyses were done by BioStat (Poland). Language translator Izabella Mrugalska provided assistance in preparing and editing the manuscript.

Author Disclosure Statement

All authors report no conflicts of interest relevant to this article.

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Adhesion of meticillin-resistant *Staphylococcus aureus* to DACC-coated dressings

• **Objective:** The aim of this *in vitro* study was to demonstrate the binding capacity of multiple meticillin-resistant *Staphylococcus aureus* (MRSA) strains and compare the binding capacity to meticillin-sensitive *Staphylococcus aureus*.

• **Method:** The binding of *Staphylococcus aureus* to a surface was assessed by bioluminescent monitoring of the bacterial ATP levels. This assay can be used as an *in vitro* diagnostic model for bacteria binding in a critically colonised wound.

• **Results:** Eleven strains of *Staphylococcus aureus* were examined including MRSA, all of which efficiently and equally adhered to the dialkyl carbamoyl chloride (DACC)-coated dressing (Sorbact; Abigo Medical AB). The binding capacity was all in the same range 0.7–2.9x10^6 CFU/cm^2. regardless of the antibiotic resistance properties of the specific strain.

• **Conclusion:** The decrease of wound bioburden of *Staphylococcus aureus* including MRSA is the result of the high binding capacity shown in this study and by earlier data. The findings in this study strengthen the held view that development of antibiotic resistance has minimal impact on the surface structures of the microorganisms in wounds.

• **Declaration of interest:** This work was supported by Abigo Medical AB, Sweden. The laboratory facilities for the MRSA work were provided by Sahlgrenska University Hospital and the Department for Clinical Bacteriology, University of Gothenburg, Sweden. No animals or humans were used in this study..

wound healing; adhesion; bacteria binding; *in vitro*; MRSA

Meticillin-sensitive *Staphylococcus aureus* and meticillin-resistant *Staphylococcus aureus* (MRSA) cause similar infections, ranging from minor infections of the skin to major wound infections.[1] Individual immunity, size, localisation and the amount, and the virulence of the microorganisms present determine whether a wound remains harmlessly colonised or leads to infection.[2] MRSA is especially troublesome in hospitals, prisons and nursing homes, where patients with open wounds, invasive devices, and weakened immune systems are at greater risk of infection than the general public. Almost half of infections in burn patients at health facilities are caused by *Staphylococcus aureus*.[3-6]

An *in vitro* study conducted over 20 years ago enabled measurement of bacterial cell surface hydrophobicity (CSH) to assess the specific surface characteristics of microorganisms and their respective contribution to binding.[7] This research group, led by Wadstrom and Ljungh, demonstrated the presence of cell surface hydrophobins influencing the ability of bacteria to colonise and bind to target. A wound dressing was designed where the microorganisms bound preferentially to the dressing rather than interacting with the wound.[8,9]

The dressing used as a surface for adhesion in this study consists of cellulose acetate-based fabric coated with dialkyl carbamoyl chloride (DACC). As a result of the coating, the dressing is afforded microbial binding properties. Microbial binding is achieved by non-specific factors such as physical hydrophobic interaction and specific adhesin factors including fibrinogen-binding protein.[10,11] Binding is a complex process that involves the surface properties of microorganisms, wound and dressing. The initial process is normally non-specific, involving physical forces as hydrophobic interaction, electrostatic interaction, van der Waals forces, gravitational or Brownian motion.[11] The secondary process involves chemical interaction, where specific adhesion becomes predominant and involves specific molecular reactions between the bacterial surface structure and substratum surfaces (DACC-dressing). The benefit of a dressing of this type is to enhance control of the wound bioburden, reducing the overall demand placed on antibiotics, without using antimicrobial substances.

The aim of this *in vitro* study was to demonstrate comparable binding of clinical *Staphylococcus aureus* strains relative to multiple MRSA strains using a DACC-coated dressing as adhesion material. The study focuses on the adhesion as a virulence factor, which should not be confused with encapsulation, absorption, or killing of microorganisms.

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Table 1. Characteristics of *Staphylococcus aureus* organisms

Target organism CCUG number	MRSA	Spa type, PFGE* type	comment
1. 2354	-	n.a	NCTC 7428, pure β toxin
2. 35603	+	n.a	QC MRSA, 1992, wound
3. 43063	+	t2363,F	1999, wound on blisters
4. 44509	+	SP	2000, wound from groin
5. 45435	+	t012, SM	2001, wound on the mouth
6. 46315	+	t044, P	2002, wound on the knee
7. 47418	+	t067, J	2003, wound on the toe
8. 53315	+	t008, K	2006, wound on the thigh
9. 56450	-	n.a	2008, wound on the elbow
10. 58494	+	t018, SP	2009, wound from heel
11. 60578	+	t044, P	2010, unspecified wound secretion

*Pulsed-field gel electrophoresis: n.a: Neither Spa type nor PFGE type available, QC=quality control

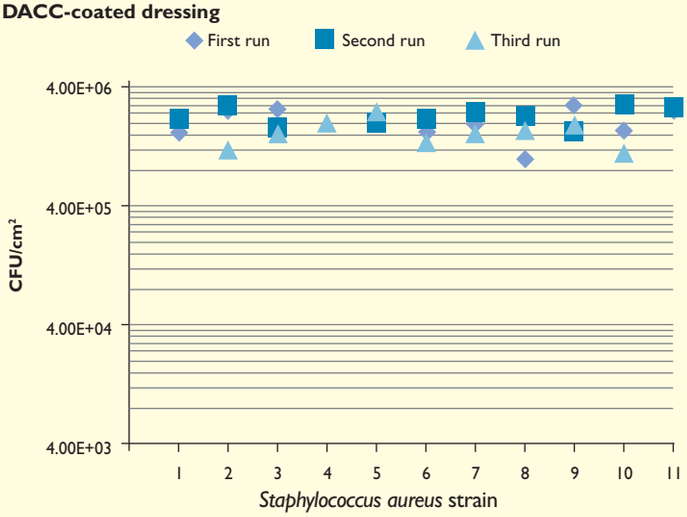
Method

Materials

The dressings used in the study consisted of a cellulose acetate-based fabric coated with DACC (Sorbact Compress, Abigo Medical AB). The same uncoated cellulose-acetate fabric was used as a control. Both the uncoated and coated dressings were sterile.

Eleven isolates were used in this study. The MRSA strains were isolated from different inpatient and outpatient wounds (Table 1) and all the strains were tested for presence or absence of Nuc-gen and MecA-gen using real-time PCR. The isolate from culture collection at University of Gothenburg, CCUG 35603, was used as control for MRSA. The strains CCUG 2354 and CCUG 56450 were used as isolates for meticillin–sensitive strains. The bacterial strains were chosen were isolated from different wounds, and had different pulsed-field gel electrophoresis (PFGE) and Spa type. The target organisms, origin are presented in table 1. All the MRSA isolates in this study are clinical and have caused infection in patients who sought treatment.

Fig 1. Adhesion of Staphylococcus aureus including MRSA to DACC-coated dressing



Storage and preparation of bacteria

Cell material from each strain was stored in Hogness freezing medium at -80°C. The bacterium were grown on 5% horse-blood agar plates for 48 hours before harvesting. Bacteria were further grown on horse-blood agar plates for an additional 18 hours to be used for adhesion experiments.

Bacterial cells were washed and centrifuged for 15 minutes (2500g, 20°C). The supernatant was discarded and the cells were re-suspended (phosphate buffer, 0.02M, pH 6.8). The turbidity of the suspension was measured with a spectrophotometer at λ=610nm and the concentration of bacterial suspensions were subsequently adjusted to $1x10^8$ CFU/ml in each of the eleven cell suspensions. The exact concentration was obtained through plating results the day after the luminometer run. The cells were grown on blood agar plates after serial dilutions and incubated at 37°C overnight. The viable number of bacteria (CFU/ml) was determined by colony counts on plates consisting of 30–300 colony-forming units.

Firefly assay of adenosine 5'-triphosphate (ATP) for adhesion

To study the binding of bacteria to a dressing surface, an inoculum of each strain was added to the wound dressing. The bioluminescence technique was used to quantify the bacterial adenosine 5'-triphosphate (ATP) levels against a reference standard curve. The enzyme in the ATP kit (Sigma-Aldrich, Sweden) reacts with the ATP in live bacteria, resulting in light emission.

Fresh bacterial suspensions were prepared and kept on ice for no longer than one hour before the assay was performed. Punched samples of fabric with a $1cm^2$ single layer of the coated dressing were incubated with 250µl of $1x10^8$ CFU/ml bacterial suspension at 20°C for one hour, while shaking at 100rpm. After adhesion, the dressing was rinsed three times with 0.02M phosphate buffered saline (pH6.8) to remove loosely adhered bacterial cells. The numbers of bacterial cells adhering to the dressing were determined by a standardised luminescence technique for ATP detection.[12] The kit (Sigma Aldrich, Sweeden) contained a lucifrase enzyme which is dissolved in 5ml of sterile water to generate a stock solution. From the stock solution, 200µl was added to untreated, flat-welled, white ATP-free 96-well polystyrene microtiter plates (Costar 3912, Corning, Sweden). The light emitted from bacterial

ATP was measured at 20°C with a luminometer (Lumistar Omega, LabVision, Sweden). The ATP reagents and samples were kept on ice during preparation. Sterile buffer was used as negative control. Unless stated each of the bioluminescence assays was run with two different samples of the strain of bacteria on three separate occasions.

Statistics

Comparison of CFU/cm² was performed using Fisher's exact test (GraphPad Prism; GraphPad Software, La Jolla, CA). A two–tailed p-value of 0.05 was defined as statistically significant.

Results

Adhesion to the DACC-coated dressing of the *Staphylococcus aureus* control strain CCUG 2354, clinical strain CCUG 56450 and nine MRSA strains in colony-forming units per dressing sample (CFU/cm²) are presented in table 2 and fig 1. There were two samples of each of the 11 bacterial strains in each run. The data in table 2 shows both sample values separated by a semicolon. The initial binding capacity demonstrated for *Staphylococcus aureus* including MRSA in the experiments was 0.7–2.9x10⁶ CFU/cm². These experiments were repeated three times as shown in table 2.

A comparison of the adhesion capacity between DACC-coated dressing material and uncoated control with the *Staphylococcus aureus* control strain CCUG 2354 with an initial concentration of 8x10⁷ CFU/ml was performed. Three parallel samples, were used for coated and uncoated control (Fig 2). The DACC-coated material bound a mean value of 1.5x10⁶ CFU/cm², whereas uncoated binding was reduced to a mean value of 6.8x10⁴ CFU/cm², which was significantly lower ($p<0.0001$; Fisher's exact test).

To illustrate the mode of action, binding of a mixed culture containing *Staphylococcus aureus* control strain (CCUG 2354), *Pseudomonas aeruginosa* (CCUG 17919) and *Candida albicans* (CCUG 32723) to the DACC-coated dressing was examined at high resolution (Fig 3). The figure shows not only binding to the material, but also aggregation between microbes. The electromicrograph was produced externally (TATAA Biocenter AB, Gothenburg, Sweden).

Discussion

The results of this study are intended to promote clinical confirmation of reducing the MRSA impact in the wound care setting. Due to the complications associated with antibiotics, there is a need for non-antibiotic management strategies as an alternative or combined therapy when absolutely necessary.

The *Staphylococcus aureus* isolates used in our adhesion experiments were collected from different wound environments and analysed by Spa typing and PFGE. The expression characteristics of the strains used could indicate different surface properties, resulting in the possibility of different adhesion capacity. Furthermore it is likely that the meticillin-sensitive *Staphylococcus aureus* and MRSA strains have different adhesins on their cell walls. All of these features, together with morphological structures, can be involved in facilitating adhesion to the DACC-coated dressing. The bacteria can also bind to the target through various receptors, to further increase the binding to the dressing material. However, recent studies of the anti-infectious agents against MRSA imply the cell surface structures con-

Table 2. Adhesion of *Staphylococcus aureus* including MRSA to DACC-coated dressing

Target organism CCUG no	Run 1	Run 2	Run 3
	x 10⁶ CFU/cm²	x 10⁶ CFU/cm²	x 10⁶ CFU/cm²
1. 2354	2.1; 2.2	2.0; 2.4	1.5; 1.8
2. 35603	1.1; 1.2	2. 8; 2.9	2. 5; 2.7
3. 43063	1.6; 1.7	1.8; 1.9	2.3; 2.8
4. 44509	1.9; 2.1	n.a	n.a
5. 45435	2.4; 2.6	1.9; 2.2	2.3; 2.4
6. 46315	1.3; 1.5	1.9; 2.5	1:7; 1.7
7. 47418	1.6; 1.7	2.2; 2.8	1.5; 2.4
8. 53315	1.7; 1.8	2.1; 2.5	0.7; 1.2
9. 56450	1.9; 2.0	1.6; 1.9	2.8; 2.9
10. 58494	1.1; 1.2	2.7; 3.2	1.7; 1.8
11. 60578	n.a	2.7; 2.8	2.6; 2.7

n.a: not available

Fig 2. Adhesion of Staphylococcus aureus to DACC-coated dressing material (n=3) and uncoated control (n=3).

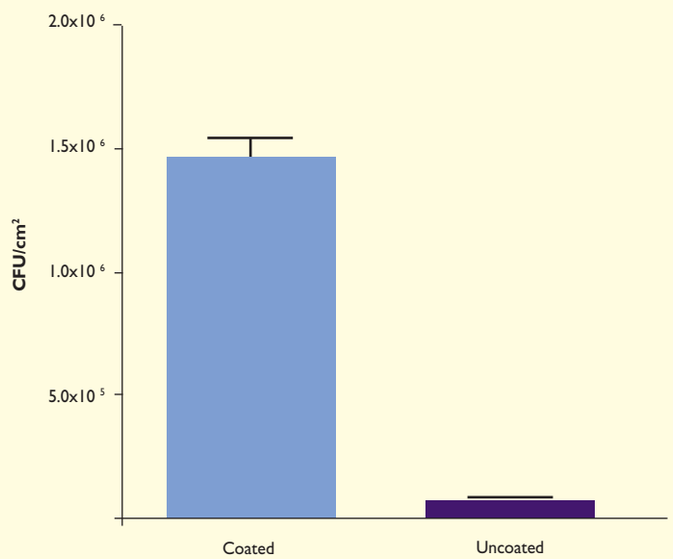
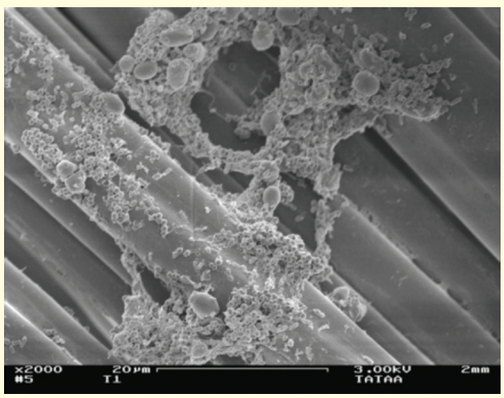


Fig 3. Adhesion of microorganisms including Staphylococcus aureus to DACC dressing



sisting of hydrophobins for both meticillin-sensitive *Staphylococcus aureus* and MRSA strains are similar.[13-15] Our binding experiments showed that the DACC-coated dressing material bound all the strains with a similar affinity, indicating that the physiochemical forces dominate over these specific chemical-binding interactions.

The binding capacity of the wound pathogens to the DACC-coated dressing demonstrated here is in line with other *in vitro* data obtained by Ljungh and coworkers.[9] We also confirm the results of the recent data showing that the microbial binding capacity of the uncoated material compared with that of the DACC-coated dressing and was significantly lower.[16] Although these studies are not evidence of a clinical effect, we anticipate the results demonstrated may improve the understanding of the clinical research and management paradigms for local wound care.

Further research in this area could include assessing the CSH of the different MRSA strains, as well as comparing a control dressing with a bacterial binding dressing on wounds to quantify the efficacy in a clinical setting. In the future, it may be possible to develop wound dressing material targeting a specific wound pathogen, where chemical binding will be the dominant force.

Conclusions

A quantitatively high and stable initial adhesion to the microbial binding dressing was detected in all experiments involving *Staphylococcus aureus* strains including the nine MRSA strains. These findings strengthen the view that development of antibiotic resistance has minimal impact on the surface structures of the microorganism from a wound adhesion perspective. In this case, the surface structures of non-antibiotic resistant *Staphylococcus aureus* are present in similar composition, as on the MRSA strains. ■

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Binding of two bacterial biofilms to dialkyl carbamoyl chloride (DACC)-coated dressings *in vitro*

• **Objective:** To date only planktonic bacteria have been shown to bind irreversibly to dialkyl carbamoyl chloride (DACC)-coated Cutimed Sorbact dressings. Therefore, this study was designed to determine whether bacterial biofilm bound to the DACC-coated dressing *in vitro*.

• **Method:** Samples of DACC-coated dressings and uncoated control dressings (supplied by BSN medical Ltd, Hull) were placed in contact with plastic coverslips on which biofilms of either *Pseudomonas aeruginosa* or methicillin-resistant *Staphylococcus aureus* (MRSA) had been cultivated for 24 hours. Dressing samples were examined by scanning electron microscopy to detect the presence of biofilm.

• **Results:** *Pseudomonas aeruginosa* biofilm bound avidly to both DACC-coated and uncoated dressing samples. MRSA bound more extensively to DACC-coated dressings than to uncoated samples.

• **Conclusion:** Biofilms of two different test bacteria bound to dressings *in vitro* with the DACC-coating on the dressings enhancing the binding of MRSA biofilm.

• **Declaration of interest:** This study was supported by BSN medical Ltd (Hull). The company had no influence on the experimental design or the interpretation of the results.

DACC; Cutimed Sorbact; biofilm; irreversible binding; MRSA; *Pseudomonas aeruginosa*

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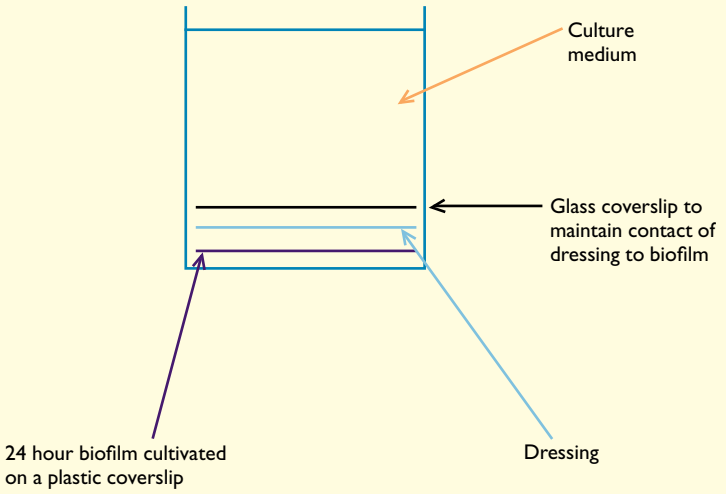
The need to reduce wound bioburden has long been recognised.[1] However, using the ability of microbial species to bind to wound dressings is a relatively recent approach to wound management that provides an antimicrobial effect without the use of an active inhibitory agent or the risk of cytotoxicity to host tissues. Bacteria exist largely in hydrophilic environments where they require water molecules for survival. Their surface layers contain both hydrophilic (water loving) and hydrophobic (water repellent) components which facilitate interaction with either hydrophilic or hydrophobic molecules, respectively. Bacterial cell surfaces contribute to hydrophobic interactions with host cells and inanimate surfaces that are important in the initiation of infections and biofilm formation.[2]

In 2006, the influence of cultural conditions on cell-surface hydrophobicity (CSH) of five planktonic bacteria (*Staphylococcus aureus*, *Staphylococcus haemolyticus*, *Escherichia coli*, *Enterobacter cloacae* and *Pseudomonas aeruginosa*) were investigated, and the binding capacity to a dressing coated with a hydrophobic fatty acid derivative called dialkyl carbamoyl chloride (DACC) was determined using *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Enterococcus faecalis*, *Bacteroides fragilis* and *Fusobacterium nucleatum*.[3] For *Pseudomonas aeruginosa,* maximum binding was observed at two hours and remained stable for 20 hours, showing that bacteria bound to the dressing did not multiply.[3] A recent investigation into the CSH of *Mycobacterium ulcerans* found it to be higher than that of *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.[4] Additionally, planktonic cultures of *Mycobacterium ulcerans* were found to bind more effectively to DACC-coated dressings than to untreated control dressings *in vitro*, allowing the authors to suggest a possible role for the coated dressing in reducing the bacterial load of Buruli ulcers.[4] Planktonic cultures of two strains of methicillin-sensitive *Staphylococcus aureus* (MSSA) and nine clinical strains of methicillin-resistant *Staphylococcus aureus* MRSA displayed equal binding capacity to DACC-coated dressings.[5] Binding of a range of wound colonising bacterial species to DACC-coated dressing has, therefore, been demonstrated in the laboratory using planktonic cultures.

Hydrophobic interaction was the rationale for a clinical study in which DACC-coated dressings were used to investigate reductions in wound bioburden.[6] In this study, quantification of bacterial burden in 20 chronic wounds treated with the DACC-coated dressings showed that of the 15 wounds with a positive clinical outcome, a significant decrease in bacterial load was found in 10 but that it was unchanged in 5. The remaining 5 patients with a negative clinical response showed a non-significant decrease in bacterial load.[6]

Since the demonstration of an association between wound chronicity and the presence of biofilm,[7,8] the need to reduce wound bioburden

Fig 1. Diagrammatic view of a test well within the microtitre plate



including biofilm has been recognised. Binding of biofilm to DACC-coated Cutimed Sorbact dressings has not yet been demonstrated, so this study was designed to investigate whether biofilms of two common wound pathogens bind *in vitro* and if the DACC-coating on these dressings promoted increased binding.

Method

Test organisms and dressings used

Pseudomonas aeruginosa and MRSA were used throughout this study. These had been isolated from different out-patients attending a local wound care clinic and stored at –80°C until required.

BSN medical Ltd provided samples of sterile Cutimed Sorbact dressings with a DACC-coating (72164-01; batch number 807093) and sterile Cutimed Sorbact dressings manufactured without a DACC-coating (72164-01 batch number 72632). Dressing samples were cut under aseptic conditions into circles with a 15mm diameter for testing.

Cultivation of 24 hour established biofilms

A starter culture of each test organism was cultivated in 10ml tryptone soya broth (TSB; Oxoid, Cambridge, UK) overnight at 37°C. Immediately before use, each starter culture was diluted in sterile TSB (1/100 dilution for *Pseudomonas aeruginosa* and 1/500 dilution for MRSA) and 2ml dispensed into wells of a 24-well microtitre plate (Nunc, Roskilde, Denmark) that contained a sterile Thermanox plastic coverslip (Agar Scientific, Stansted, UK). In each plate, three wells contained only 2ml TSB and coverslip to act as a negative control to test for sterility and non-specific binding, and three wells contained only 2ml diluted inoculum as a positive control for untreated biofilm. All plates were incubated at 37°C for 24 hours to allow biofilm to establish on the coverslips.

Fig 2. These images are examples of those the used to assess the extent of biofilm coverage of dressing samples (see Table 1). No biofilm present (a), 1–30% of dressing covered by biofilm (b), 30–60% of dressing covered by biofilm (c), 61–90% dressing covered by biofilm (d), 91–100% dressing covered by biofilm (e)

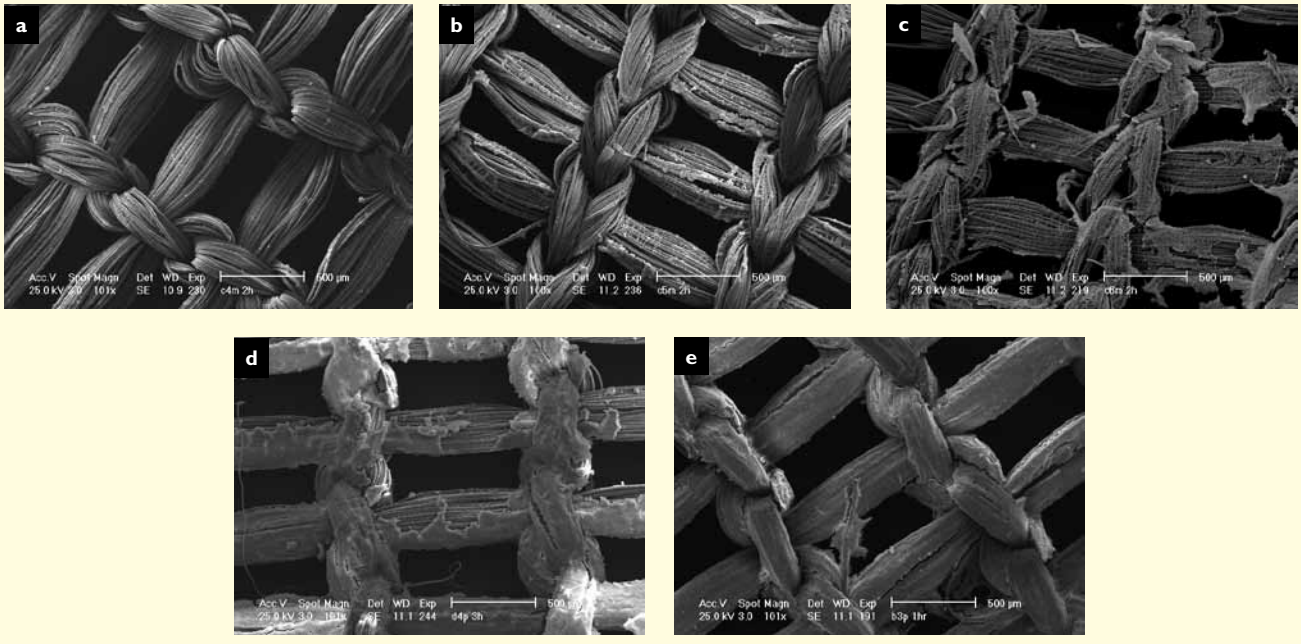


Table 1. Scoring system used by trained volunteers to evaluate the extent of coverage of biofilm associated with dressing samples

Score	Biofilm covering of the wound dressing	Example image
0	No biofilm visible	Fig 2a
1	Limited coverage and most of the dressing fibres are still visible (1–30% of the dressing covered)	Fig 2b
2	Moderate coverage (31–60% of the dressing covered)	Fig 2c
3	Marked coverage but some parts of the dressing still visible (61–90% of the dressing covered)	Fig 2d
4	Extensive coverage with hardly any parts of the dressing visible (91–100% of the dressing covered)	Fig 2e

Binding of 24 hours established biofilm to dressing samples

A circular dressing sample was aseptically introduced in selected wells, followed immediately by a sterile glass coverslip to ensure contact between dressing and biofilm and to prevent the dressing floating away from the biofilm layer (Fig 1). DACC-coated and uncoated dressings were tested in duplicate in the same microtitre plate; positive controls (no dressings) and negative controls (no bacteria) were included in all plates. Plates were incubated at 37°C and at known time intervals (normally up to 3 hours) and wells were sampled to retrieve the dressing, making sure that the orientation of the sample was known (i.e. surface in contact with the biofilm). Biofilm on the surface of the dressing was visualised by scanning electron microscopy (SEM).

Scanning electron microscopy of dressing samples

Dressing samples were transferred to wells in fresh microtitre plates containing 200µl 2.5% glutaraldehyde for 5 minutes to fix the attached biofilm. After gentle washing in phosphate buffered saline (PBS; Oxoid, Cambridge, UK) and storage overnight at 4°C, fixed samples were treated with 1% osmium tetroxide for 45 minutes, dehydrated in each of 50, 70 and 90% ethanol, followed by three changes of absolute alcohol for 10 minutes. Fixed dressings were then mounted onto pins dried in a critical dryer, coated by gold spluttering and examined in a 5200LV Jeol scanning electron microscope (Jeol Ltd, Hertfordshire, UK). For each sample in every experiment at least four representative images were captured, usually three at low magnification (typically 100X) and at least one at a higher magnification size (between 200 and 10,000X)

Images of the dressing samples were evaluated for the extent of biofilm coverage by six volunteers. These were postgraduate biomedical science students and research technicians who had undergone a training programme using suitable sample images and a scoring system (Table 1 and Fig 2). For each time point and each test organism, three images coded to ensure anonymity were scored between 0 (no binding) and 4 (extensive binding of biofilm to dressing) by each volunteer, who worked independ-

Fig 3. Biofilm of *Pseudomonas aeruginosa* bound to dressing samples after one hour contact. The dressing surface in direct contact with the biofilm established on the plastic coverslip in test well (a). The surface not in contact with biofilm in the test well (b)

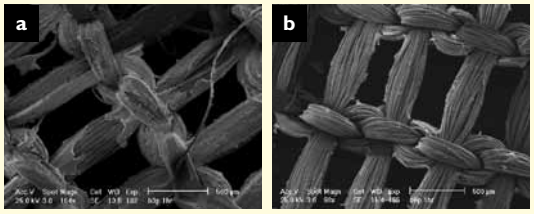


Fig 4. Binding of *Pseudomonas aeruginosa* biofilm to dressing samples. Uncoated after 1 hour contact (a), uncoated after 3 hour contact (b), DACC-coated after 1 hour contact (c), DACC-coated after 3 hour contact (d)

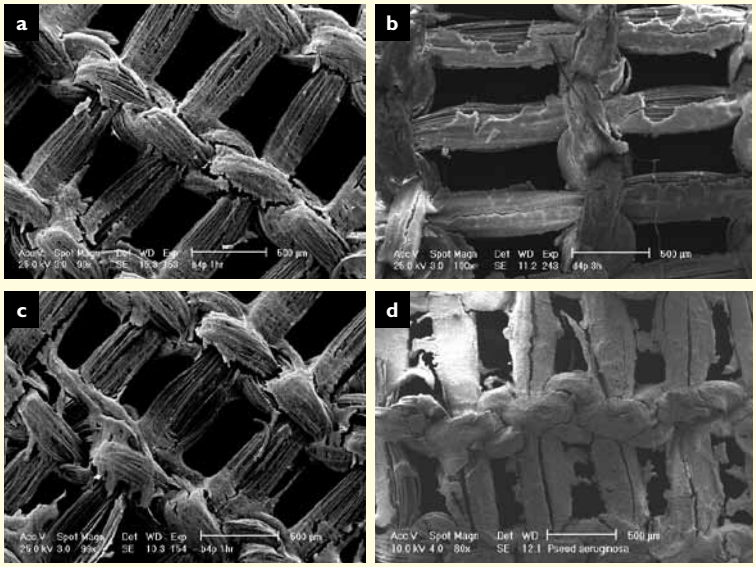


Fig 5. Binding of MRSA biofilm to dressing samples. Uncoated after 1 hour contact (a), uncoated after 3 hour contact (b), DACC-coated after 1 hour contact (c), DACC-coated after 3 hours contact (d)

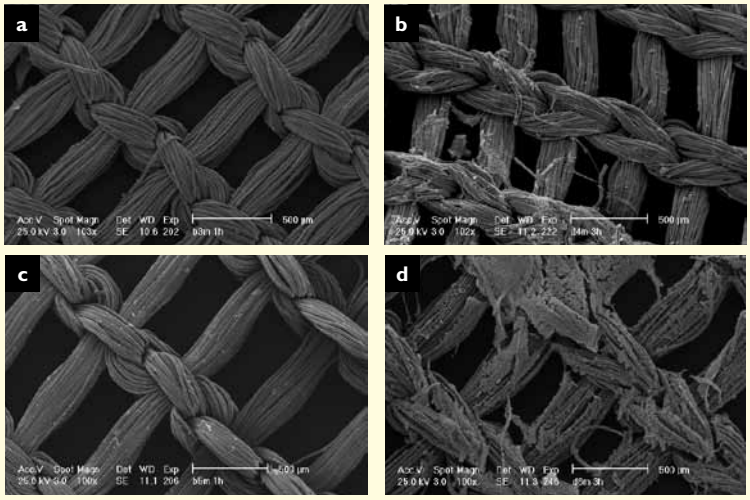
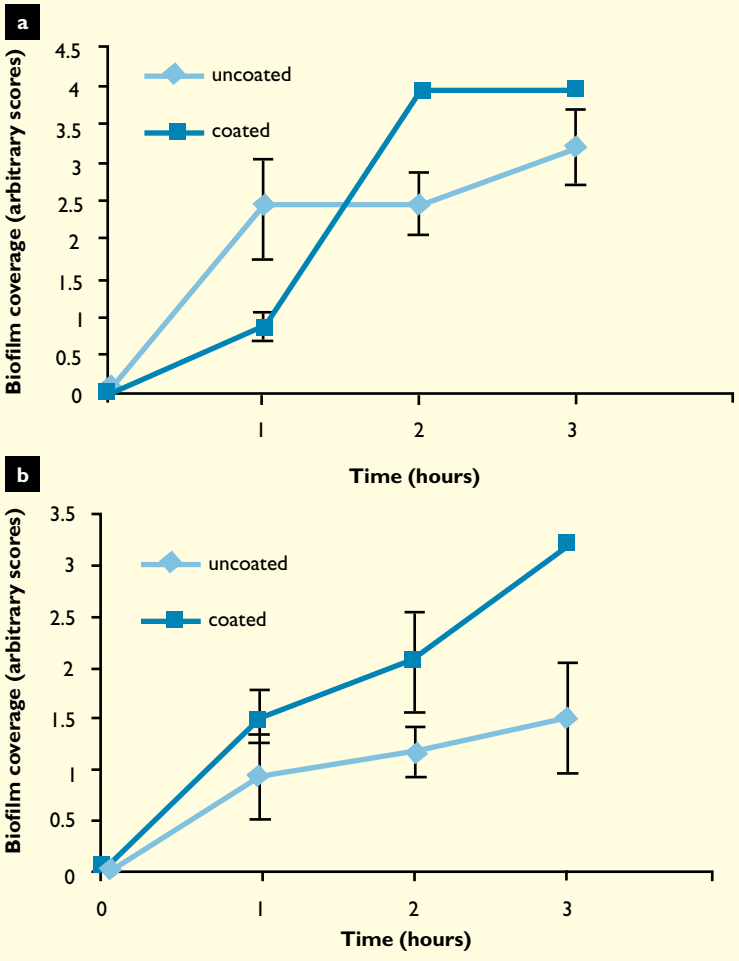


Fig 6. Biofilm coverage of dressing samples *Pseudomonas aeruginosa* (a) and MRSA (b)



ently. Mean scores and standard deviations were calculated and plotted versus time. Experiments were performed on two occasions.

Results

The presence of biofilm on dressing samples was determined using the surface that had been in contact with the biofilm established on the plastic coverslip, rather than the distal surface that had been in contact with the glass coverslip (Fig 1). It was seen that biofilm transferred from the plastic coverslips directly to dressing samples (Fig 3a) and did not migrate extensively through the dressing sample to the distal surface during the contact times tested here (up to 3 hours) (Fig 3b). Dressing samples exposed to *Pseudomonas aeruginosa* biofilm indicated rapid and extensive acquisition of biofilm (Fig 4); the extent of biofilm associated with uncoated (Fig 4a and 4b) and DACC-coated dressings (Fig 4c and d) showed no marked differences. Binding of MRSA biofilm to dressings was initially (Fig 5a and 5c) at a slower rate compared to *Pseudomonas aeruginosa* (Fig 4a and 4c). After a 3-hour contact period the coverage of uncoated dressing samples by MRSA biofilm (Fig 5b) was not as extensive as that of DACC-coated samples (Fig 5d), suggesting that the presence of the hydrophobic fatty acid derivative on the dressing surface did enhance biofilm binding. These observations were supported by the dressing coverage evaluations performed by the volunteer group (Fig 6).

In order to determine whether the bacterial cells attached to DACC-coated dressings were present as planktonic cells or as biofilms, some images at higher magnification were collected from samples tested with each of *Pseudomonas aeruginosa* and MRSA, biofilm structures were evident (Fig 7 and Fig 8, respectively).

Discussion

Using SEM to observe the extent of binding of established biofilm it was found that DACC enhanced the binding of MRSA biofilm compared with uncoated dressing samples (Fig 5d and 6a); this is in line with previous work on the binding of planktonic staphylococci to DACC-coated dressings.[5] *Pseudomonas aeruginosa*, however, bound similarly to coated and uncoated dressings. These differences probably reflect the distinct adhesins present on the surface of each species and the sticky nature of the extracellular polymeric material produced by *Pseudomonas*. Binding of established biofilms to dressing samples started within an hour of contact time for both test organisms. This concurs with the observation made by Ljungh et al., using planktonic *Pseudomonas aeruginosa,* that maximum binding to the DACC-coated dressing occurred at 120 minutes *in vitro*.[3]

The ability of some wound dressings to sequester

and immobilise microbial cells from simulated wound fluid *in vitro* has been described and benefits to infection control recognised.[9] Although the findings of this small laboratory study suggest a potential for DACC-coated dressings to lower the surface bioburden of wounds by binding biofilms as well as planktonic bacteria, it can only be confirmed *in vivo*. Recently, two pertinent studies have demonstrated a reduction in wound bioburden levels following the use of a DACC-coated dressings, although the presence of biofilm in neither study was tested. In one, using traditional culturing techniques of wound swabs to monitor the bioburden of aerobic bacteria in hard-to-heal leg ulcers, application of either Aquacel Ag or Cutimed Sorbact changed daily for a total observation period of four days, showed significant reductions in bioburden.[10] In the other, a molecular approach using punch biopsies collected weekly from chronic leg ulcers treated twice a week with DACC-coated dressing over a four-week period showed a significant decrease in the bacterial load of 10 out of 15 healing wounds, but no change in 5 out of 5 non-healing wounds. Clinical observations indicated that DACC-coated dressing resulted in completely successful therapy of 7 out of 20 patients and an improvement for 9 further patients. However, an analysis of information on bacterial load obtained from wound swabs taken from the same patients did not correlate with clinical outcome.[11] This raises the importance of considering the differential effects of topical interventions on bacterial species unequally distributed throughout the wound environment.[12] Biofilm is not universally located at the surface of the wound,[13] and it may be embedded within deeper tissue where it may not be affected by a therapy confined to the wound bed. The capacity of topical antimicrobial interventions to control biofilm in deep tissue must, therefore, always be evaluated clinically.

The fact that biofilms are especially tolerant to antibiotics[14] explains why some wounds fail to respond to antimicrobial interventions. Until effective antibiofilm agents are developed, the ability of a dressing to bind biofilms provides a non-invasive means to remove biofilm from the surface layer of a wound without sharp debridement or potentially cytotoxic chemical interventions. Another advantage of this approach is the diminished risk of the emergence of dressings-resistant species.

Limitations

An important limitation of this study is that it contains *in vitro* data, which is not necessarily transferable to the clinical situation. We used pure cultures of two representative bacteria that had been isolated from out-patients with chronic wounds attending a local hospital and cultivated them in microtitre plates for 24 hours before contact with dressing samples. Under these 'artificial conditions', the biofilms generated would not have been mature and would have behaved differently if they had been established for longer periods. There are many laboratory models for the study of biofilms, but none can accurately reproduce the complex conditions within a wound. Many experiments have used *Pseudomonas aeruginosa* cultivated in flow chambers where it grows to produce mushroom-like structures, but the relevance of these systems to human chronic infections has been questioned,[15] since such structures have not yet been observed in wounds.[16] Most chronic wounds are characterised by polymicrobial communities of microbial species *in vivo*[17–19] and mixed cultures are used in some experimental models.[20–22] Animal models can also provide more realistic conditions, even if confined to pure cultures.[23]

Laboratory investigations may help to elucidate mechanisms of action, but standardised methods for evaluating anti-biofilm agents are yet to be devised. However, it is clear that only clinical observations can establish the efficacy of antimicrobial interventions.

Fig 7. *Pseudomonas aeruginosa* biofilm attached to DACC-coated dressings at 1000x magnification (a) and 10000x magnification (b)

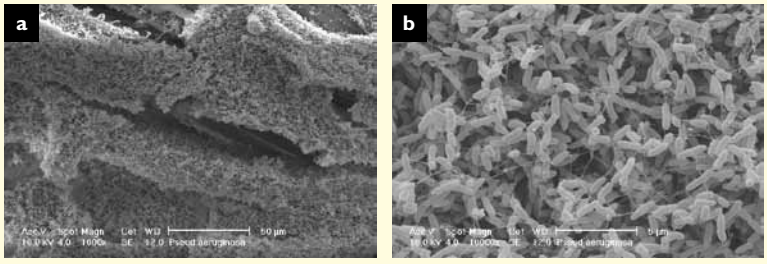
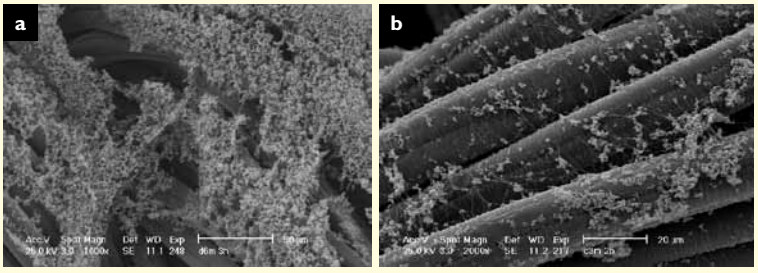


Fig 8. MRSA biofilm attached to DACC-coated dressings at 1000x magnification (a) and 2000x magnification (b)



Conclusion

This is the first demonstration that DACC-coated dressings bind MRSA and *Pseudomonas aeruginosa* biofilms *in vitro*. Whether this occurs widely *in vivo* has yet to be demonstrated, but this will only be known after the development of a routine biofilm diagnostic test that can be used before and after the clinical use of these dressings. ■

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EWMA Journal

Volume 14
Number 3
April 2014

Published by European Wound Management Association

VERUM – A European Approach for Successful Venous Leg Ulcer Healing:

Implementation of a Comprehensive Therapy Concept (VERUM13) in Daily Practice

PRESSURE ULCERS IN SPAIN

VERUM – A European Approach for Successful Venous Leg Ulcer Healing: Implementation of a Comprehensive Therapy Concept (VERUM[13]) in Daily Practice

ABSTRACT

Background: Successful healing of venous leg ulcers can only be achieved with a combination of compression therapy and moist wound healing[1]. Therefore, this treatment strategy is recommended by therapy guidelines; however, implementation in daily practice is thought to be, at the least, complicated or even impossible.

Aim: The main aim of this study was to determine whether a clear treatment strategy is helpful for the implementation of a holistic therapy concept. In order to generate additional information about the practicability and implementation of holistic therapy concepts necessary for successful healing of venous leg ulcers, a comprehensive therapy concept consisting of compression therapy and moist wound care was implemented in daily medical practice in different countries.

Methods: Sixty-three patients from Germany, Italy, and Austria were treated for 12 weeks in daily medical practice with phase-adapted moist wound dressings, bacteria-binding wound dressings, and short-stretch compression bandages in the initial oedema phase, followed by compression treatment using a compression stocking system with high stiffness characteristics.

Findings: Approximately 85% of wounds were significantly reduced in size, and 53% of wounds healed completely within 12 weeks. The patients' well-being was substantially improved, leading to high compliance. As the majority of patients had not experienced any progress in healing for extended periods of time due to their complicated health situation or ineffective treatment, these results were rated very positively by patients and physicians. In addition, the bacteria-binding wound dressing allowed infected wounds to be treated without antibiotics.

Conclusion: Holistic therapy concepts offer significant advantages and can be successfully implemented in daily practice independent of national or local wound care traditions.

INTRODUCTION

Clinical data indicate that compression accelerates the healing process of venous leg ulcers[2], and wound-healing rates are far superior in patients treated with compression than in those treated without compression[3,4]. Thus, to improve the healing process, clinical guidelines recommend treatment of venous or mixed venous ulcers (0.6> ABI <0.9) with high pressure of 30 to 40 mmHg at the ankle[5]. On the other hand, 50% of all patients with venous leg ulcers heal completely within 3 months following treatment with moist wound healing, and 70% of the cases within 1 year with this strategy[6]. Therefore, the concept of moist wound management in combination with compression is considered the most effective approach; yet, these findings do not receive adequate attention in actual daily practice[7,8,9,10].

In Germany in 2002, only an estimated 10%-20% of patients with chronic wounds received moist wound therapy[8], and as many as 25% of patients with venous leg ulcers failed to receive compression therapy[7]. These findings were confirmed by a survey of 45,975 patients on the use of compression therapy in combination with contemporary moist wound management, as the study showed that patients with chronic leg ulcers did not always receive optimal treatment in Germany[8].

Even though the general consensus is that successful healing does not depend on the quality of a single product alone but rather on the interaction of all products within a comprehensive therapy concept, such concepts are not consistently implemented in daily practice. Most likely, this occurrence is not due to the complexity of the therapy concepts alone, which can make their implementation difficult, but rather due to the lack of previous experience with the combined use of multiple wound-healing and compression products.

In an effort to gain further experience in the implementation of complex therapy concepts for treatment of venous leg ulcers, 63 patients in Germany, Italy, and Austria were treated with compression therapy and moist wound dressings. The objective of this multicentre evaluation was not only to assess the performance and ef-

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Conflict of interest: The article has been supported by a research grant from BSN.

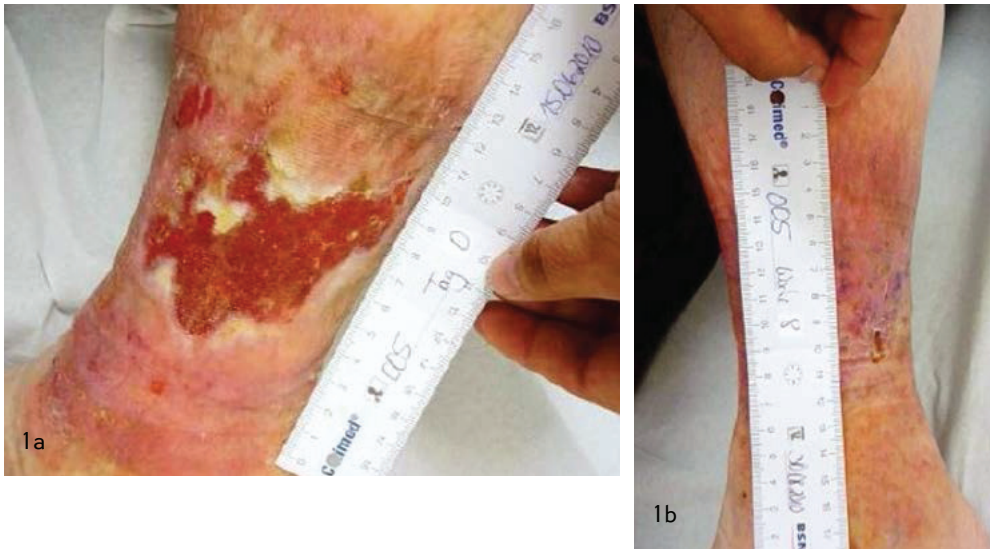

Figure 1: A 6-month-old Ulcus cruris venosum of a 74-year-old patient who was also diagnosed with other risk factors including contact sensitization and obesity. The ulcer showed signs of infection and red wound edges. Treatment was initiated on 15th June 2010 (a). Full wound healing was achieved on 30th August 2010 (b).

fectiveness of the wound care and compression products but also to investigate the efficacy of the overall therapy concept under conditions of routine medical care.

METHODS

Region

The multicentre case studies were conducted on a non-interventional basis at several phlebological surgeries and wound care centres in Italy (Roberto Brambilla, Istituti Clinici Zucchi, Monza; Daniele Aloisi, Poliambulatorio Mengoli, Bologna; and Marco Fioruzzi, Policlinico San Marco, Zingonia), in Germany (Dr. Iris Weingard, Venenzentrum Freiburg, Freiburg; Dr. Thomas Heisterkamp, Dermatologisch-Phlebologische Praxis Gescher, Gescher;, and Dr. Edith Janthur, Venenzentrum SAARLOUIS, Saarlouis) and in Austria (Peter Kurz, Wund Pflege Management GmbH, Bad Pirawarth).

Time Frame

In accordance with the predefined observation schedule, the baseline visit was followed by a maximum of five follow-ups within a 12-week period. The total period of observation varied from 6-12 weeks, depending on the status of the individual patient and wound-healing progress.

Aetiology

Only patients with ulcers of venous aetiology were included. Many patients also suffered from other diseases, including diabetes (15.9%), cancer (7.9%), and hypertension (11%). The majority of the patients had additional risk factors, such as allergies. Every patient had at least one chronic ulcer. According to the contra-indications, patients with arterial leg ulcers, recent deep vein thrombosis, or cardiac insufficiency were excluded. The patient ankle-brachial index (ABI) differed from 0.8 to 1.1. None of the patients showed any signs of arterial occlusive disease. In 31.7% of cases, the body mass index (BMI) was > 28 kg/m^2 (obese patients). Patient age varied between 41 and 92 years, and 58.7% of the patients were women (37/63).

Wound Status

Only venous leg ulcers of less than 1 year were included. The size of the ulcers varied from 10 to 90 mm in length and from 5 to 98 mm in width at baseline (length, 28.3 ± 21.6 mm (mean ± standard deviation); width, 16.7 ± 16.8 mm; resulting wound surface area, 5.1 ± 12.0 cm^2). Approximately 43% of the wounds showed signs of infection/biofilm (redness, itching, pain, or odour), and 80% of the wounds exhibited red wound edges.

Data Management

During this evaluation, wound status and healing was documented on the basis of clinical standard parameters. Initial set up of the protocol and questionnaires as well as the data collection was supervised by an external clinical laboratory. The wound status was documented by photo and with a description at the beginning of the study (baseline) and at each subsequent visit. Relevant parameters included the size and appearance of the wound (phases of wound healing, exudation, and signs of infection) as well as the peri-wound skin. Complete healing was defined as full closure of the wound. Furthermore, the evaluation included a patient Quality-of-Life (QoL) survey that was based on the Tübingen Questionnaire[11], in which patients were asked about the degree of impairment they experienced in their routine activities, such as lifting and carrying heavy objects, standing for extended periods of time, light/heavy household and gardening chores, and walking and climbing stairs.

At the final visit, the attending physician provided a final assessment of the outcome as well as the therapy concept. The computer-aided documentation during the study was performed by the investigators and evaluation staff at the centres. The data were evaluated by means of descriptive statistics.

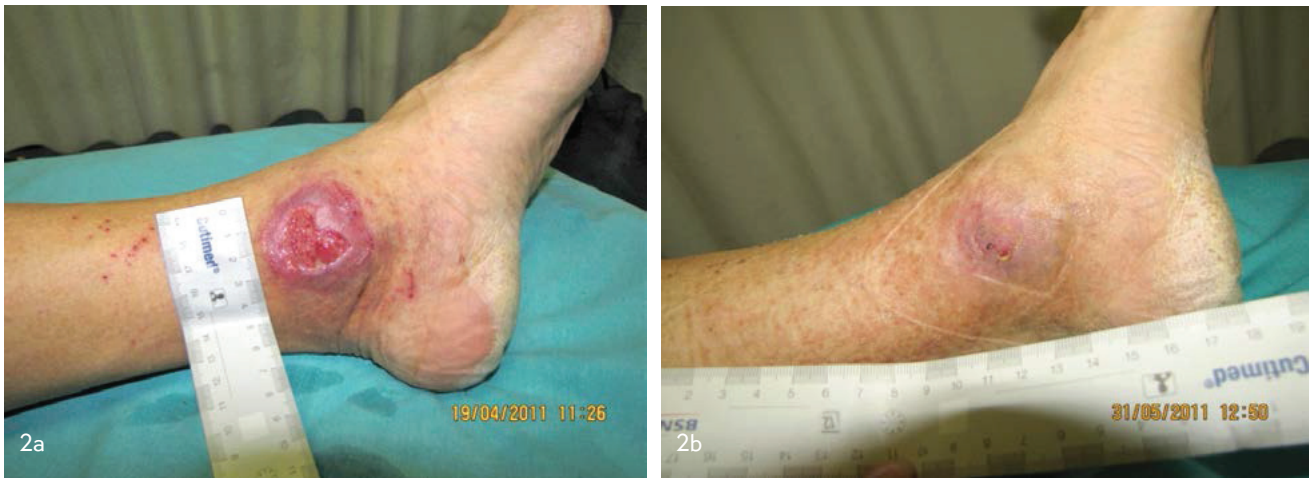

Figure 2: A 3-month-old Ulcus cruris venosum of a 59-year-old patient who was diagnosed with disturbed perfusion. The ulcer showed signs of infection and red wound edges. Treatment was initiated on 19th April 2011 (a). Wound closure was achieved on 31st May 2011 (b).

Wound Treatment and Compression Therapy

Before applying the high stiffness compression stocking system (JOBST® UlcerCARE™, BSN medical GmbH) the oedema had to be reduced to allow selection of correct stocking size. For this purpose short-stretch compression bandages (in Germany and Austria, Comprilan was used, and in Italy, Comprilan or Tensoplast [BSN medical GmbH] was used) were applied for up to one week depending on the extent of the oedema. The bandages were applied by experienced staff. According to the local protocol, the wounds were always cleaned, and the wound edges protected with a polymeric solution (e.g., Cutimed® Protect, [BSN medical GmbH]) before application of the wound dressing and compression. For exuding wounds, a superabsorbent polyurethane foam dressing (Cutimed® Siltec range, BSN medical GmbH) was used. In the case where signs of infection were noted, a bacteria-binding dressing (Cutimed® Sorbact® [BSN medical GmbH]) was placed directly on the wound and then covered with the foam dressing. In these cases, after normalisation of the wound, the bacteria-binding dressing was applied as a preventive measure. At the beginning, the wound dressings were changed daily, while later, the dressings were changed less frequently depending on the status of the wound (e.g., signs of infection or wound fluid). At subsequent visits following reduction of the oedema, wounds were treated similarly, but a compression stocking system (40 mmHg) was applied instead of the compression bandages. Instructions for use included use of the liner for 24 h and the upper-stocking for 12h. All wounds were treated regularly depending on the status of the wound but the complete questionnaire and patient survey were assessed, and pictures were taken only after 2 weeks (optional) and after 4, 8, and 12 weeks.

RESULTS

At the baseline, the wound status of the majority of leg ulcers was rather complicated: 82% of the wounds showed inflammation, 4% were covered by necrosis, and 59% were covered by slough. Heavy exudate was reported in 24.1% of the ulcers, and moderate exudate levels were observed in 75.9% of the wounds. In addition, 93% of all patients showed a pronounced redness of the wound edges.

During routine examination, infections were suspected in 48% of the patients due to the presence of classical signs, including redness, pain, and odour. In cases of suspected infection, a bacteria-binding dressing was applied during the observation period. The intended therapy was, therefore, implemented without the use of antibiotics. Furthermore, an overall reduction of redness was observed over the course of the wound treatment in these cases.

Individual differences in wound size reduction (length, width, and depth) were observed after 2, 4, 8, and 12 weeks according to analysis with the Wilcoxon test together with photo documentation. In 85% of cases, either a reduction in wound size or complete healing of the wounds was achieved. In cases where complete healing was not achieved, wound size reductions ranged from 43.8% to 92.4%. Examples are shown in Figures 1 and 2.

In addition, 65.5% of the patients reported illness-related limitations in daily activities, and 24.1% even characterized their situation as mostly or completely impaired. The individual illness-related limitations in daily activities varied, likely depending on the severity of the oedema, pain, and wound status. The analysis of the Tübinger Questionnaire revealed a shift from high to low impairment during the period of the observation and from low to no impairment in tasks such as lifting objects, walking, standing, and household and gardening chores. Spontaneous reports of improvement in general were also noted, as patients experienced substantial improvement in ▶

their wound status and well-being. These improvements resulted in a high degree of patient compliance. Only one patient did not comply with the programme, and none of the other patients asked for premature discontinuation of treatment.

Physicians rated the use of a stocking system very positively but pointed out that oedema reduction by means of compression bandages is very important to ensure an optimal fit of the stocking system. As in typical clinical practice, treatment adaptations were necessary for individual patients. Three patients experienced an increase in swelling after initial reduction of their oedema, rendering the compression system too tight. One patient developed an adverse reaction to one of the components of the wound dressing (redness and itching in the contact region of the wound dressing). Two patients developed an infection during the observational period and had to be treated systemically. In two other cases, no healing progress was achieved within 12 weeks. This scenario indicates that the patients were likely resistant to this therapy, and the patients were thus treated differently. Another patient experienced a massive deterioration of his general health status (acute erysipelas) at the beginning of the observation period, resulting in additional treatment; however, no causal relationship with the therapy concept could be established. One patient was considered a drop-out, as he failed to follow the physician's instructions by switching to a different product for one week without permission. As a result, he did not wear the compression stocking system as recommended, which resulted in deterioration in wound status.

Overall, the therapy concept was rated very positively. Both patients and physicians reported that they would use it again. In combination with close monitoring and guidance of the patients, the therapy concept resulted in a high degree of patient satisfaction and compliance.

DISCUSSION

Currently, the combination of moist wound management and compression is acknowledged as the most effective approach for treating venous leg ulcers[3,4]. Therefore, it is surprising that this therapy concept is given little attention in actual daily practice, even in highly developed countries such as Germany[7,8,9,10]. To our knowledge, data regarding the implementation of this approach into daily practice in other European countries are not available, and thus, this lack of implementation is presumably not an issue only in Germany. Insufficient knowledge of the selection of compatible compression and wound management products may underlie the lack of implementation of this fundamental scientific knowledge in daily practice.

The scope of this evaluation was to determine whether a clear treatment concept is helpful for the implementation of a holistic concept. Therefore, a set of well-adapted products was selected, and application of these products was carefully explained. In our opinion, it is important that the wound care products work reliably under compression. The choice of super-absorbent polyurethane foam dressings with skin-friendly silicone was based on the fact that this type of wound dressing maintains a moist environment in wounds even under compression under 40 mmHg. Indeed, we observed very good fluid handling by the wound dressings (moist wound-healing conditions with no maceration). In addition, atraumatic dressing changes (no pain and no sticking to the wound) were achieved. We also recommend protection of the wound edges for additional safety.

Since wound infections hinder progress in wound healing in our opinion antimicrobial control is a very important part of the treatment concept. Therefore, we included a bacteria-binding wound dressing, which binds bacteria irreversibly via a physical mode of action[12]. The bound bacteria are inactive and unable to replicate and are removed with each dressing change, reducing the overall bacterial load without the risk of cytotoxicity or bacterial resistance[12]. Our results show that the bacteria-binding dressings work well in concert with the foam dressings even under 40 mmHg. Therefore, the bacteria-binding dressing is a helpful tool for controlling wound infections.

Despite the prevalence of different local treatment protocols, all physicians implemented the treatment concept successfully, and the high number of different products presented no hindrance to implementation of the treatment into daily practice. Positive healing results in 85% of the cases confirmed previous scientific evidence that the combined use of moist wound management and compression systems provides an adequate therapy concept for treating venous leg ulcers[3]. As the majority of patients had not experienced any progress in healing for extended periods of time before this study, even small improvements were considered a success.

In the many cases where an infection was suspected, the achieved wound healing results can be considered very positive. Also, in these cases, the therapy could be followed without interruption or the use of antibiotics (with exception of two patients). Furthermore, the compression stocking systems, which were easy to don and were operator-independent, were appreciated by both patients and physicians.

In conclusion, we observed that a clear and easy-to-understand therapy concept increases patient satisfaction and QoL and leads to a high patient compliance and acceptance by physicians. With a trend toward shortened hospitals stays and ambulant treatment of wound patients, the need for simplified treatment concepts increases. Our example encourages implementation of this therapy concept into daily practice, and additional clinical evidence in support of such concept is warranted in order to increase the number of adequately treated venous leg ulcers.

Implications for Clinical Practice

- This therapy concept together with close monitoring and guidance of patients achieves good healing results.
- Even complex therapy concepts can successfully be integrated into daily practice.
- The therapy concept could be implemented independently of local treatment traditions.
- The therapy concept improved patient Quality-of-Life and as a result patient compliance.

Further Research

- Further case studies are planned in the UK and USA to support these observations.
- Further case studies with bigger wounds are planned, keeping in mind the increasing number of ambulant patients with big wounds and the earlier discharge of patients into self-management in the future.

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13. **Abbreviations** VERUM Venous Return Ulcer Management

Venous Leg Ulcer Therapy

Cutimed® Sorbact®
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JOBST® UlcerCARE™

Teaming up for more success.

A European case study of BSN medical showed that the combination of Advanced Wound Care and Compression Therapy significantly improved 85 % of venous leg ulcers and completely healed 53 % of all cases within 12 weeks.

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Published in EWMA Journal 2013, pages 19-23; Volume 13 No 2, October 2013

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Evidence is building to support using a DACC-coated antimicrobial wound contact layer with NPWT

KEY WORDS

- DACC
- Exudate management
- Infection
- Negative pressure wound therapy

Heavily exuding, infected cavity wounds requiring negative pressure wound therapy (NPWT) can become chronically inert over time, resulting in complex clinical, financial and personal challenges to healthcare workers and patients. This evaluation explored the benefits of utilising a DACC-coated antimicrobial wound contact layer in conjunction with NPWT in 10 patients with heavily exuding, infected wounds. The results demonstrated positive outcomes in regards to non-adherence, atraumatic application and removal, reduction in bacterial burden and exudate levels and timely downgrading from NPWT to conventional dressing therapies.

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Heavily exuding, infected wounds can incorporate a myriad of wound groups, such as pressure ulcers, surgical incision sites, venous leg ulcers, burns and traumatic lesions (Gray et al, 2008).

Despite the use of modern, innovative dressings, some wounds can take a long time to heal, fail to heal, or recur, causing significant pain and discomfort to the individual. It is well documented that if infected, highly exuding wounds are not brought under control, healthcare costs increase, carer burdens rise, and patients' quality of life decreases (Bottomley, 2007; Guy and Grothier, 2012).

This article evaluates the use of an antimicrobial, DACC-coated wound contact layer to replace a conventional wound bed liner in conjunction with negative pressure wound therapy (NPWT) to promote an optimum environment for healing in 10 patients with static, exuding, infected wounds.

NPWT

NPWT has facilitated the management of various acute and chronic wounds which are at risk of infection and are failing to heal. NPWT devices perform using the same general principles – a foam or gauze is deployed within the wound cavity and covered with an adhesive film to create a seal which is connected to a vacuum device (Gregor et al, 2008; Bateman, 2013).

To enhance wound healing, it is recommended that the wound bed is prepared adequately and where possible, necrotic tissue is removed prior to application and a protective wound bed liner is utilised where appropriate (Vowden et al, 2007; Jeffery, 2012).

NPWT increases blood flow to the wound bed, improving waste product removal, increasing granulation tissue within the cavity, aiding epithelial cell migration, and ultimately leading to wound closure (Morris et al, 2007; Orgill et al, 2009).

Removal of excess exudate containing inhibitors such as cytokines and proteinases alongside effective mechanical action, wound contraction and reduction are induced, providing an optimum environment for wound healing to occur (Orgill and Bayer, 2011).

Caution should be taken when employing NPWT on wounds with contraindications to the mechanical effects of the device, such as malignancy, visible underlying blood vessels and risk of haemorrhage (Guy and Grothier, 2012).

Many NPWT device packs provide the clinician with a non-antimicrobial wound contact layer to act as a barrier to prevent adhesion of the accompanying foam or gauze to ensure atraumatic

Table 1. Patient demographics.

Patient	Gender	Age	Wound type	Location	NPWT duration (weeks)	Confirmed bacteria prior to evaluation
1	Female	52	Surgical	Left hip	12	*Staphylococcus aureus*
2	Male	68	Surgical	Right knee	4	*Pseudomonas aeruginosa*
3	Male	44	Diabetic foot ulcer	Right heel	4	*S aureus*
4	Male	28	Self harm lesion	Left forearm	3	*P aeruginosa*
5	Female	51	Burn	Right hand	4	MRSA
6	Female	68	Surgical	Left hip	23	*Candida albicans*
7	Male	55	Venous leg ulcer	Left calf	12	*S aureus*
8	Male	44	Surgical	Right forearm	3	*P aeruginosa*
9	Female	52	Trauma	Abdomen	2	Strep A and B necrotising fasciitis
10	Female	47	Surgical	Abdomen	1	*P aeruginosa*

removal and therefore protection of underlying structures (Guy and Grothier, 2012).

It is within this evaluated patient group that a low adherent liner was required to primarily protect the underlying structures and one that also provides additional antimicrobial benefits which would possibly benefit the static nature of the wounds.

Previous clinical evaluations exploring the use of DACC-coated dressings within NPWT have highlighted positive outcomes in regards to both atraumatic and antimicrobial properties and are therefore safe to use in this patient population (Bateman, 2013; Jeffery, 2014).

INFECTED, EXUDING WOUNDS

Infection status occurs within tissue when bacteria quickly multiply and overwhelm the patients' natural defence mechanisms, resulting in an increase in pain, inflammation, erythema and systemic fever which delay wound healing (NICE, 2008). Bacteria will initially contaminate the wound through either endogenous or exogenous processes at the time of the tissue injury with variance in contamination (Bateman, 2013).

Jeffery (2014) emphasises that the presence of devitalised tissue alongside excessive fluid will encourage bacterial reproduction, resulting in more severe symptoms. Mangram et al (1999) and Milne et al (2012) concur, stating that infection and excess exudate will have a negative effect upon tissue healing and progression if not managed in a timely manner.

DACC-COATED CUTIMED® SORBACT® DRESSINGS

Cutimed® Sorbact® antimicrobial dressings are coated with DACC, a fatty acid derivative which, in the presence of moisture, attracts and binds bacteria and fungi to the dressings, reducing the overall concentration of microbes in the wound. This innovative mode of action effectively removes microbes that otherwise may result in delayed wound healing (Meberg and Schøyen, 1990).

The dressings can be used as a wound filler, as in Jeffery's (2014) clinical work, or as a liner, as in this evaluation. The dressings can be placed within many wound types, are effective on all common wound pathogens and fungi and can be utilised by any patient group.

METHOD

Ten patients were referred to the wound care service with exuding, infected wounds, confirmed with a wound bed swab taken one week prior to the introduction of Cutimed Sorbact. All wounds were being treated with Trust-agreed NPWT, but wound progression had stagnated.

Verbal consent was obtained by all patients following discussion and agreement from the respective managing consultants. The evaluated patients had a broad range of demographics, wound types and bacterial infection (*Tables 1* and *2*).

A DACC-coated Cutimed Sorbact swab was used to line the wound bed and walls, replacing the conventional liner, in conjunction with ongoing NPWT.

This product was chosen due to its antimicrobial benefits and low adherence properties. It was familiar to the clinician from experience with its use in patients with similar wounds, where it had shown to successfully reduce bacterial burden and lead to timely downgrading to conventional dressing therapies (Bateman, 2013).

The normal regime of cleansing with sterile saline utilising a non-touch aseptic technique and vacuum closure was adhered to. The NPWT device regime continued with twice-weekly gauze filler changes, but the DACC-coated liner remained *in situ* and was only replaced every 7 days.

Wound assessment and documentation was undertaken by the senior wound care lead prescriber, who did not undertake the dressing care, in order to ensure that the data collection process was consistent, objective and accurate.

Table 2. Summary of patient demographics.

Number of patients	**10**	
Gender	Male	50%
	Female	50%
Age range	28–68 years	
Mean age	51 years	
Wound types	Surgical	50%
	Burn	10%
	Ulcer	20%
	Trauma	20%
Confirmed bacteria within wound in 7 days prior to start of evaluation	*Staphylococcus aureus*	30%
	Pseudomonas aeruginosa	40%
	Strep A and B necrotising fasciitis	10%
	MRSA	10%
	Candida albicans	10%
Pre-evaluation wound and NPWT treatment duration	7–161 days (1–23 weeks) (mean 48 days)	Total NHS days = 476 (68 weeks)

RESULTS

Although the evaluation referred to a small cohort (*n*=10), the results were very positive in all regards to the key outcomes, despite the variation in wounds, patient demographics and microbiology. The results also reflected previous clinical evaluations (Bateman, 2013; Jeffery, 2014).

The results clearly demonstrate an overall success in exudate reduction by week two in all patients (*Table 3*).

Negative microbiology was achieved in 60% of patients at week 1 (*n*=6) and in all patients (*n*=10) at week 2. Mean time to negative microbiology was 10 days.

Stasis was reversed, with wound size reduced for all patients from week 1 to week 3 (*Table 4*). Although this product is not promoted as an alternative to other antibiotic therapies, it demonstrated a positive and effective presence in all the wounds, consistent with previous work in this area (Jeffery, 2014).

There was a reduction in NPWT duration after the deployment of the DACC-coated wound contact layer Before the dressing was used as a liner, the total number of days all patients were under NHS care for these wounds was 476. When DACC was added to the NPWT regimen, this fell to 266 days, a 44% reduction. Treatment duration with the DACC-coated dressing and NPWT ranged from 14–56 days, with a mean treatment time of 27 days.

Table 5 demonstrates the reduced time from advanced wound management to conventional

Table 3. Exudate reduction.

Pre-DACC	***n***	**%**	**End of treatment**	***n***	**%**
High	8	80%	High	0	0%
Moderate	2	20%	Moderate	3	30%
Low	0	0%	Low	7	70%

Table 4. Wound depth reduction

Patient	Pre-DACC (cm)	End of treatment (cm)	% reduction
1	2	1.5	25%
2	3	1	67%
3	1	0.5	50%
4	2	1.2	40%
5	2	1.3	35%
6	8	3	63%
7	2	1.8	10%
8	2.5	2.2	12%
9	4	1	75%
10	4.5	3.5	22%

care with a soft silicone foam dressing. The reduction in NHS healthcare days spent on providing NPWT signifies savings in cost and nursing resources, and increasing discharge to other healthcare environments in which wound management can occur.

CONCLUSION

Within this 10 patient evaluation, the use of DACC-coated Cutimed Sorbact dressings, when used as a low adherent wound contact layer, in conjunction with NPWT, has demonstrated benefits relating to non-adherence, atraumatic wound bed protection, reduction in exudate levels and subsequent wound bed size reduction.

The implementation of this low-cost dressing which could be left in place for 7 days, has enabled clinicians to downgrade wound care from advanced level to basic level dressing regimens by 50%, which ultimately has a positive benefit for healthcare costs and resources.

The author suggests further exploration in this area, particularly in regards to patient and clinician experiences alongside financial and procurement outcomes.

It is proposed that this product be made available through inclusion on regional and national dressing formularies. It is apparent that this innovative product is a welcome addition to the clinician's wound care toolbox in the ever challenging arena of infected, exuding wounds. WUK

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Table 5. Downgrading to conventional foam

Patient	NPWT duration pre DACC (weeks)	NPWT duration post DACC (weeks)
1	12	3
2	4	5
3	4	3
4	3	2
5	4	3
6	23	8
7	12	2
8	3	2
9	2	8
10	1	2

Cutimed Sorbact/ Article:	Source:
Management of the infant with epidermolysis bullosa	Jacqueline Denyer, RGN, RSCN, RHV Nurse Consultant, Epidermolysis Bullosa (Paediatric) Great Ormond Street Hospital, London, UK *Infant,* Vol 5, Issue 6, 2009

KEY FINDING:

Cutimed® Sorbact® dressings can be an effective treatment for treating blisters and wound management in epidermolysis bullosa, particularly for reducing the bacterial load.

WOUND CARE CONCERN:

Epidermolysis bullosa (EB) is an umbrella term for a group of inherited skin disorders in which the common factor is marked fragility of the skin and mucous membranes. There are many different types of EB and the effects vary from blistering of the feet and hands in warm weather to death in early infancy in the most severe form. Infection and critical colonization can occur frequently with blisters and precipitate the development of chronic wounds.

CONCERN BASIS:

- Chronic wounds are evidenced by high bacterial count, more than one bacterial strain, the presence of biofilms and sometimes drug-resistant organisms, which can all delay healing
- Chronic wounds often produce high levels of exudate, which can cause maceration, inflammation and wound extension, creating additional wound management challenges
- Antimicrobial dressings introduce chemical agents (e.g. silver) to the wound which could create bacterial resistance or adverse side effects

WOUND CARE SOLUTION:

- Preventing colonization and reducing bacterial load is key to avoiding chronic wounds in cases of EB
- Raised plasma silver levels were found following use of silver impregnated dressings (no longer recommended for infants at this facility)
- Effective antimicrobial agents include Crystacide Cream and medical grade honey (ointments and impregnated dressings)
- Cutimed® Sorbact® dressings remove bacteria via hydrophobic interaction to bind bacteria to the dressing and have proven useful in treating blisters in cases of epidermolysis bullosa, however care needs to be take to ensure the dressing itself doesn't further traumatize the fragile skin via friction, application methods, etc.

SIGNIFICANT CASE FINDINGS:

(n/a - no cases presented in article)

For detailed descriptions of the different types of EB and the associated prognosis, risks and treatments to each, please see complete article.

Safe, long-term management of bioburden that helps promote healing Evidence review of DACC technology

Unlike common antimicrobial dressings, the Cutimed Sorbact range does not kill pathogens, but instead binds them to its surface, so they can be safely removed at dressing change. As a result, it can be used long term with minimal risk of side effects. This supplement describes the evidence base on the efficacy of DACC technology

The purpose of this supplement is to review the evidence on the use of Cutimed Sorbact in the treatment of chronic wounds. The evidence comprises laboratory tests and clinical studies including comparative studies, case series and case reports. It is generally accepted that clinicians need to ensure that wound management is evidence based,[1] although, in general, little comparative data is available on the efficacy of modern wound management devices. Most randomised controlled trials (RCTs) usually measure the effect of a treatment/product on wound closure, whereas the use of endpoints that record intermediate aspects of healing, such as a viable wound bed or the elimination of infection,[1] are generating increasing interest and gaining in popularity. Such endpoints may be more relevant to clinicians, who often choose a product/treatment that will achieve an intermediate goal on the way to the final outcome of a healed wound. To close this gap, case series and case studies can provide valuable evidence that is of direct clinical relevance. This supplement contains a wide selection of data, including a large number of case series and case studies, many of which focus on reduction of infection as well as healing.

Bioburden: a cause of wound chronicity

A critical component of wound healing (and thus an important intermediate endpoint) is the management of wound surface bioburden. All wounds have bacteria on their surface. The presence of free-floating, non-replicating planktonic microorganisms on the wound surface is referred to as contamination. This is essentially a benign state where transient microorganisms do not induce a host response or delay healing.[2] These planktonic bacteria evaluate the local environment and may attach to the wound bed and/or each other, or continue in a planktonic state. Following confirmation that the local environment is able to sustain microbial growth, irreversible attachment occurs. The bacteria divide and form larger groups of multiple species of microorganisms on the wound surface and a state of colonisation is reached.[2] This represents microbial habitation and replication, which is largely unrecognised by the host and does not delay healing.

Microbes within the colony attach to the wound bed and anchor themselves to the tissues. They then begin to secrete an extracellular polymeric substance (EPS) that surrounds and protects the colony from the host's immune system and extrinsically applied antimicrobial agents. Once the microbial colony reaches a certain critical mass or quorum, the cells begin to secrete 'quorum-sensing molecules' that attract other microbes to the biofilm. As the biofilm grows, it begins to expand into a multispecies community by including other microorganisms on the wound surface. A critical point is reached when the biofilm triggers a local inflammatory event from the host's immune system.[3] Exudate is produced in the wound bed and provides the biofilm with a source of fluid and nutrients.[4] Once the biofilm's microbial population has reached a certain size or density, individual bacterial cells escape from the colony where they revert to a planktonic phenotype and float away from the main biofilm (Fig 1). These small entities may reattach at another location and form new biofilms of their own. At this time, they are most susceptible to topical and systemic antimicrobials.[5]

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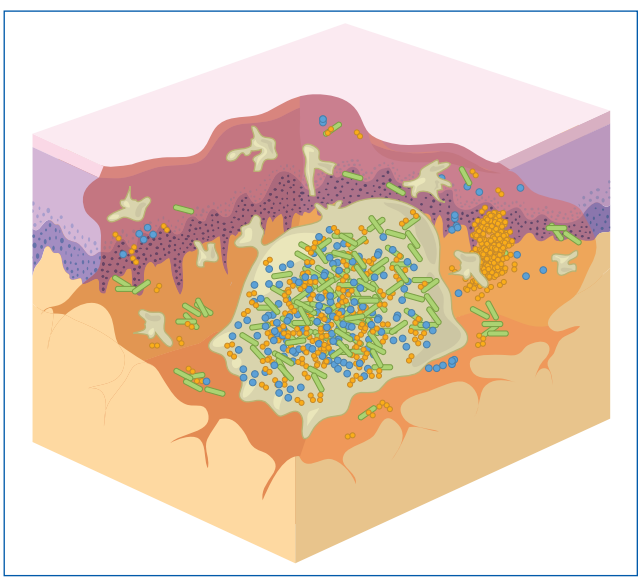
Microbes bind to both each other and the host extracellular matrix to protect themselves from marauding neutrophils and macrophages. While these predatory cells can engulf and destroy individual planktonic bacteria, they have difficulty penetrating the surface of a mature biofilm. Bacteria within a biofilm decrease their rate of cell division and invasive exotoxin production, and thereby conserve energy. The biofilm will gradually increase in size and constantly stimulate an inflammatory response from the host in order to provide a steady flow of fluids and nutrients to the colony. In this way, the biofilm ensures its survival and remains a constant threat to the host.[6]

Characterisation of biofilm is currently at an indeterminate stage, despite the recognised and underlying prolonged inflammation associated with wound biofilm infection.[7]

When the mature biofilm releases planktonic bacteria into a host environment, these microorganisms revert to a more aggressive form of behaviour: they divide rapidly and emit exotoxins that allow the microorganisms to spread within the host and form new biofilms. Alternatively, if large enough numbers of these bacteria are released into the bloodstream, they can cause an acute infection, where the host immune system responds with the generation of the classical signs of infection: pain, fever, swelling (oedema) and redness (erythema). Ascending cellulitis from the site of the wound is a potential development as the bacteria and their exotoxins migrate along lymphatic channels in a proximal direction.[8] At this point, fever, chills, night sweats and a general malaise will quickly overwhelm the patient, who will become very ill.

A number of therapeutic interventions is available to address biofilm infection and help create an environment, whereby the wound can progress towards granulation tissue formation and epithelialisation. However, bacteria have adapted to overcome our attempts to reduce their numbers by developing innate and induced resistance to antibiotics and the ability to form biofilms that are impervious to topical antiseptics.[9,10]



Peter Lamb

Fig 1. Biofilm in the wound bed: individual bacterial cells escape from the colony and revert to a planktonic phenotype.

Box 1. Difference between polar and non-polar molecules

Polar

Molecules with a partial positive electrical charge at one end and a partial negative charge at the other. Water is a polar molecule

Non-polar

The electrons are distributed more symmetrically because asymmetrical distribution would lead to partial charges and polar molecules. A rule is that substances dissolve in like substances. Therefore, non-polar substances such as oil cannot dissolve in water as it is a polar molecule

Cell surface hydrophobicity: a key component of biofilm formation

A surface is hydrophobic if it repels or does not mix with water. A hydrophobic effect occurs when non-polar substances aggregate in aqueous solution and repel water molecules. A common, everyday example of this is the separation that soon occurs after water and oil are mixed together. The difference between polar and non-polar is described in Box 1.

Hydrophobic reaction plays an important role in biofilm formation. It is well known that bacteria flourish in a moist environment, e.g. biofilm formation on water pipes, indwelling catheters, and wound colonisation.[11,12] When

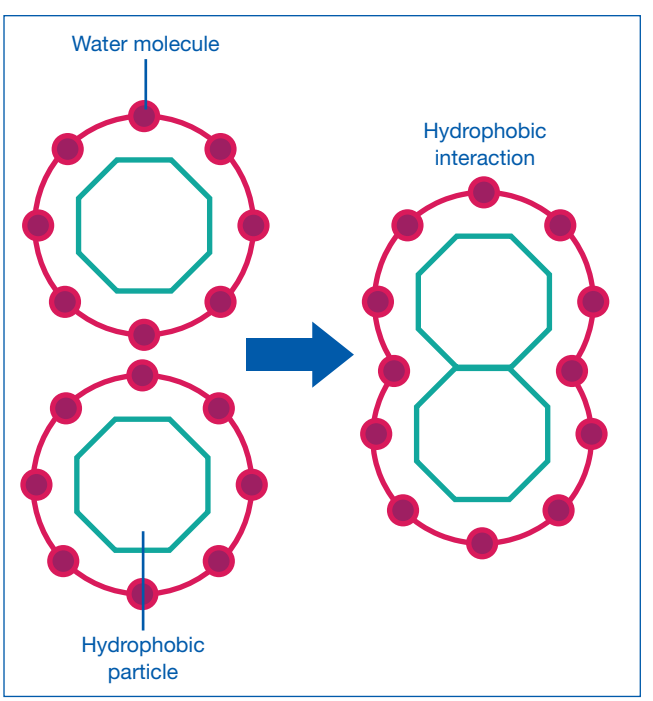


Fig 2. The principle of hydrophobic interaction.

in wet surroundings, many pathogenic microorganisms use hydrophobic interaction to attach to a surface, such as damaged wound tissue or other bacteria, as part of the process of biofilm formation.[11,13,14] These hydrophobic interactions take place when cells exhibit cell-surface hydrophobicity (CSH). The cells, which are located on the extracellular matrix (ECM) of the damaged tissue, bond together and expel water molecules. In this way, the cells aggregate and are held together by the surrounding water molecules (Fig 2). Pathogenic bacteria that exhibit CSH rely on these hydrophobic properties to attach to the wound bed, form colonies[15] and initiate the infective process.[16] Aerobic bacteria such as *Staphylococcus aureus*, including the meticillin-resistant types, and *Streptococcus* spp, anaerobes such as *Peptostreptococci*, and numerous fungi and yeasts all exhibit varying degrees of CSH.[17-19] The extent of CSH varies between bacterial species, as well as between members of the same species. There is also evidence that bacteria within a wound may respond to various environmental conditions by altering their degree of CSH, as well as their toxin production.[20]

The chronic wound with its biofilm, poor wound-bed circulation and subsequent lack of adequate oxygenation provides the ideal environment for the development of CSH in many wound pathogens.[20] The risk of systemic antibiotic toxicity and/or the increased risk of selection for resistance means that antibiotic therapy should be reserved for the treatment or prevention of systemic infection. This, and the cytotoxicity commonly seen with many topical antiseptics, encouraged the development of a new technology to address the problem.[21]

Introducing a new paradigm

As more became known about CSH of bacteria, the possibility of using this characteristic against pathogens became attractive. The use of CSH to bind bacteria to dressing fibres and then remove them from the wound at dressing change introduced a paradigm shift in wound bed management. It was clear this might not only make it possible to prevent biofilm formation, but also to reduce inflammation by eliminating the endotoxin release triggered by the cell-wall disruption of bacteria killed by the use of antiseptics and antibiotics. Common antiseptics and antibiotics work in several ways to kill bacteria. After the bacterial cell wall is ruptured, intracellular antigenic material and cell wall endotoxins are released into the wound fluid. Following the deaths of millions of bacteria, the wound fluid becomes decidedly inflammatory. Trapping bacteria onto a hydrophobic material therefore becomes an attractive way of managing wound bioburden and improving healing.

Hydrophobic dressings that bind bacteria are not commonly referred to as antimicrobials because the microorganisms are not killed by the hydrophobic interaction, but instead are simply collected for removal. The use of a highly hydrophobic dressing material that reduces the microbial load offers an attractive alternative to non-antibiotic treatments or can be used as an additional measure when reducing antibiotic usage in superficial infections.[16,22]

DACC technology

The Cutimed Sorbact range of dressings contains dialkylcarbamoyl chloride (DACC), which is a synthetically produced derivative of a naturally occurring hydrophobic fatty acid found in a spider's web. (Spider silk is partly hydrophobic, which explains why cobwebs cannot be wetted, but instead form droplets of water on their surface.) Cutimed Sorbact dressings are coated with DACC, which mediates the irreversible binding of bacteria that exhibit CSH (Fig 3).

Large numbers of adherent or 'trapped' bacteria can then be removed from the wound at each dressing change (Fig 4). They are removed without disrupting the bacterial cell wall, thereby avoiding the resultant increase in inflammation observed with traditional antibiotics or antiseptics.[22]

Numerous bacteria and fungi that exhibit CSH have been shown to attach to the DACC-coated material, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Candida albicans* and the dermatophyte *Trichophyton rubrum*.[16,23,24] DACC-coated dressings rapidly and effectively bind *Staphylococcus aureus* and *Pseudomonas aeruginosa* within 30 seconds of contact and continue to exhibit effective binding of bacteria for 2 hours.[16,23] Once bound to the DACC coating, bacteria exhibit a decreased rate of replication, slower metabolism, and decreased production of bacterial toxins.[16]

The passive 'trapping mechanism' exhibited by DACC-coated dressings avoids the risk of microbial resistance seen with antibiotics and some antiseptics, which gives it a significant advantage over common antimicrobial dressings. They also avoid release of large amounts of antigenic, inflammatory, intracellular contents into the exudate commonly seen with use of antibiotics and antiseptics.

Cutimed Sorbact differs from silver, iodine, or PHMB-impregnated dressings in that it does not release chemicals into the wound to kill bacteria or kill bacteria absorbed into the dressing. Because there is minimal risk of sensitisation, allergy, systemic absorption, cytotoxicity, microbial resistance, or skin or wound discolouration, The dressing range can be used on patients who are sensitive to other wound dressings and can be safely used for microbial prophylaxis on a long-term basis or until there are no clinical signs of infection and the wound is granulating. It is also safe to use on infants, children, adolescents and the elderly.

The Use of a Novel Dressing Cutimed® Sorbact® in Managing an Infected Wound in a Neonate

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Introduction

Very little wound care research is carried out in children, especially neonates. This is partly due to assumptions that the process of wound healing is the same for children as in adults, but also due to concerns over legal and ethical issues in carrying out research in this population[1].

While it is known that wound healing occurs more rapidly in paediatrics and is generally uneventful, this can easily be compromised by malnutrition, hypotension, oedema or infection. Neonates are at particularly high risk of sepsis which can originate from a bacterial colonisation and proliferation from a wound.

Development of a wound infection after implantation of an epicardial pacemaker is quite a serious complication. The sepsis could lead to the development of a bacteraemia, which in turn could lead to persisting infection due to the foreign body (i.e. the pacemaker lead and generator). Therefore prompt treatment both locally and systemically is required. Once the protective layer of the skin is broken, bacteria may spread rapidly, leading to infection of the deeper tissues, including the sternum. The consequences of such events could be the development of osteomyelitis, and the possibility of endocarditis.

Method

A premature neonate (38 weeks gestation) who was born with congenital complete heart block underwent an implantation of a pacemaker at three days of age. The patient was transferred back to the local hospital two days following surgery, after making an uneventful recovery.

However, three days later the patient was readmitted with a diagnosis of sepsis or necrotising enterocolitis. On admission it was noted that she had a discharging sternotomy wound. It was found the patient was septic. Subsequent investigations revealed that she was MRSA positive for skin and wound swabs. Blood cultures were negative. She was commenced on antibiotics (Vancomycin) and a referral was made to the Tissue Viability service for advice on wound management.

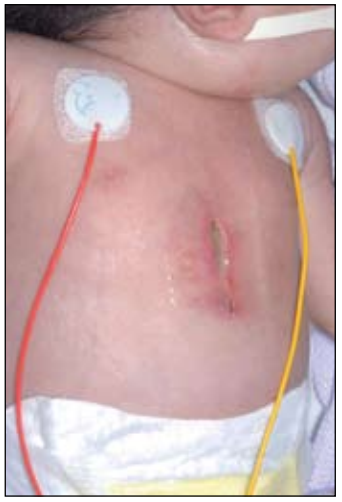
An assessment by the Tissue Viability Nurse Consultant identified the patient as having a critically colonised wound (based on the wound dehiscence, the presence of slough and necrotic tissue, MRSA positive wound swab but minimal local erythema and oedema). The patient was at high risk of further deterioration with severe consequences as described above due to her current sepsis and immuno- compromised state. Trust guidelines on the prevention and management of wound infection advocate the use of a topical antimicrobial as an adjunct to the systemic antibiotics. The dilemma was a choice of product suitable for use with a premature neonate.

While the epidermal and dermal layers of an infant this age are similar, they still remain extremely fragile. The absorption of chemical agents through the skin and wound bed has not been sufficiently studied to enable product manufacturers to provide assurance on safe use in this age group. A novel dressing was chosen Cutimed Sorbact, which binds to bacteria irreversibly without an active chemical agent. The hydrophobic interaction and the special coating of the dressing help to remove the bacterial[2].

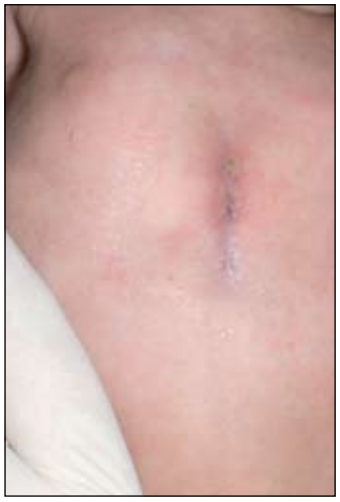
The dressing was applied to the wound in a double layer and covered with a film dressing. This was changed twice a week. She was discharged back to the local hospital twenty five days after her initial readmission.

Results

The use of Cutimed Sorbact was successful in the management of this neonate following MRSA colonisation of her surgical stenotomy wound. An initial photograph taken on 8.10.09 shows the extent of the wound. The wound was being treated with Cutimed Sorbact at this time. The final photograph was taken at the surgical out patient clinic following completion of treatment.



Initial photograph showing extent of wound



Photograph following completion of treatment

Conclusion

An antimicrobial dressing which enhances the normal physiological process of wound healing without affecting fibroblast formation or potential absorption into the tissues has a benefit in reducing the risk of infection[3]. Cutimed Sorbact has very strong hydrophobic properties due to the dialkylcarbamoylchloride (DACC). This physical interaction leaves very little possibility of development of resistance to micro-organisms. The use of this dressing in with this patient contributed to a successful outcome from her infection.

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