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Can dressings soaked with polyhexanide reduce bacterial loads in full-thickness skin grafting? A randomized controlled trial.

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ABSTRACT

Background: Polyhexamethylene biguanide (PHMB)-based antiseptic solutions can reduce bacterial loads in different clinical settings and are believed to lower risk of infections.

Objective: To assess the efficacy of a PHMB-based solution in lowering bacterial loads of full-thickness skin grafting (FTSG) wounds and the risk of SSIs.

Methods: In this double-blinded clinical trial, 40 patients planned for facial FTSG were randomized 1:1 to receive tie-over dressings soaked with either PHMB-based solution or sterile water. Quantitative and qualitative bacterial analysis was performed on all wounds before surgery, at the end of surgery, and 7 days postoperatively. In addition, all patients were screened for nasal colonization of S. aureus.

Results: Analysis of wounds showed no statistically significant difference in bacterial reductions between the groups. The SSI rates were significantly higher in the intervention group (8/20) than in the control group (2/20) (P=.028). Higher postoperative bacterial loads were a common finding in SSIs (P=.011). This was more frequent when S. aureus was present postoperatively (P=.034), intraoperatively (P=.03), and in patients with intranasal S. aureus colonization (P=.007).

Limitations: Assessment of SSIs is largely subjective. In addition, this was a single-center study and the total number of participants was 40.

Conclusion: Soaking tie-over dressings with PHMB-solution in FTSG had no effect on postoperative bacterial loads and increased the risk of SSI development. The presence of S. aureus intranasally and in wounds preoperatively and postoperatively increased postoperative bacterial loads, which in turn resulted in significantly more SSIs.
Key words: Surgical site infections; dermatologic surgery; pathogenesis; prevention; wound infection; bacteria; *S. aureus*

Classifications:
- 212: Bacterial infections
- 790: Evidence-based medicine
- 1239: Infection
- 1660: Microbiology
- 2170: Prevention
- 2520: Surgery
- 2780: Wounds & wound healing
Capsule summary:

- PHMB as an antiseptic has gained popularity in different clinical settings but hasn’t yet been studied in full-thickness skin grafting (FTSG).

- This trial showed that adding PHMB to tie-over dressings had no effect on reducing bacterial loads in wounds and resulted in more surgical site infections.

- Use of PHMB in FTSG as a method to prevent SSIs is questionable, and further clinical studies are warranted.
INTRODUCTION

Polyhexamethylene biguanide (PHMB) is a polymer used as a disinfectant and antiseptic.\(^1\)\(^-\)\(^6\) In recent years, it has gained popularity and has been used safely in different clinical settings such as in intraoperative irrigation during nail surgery\(^1\), treatment of burns\(^2\), orthopedic surgery antisepsis\(^6\), wound dressings\(^3\), prevention of infections in peritoneal catheters\(^4\), and in combination with negative-pressure wound therapy (NPWT) where it has been shown to be better than NPWT alone in treating infected wounds.\(^7\)

The advantages of PHMB include broad antibacterial activity, good cell and tissue tolerability, low risk of contact sensitization, promotion of wound healing, and no development of bacterial resistance.\(^2\) In addition to having an effect on Gram-negative bacteria\(^8\), it also has effects against methicillin-resistant *Staphylococcus aureus* (MRSA).\(^9\) The microbicidal effect of PHMB is comparable to that of chlorhexidine\(^10\), but does not contain the toxic substituents found in chlorhexidine.\(^11\)

In this study we investigated whether a PHMB-based antiseptic solution added to tie-over dressings used in full-thickness skin grafting (FTSG) could reduce bacterial load of wounds. This is a factor believed to have a role in the development of surgical site infections (SSIs) as previously published by our group.\(^12\) We hypothesized that a reduction in the bacterial load would lower the risk of SSIs. We were also interested in examining the presence of *S. aureus* intranasally and wanted to study its relevance for SSIs. Recent studies have indicated that nasal colonization with *S. aureus* is an important risk factor for development of SSIs.\(^13\)\(^-\)\(^15\) By analyzing bacterial quantities
and species at different stages of surgery, we sought to improve our understanding of
the development of SSIs and its complex pathogenesis.

METHODS

Study Design
We conducted this prospective, double-blinded, randomized, placebo-controlled trial
between September 2014 and September 2015 at Lund University Hospital, Sweden.
This single-center study was approved by the ethical committee in Malmö/Lund,
registration number (2013/762) and registered with ClinicalTrials.gov
(NCT02253069). All patients over age 18 planned for facial FTSG were allowed to
participate in the trial. We limited inclusion to surgery localized to the face because
bacterial loads are known to vary from one anatomical site to another. All grafts
were harvested from the neck region. Exclusion criteria were diabetes, treatment with
antibiotics within the last four weeks prior to surgery, and planned antibiotic therapy.
Written informed consent was obtained from all patients before enrollment. The same
nurse prepared all patients for surgery, which included using a 0.5% chlorhexidine
solution for preoperative skin preparation. Four dermatologists performed surgery
under routine sterile conditions. One principal investigator was in charge of collecting
bacterial samples and assessing wounds postoperatively.

Power analysis and randomization
In a previous in vitro study, a reduction of >5 log_{10} was achieved with a concentration
of 0.02% PHMB against S. aureus. We hypothesized that application of 0.1%
PHMB as found in the commercially available Prontosan® Wound irrigation solution
(B. Braun Medical, Switzerland) would at least reduce bacterial load in wounds by
half versus placebo. To get 80% power with an \( \alpha \)-value of 0.05, it was calculated that 16 patients were required in each group. By including 20 patients in each group in this trial to allow for dropouts, noticeable differences in bacterial reduction would be detected. Patients were randomized according to a list generated using QuickCalcs (www.graphpad.com/quickcalcs).

**In vitro antibacterial assay**

Prior to this trial, *in vitro* experiments were performed to assess antibacterial activity of PHMB. See Supplementary Methods.

**Intervention**

At the end of each surgery, once the skin graft had been sutured to the wound, a tie-over dressing was cut from Mepilex\textsuperscript{®}. It was then soaked with either Prontosan\textsuperscript{®} solution or sterile water (see Supplementary Methods for details) according to the randomization protocol.

**Follow up**

All patients were planned for a single follow up 7 days after surgery. Skin grafts were assessed in terms of redness, edema, discharge, graft take, and pain resulting in an overall assessment by the blinded principal investigator classifying a wound as "infected" or "non-infected". No scoring system was used for this purpose. Digital photographs were taken of all wounds pre- and postoperatively.

**Bacterial load analysis**
Bacterial samples were blindly collected from each patient using Eswabs (Copan, Brescia, Italy). Swabs were taken in a controlled manner by swabbing in a circular motion for 10 seconds. This was done at 3 different phases. Before surgery (BS) prior to antisepsis, the skin area containing the suspected neoplasm planned for excision was swabbed to establish the starting bacterial load level. Next, at the end of surgery (ES), the skin graft sutured to the wound was swabbed to establish a second starting load level. A final swab was taken from the wound one week after surgery (1W) after removal of the tie-over dressing.

Each swab was analyzed quantitatively by counting CFU per cm$^2$ of area swabbed as well as the type of bacteria present. Bacterial quantification was done by serially diluting each swab to 3 different concentrations plating each concentrate onto a Todd-Hewitt agar plate using sterile glass beads and incubating all plates in 5% CO$_2$ at 37°C for 24 h. The CFU were then counted and were usually between 30 and 300 CFUs. The CFU was divided with the swab area to measure bacterial loads in CFU/cm$^2$. Bacterial species were determined via matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry.

**Intranasal swabs**

Before surgery, an Eswab was rotated in the patient’s naris that was closest to the neoplasm planned for excision. Typing was performed using MALDI-TOF to detect presence of *S. aureus*. No quantification was done on these swabs.

**Statistics**

Statistical analyses were performed with SPSS v.22 software (SPSS Inc., Chicago, IL). Bacterial load reduction was determined by using the following formulas:
CFU(1W)-CFU(BS), CFU(1W)-CFU(ES), CFU(1W)/CFU(BS), and CFU(1W)/CFU(ES). All median values obtained were compared using a Mann-Whitney U test to examine if differences existed between the groups. Differences in categorical variables were determined using the chi-square test. Differences in continuous variables were estimated using Student’s t test. Statistical significance was set at $P < .05$.

Outcome measures

Our primary measure was to compare bacterial load reductions in both groups. The development of SSIs was a secondary outcome in this trial, and the tertiary outcome was the intranasal presence of *S. aureus* and examining its relevance for the bacterial dynamics of surgical wounds.

RESULTS

Our *in vitro* trials showed that only dressings soaked with PHMB inhibited growth of both *S. aureus* and *S. epidermidis* (Supplementary Figure 1). This was in accordance with previously published studies demonstrating antibacterial properties of PHMB against various skin bacteria. As for this trial, there were no significant differences in patient characteristics in each group in terms of age, sex, wound location, and tumor excised (Supplementary table 1). Most wounds were located on the nose, which is known to be the most common site of skin malignancies. No significant differences were noted among the groups in bacterial load levels measured before surgery, at end of surgery, and after one week. (Supplementary Table 2). No significant differences were detected between the groups in terms of bacterial reduction via the four calculations described in Methods (Supplementary Table 2).
A total of 10 wounds were assessed as infected to give an overall SSI rate of 25% in this study. Eight of these wounds belonged to the intervention group, which had a statistically higher rate of infection (chi-square 4.8, \( P = .028 \)). Statistical analyses showed that patient characteristics such as gender, age, and wound location did not correlate to SSI rates in this study. All patients with SSIs had a significantly higher bacterial load measured postoperatively after one week as illustrated in Figure 1A. When \( S. \) aureus was isolated from wounds postoperatively after one week, patients had a significantly higher bacterial load (Figure 1B). The presence of \( S. \) aureus intranasally before surgery was also associated with a higher postoperative bacterial load (Figure 1C). Whether coagulase-negative staphylococci (CoNS) were isolated from wounds postoperatively or not had no effect on postoperative bacterial loads, although a higher spread in the total CFUs was observed (Figure 2A). The presence of \( S. \) aureus at the end of surgery in patients resulted in significantly higher postoperative bacterial loads (Figure 2B).

Typing of all strains isolated from swabs revealed that CoNS and \( S. \) aureus were the predominant species (Table 1). The number of species successfully isolated from all patients was highest in in the swabs before surgery (27 different species) and lowest one week after surgery (8 species). Four out of 10 infected wounds contained \( S. \) aureus.

**DISCUSSION**

SSIs in dermatologic surgery result in unnecessary health costs as well as added pain, discomfort, and dissatisfactory cosmetic outcomes for patients.\(^{22,23}\) Furthermore, the
use of preventative measures such as antibiotic prophylaxis, although sometimes warranted, can contribute to the emergence of resistant bacterial strains and give unwanted side effects, such as allergic reactions in patients. Effective evidence-based measures are therefore highly needed—especially in FTSG surgery, which is normally associated with a higher rate of SSI.

In this randomized controlled trial, we tested the efficacy of PHMB in preventing SSIs. Our results show that PHMB had no effect on reducing postoperative bacterial loads. Surprisingly, adding PHMB to tie-over dressings resulted in a significantly higher risk of SSI. Previous studies have shown that applying a certain antibacterial agent locally to wounds can suppress the growth of certain bacterial species, which can cause an overgrowth of other species that might be harmful. Although speculative, it is possible that PHMB, by reducing the commensal flora, i.e. the microbiome, could give rise to an increased colonization of \textit{S. aureus} or other pathogens. Indeed, there appeared to be a higher spread in the bacterial levels when \textit{S. epidermidis} was absent postoperatively (Fig. 2A), and Gram-negative bacterial species were particularly detected in the PHMB-treated group one week after surgery (Table 1), findings suggestive of possible microbiome changes induced by PHMB. Clearly, the limited number of patients enrolled in this study makes it impossible to draw any firm conclusions on the protective role of commensals and the role of PHMB. However, it is worth noting that the microbiome has recently been attributed with important roles in protection against infections. For example, \textit{Staphylococcus epidermidis} can produce antimicrobials, which can keep potential pathogens at bay. \textit{S. epidermidis} can also activate toll-like-receptor-2 (TLR2) signaling and induce
antimicrobial peptide expression, thus enabling the skin to mount an enhanced response to pathogens.  

We found 27 different bacterial species before surgery making it impossible to analyze which particular species could be responsible for increasing the risk of SSIs from a statistical point of view. A quantification of each particular species would be necessary to investigate this further. Here, only the total quantity of all bacteria in a swab was measured. Nevertheless, it was interesting to note that the variation of bacterial species was highest prior to surgery and lowest postoperatively in both groups. Yet in 24 out of 40 patients, bacterial loads were higher postoperatively than preoperatively. It appears that certain species exhibits a stronger tendency to grow directly after surgery. Further studies in larger patient groups are needed to verify this observation. Another result was that the bacterial species observed here agreed well with previously published studies showing that most frequently isolated species from wounds are *S. aureus* and CoNS.

In this trial, we established two different starting bacterial loads due to the nature of FTSG surgery where skin is moved from one anatomical site to another. Comparing postoperative bacterial loads present on a graft to the presurgical swab taken on anatomically different skin would be unfair. We therefore compared the postoperative bacterial loads levels with the levels observed before and at end of surgery. Our analyses showed that the PHMB-based dressing had no effect on reducing postoperative bacterial loads. Indeed, there was actually a tendency towards higher loads one week after surgery in the intervention group compared to the control group. The extensive variety of bacterial species found preoperatively (27 different species)
is yet another interesting finding. We could only compare these data to the variety present postoperatively (8 different species). Thus, this difference could again be attributed to the anatomical skin flora variations *per se* at the donor sites or to the microbiome and host defense changes as mentioned above. Another theory in line with a recent publication $^{31}$ is that the presence of a neoplasm in the swab taken preoperatively is somehow related to a high bacterial variety.

We validated our previously published findings $^{12}$ and showed that a total postoperative bacterial load correlates positively to wound infection. Furthermore, postoperative bacterial loads were shown to be significantly higher when *S. aureus* was present in wounds intra- and postoperatively as well as in patients who had a nasal colonization with *S. aureus* detected prior to surgery. However, there was no direct relationship between presence of *S. aureus* in wounds, or intranasally, and SSIs. Still, *S. aureus* appears to continue to be one of the key pathogens involved in the development of SSIs. The presence of CoNS in wounds on the other hand seems to reduce the tendency towards developing an SSI by a reduced postoperative bacterial load. However, this observation was not statistically significant ($P=.08$) as shown in Figure 2a. Although speculative, it is thus possible that an expanded preoperative screening of bacteria present preoperatively—not only in the nares, but also at the surgical site—could aid in the prediction of SSIs. It is also possible that boosting of the "healthy" microbiome—including *S. epidermidis*—could be beneficial for wound healing outcomes and in ongoing *in vitro* based experiments. Thus, we therefore are currently evaluating the effects of both commensal and pathogenic bacteria in skin models.
A limitation of our study is that one of our outcomes (diagnosis of SSIs) was dependent on a subjective assessment of a single investigator. Studies have shown both inter- and intra-observer variations when diagnosing SSIs. These show the importance of finding a more objective method of diagnosing SSIs in the future. Nevertheless, the SSI scoring was performed in a blinded fashion to avoid potential bias between the groups. Other limitations were that this was a single-center study and that the total number of participants in the study was 40.

**CONCLUSION**

We used PHMB as a novel disinfectant to prevent SSIs in FTSG. PHMB appeared to increase the risk of SSIs at least in the experimental setting used here. In light of the emergence of new resistant bacterial strains that cause SSIs, there is a need for further research that can define preventative methods to improve outcomes. Measures that lower bacterial loads, prevent *S. aureus* regrowth in wounds and abolish intranasal colonization are important and ongoing.

**Acknowledgments**

We are greatly indebted to Mina Davoudi, Emma Matsson, Ann-Charlotte Strömdahl, and Dr. Ingrid Siemund for their efforts in conducting the study. We also wish to thank the nursing staff (Eva Jacobsson, Helene Palmqvist, Susanne Erdmann) and Åse Jönsson at our clinic for valuable assistance making this trial possible.
Abbreviations used:

SSI: Surgical site infection

FTSG: Full-thickness skin grafting

PHMB: Polyhexamethylene biguanide

NPWT: Negative-pressure wound therapy

MRSA: Methicillin-resistant *Staphylococcus aureus*

CFU: Colony-forming-unit

MALDI-TOF: Matrix-assisted laser desorption/ionization time-of-flight

TLR2: Toll-like-receptor-2

CoNS: Coagulase-negative staphylococcus


Figure 1. Postoperative bacterial loads after one week shown for each patient group (controls and PHMB) or all patients combined. (A) Differences between wounds classified as infected and non-infected. (B) Differences in regard to presence of *S. aureus* in wounds at one week after surgery. (C) Levels correlated to presence of *S. aureus* intranasally. Outliers in all plots are indicated by an asterisk (*). Solid bars depict interquartile range and the hash marks show the total range. A difference in median CFU/cm² calculated using Mann-Whitney’s test with a *P* value of <.05 is regarded as statistically significant.
Figure 2. Bacterial loads at one week after surgery measured in all patients whether (A) CoNS were isolated postoperatively and whether (B) S. aureus was isolated at end of surgery. The outliers were expressed with an asterisk (*). Solid bars depict interquartile range and the hash marks show the total range. Calculations of median CFU/cm² values using a Mann-Whitney test with a P value of <.05 were regarded as statistically significant.
Table 1. Bacterial species isolated before surgery (BS), after one week (1W), and intranasally (Nasal). Each row represents a patient. An asterisk (*) in the beginning of each row indicates patients developing an SSI.
SUPPLEMENTARY DATA

METHODS

In vitro antibacterial assay

Todd-Hewitt (TH) agar plates were streaked with *S. aureus* ATCC 29213 and *S. epidermidis* ATCC 14909. Each plate contained $1 \times 10^5$ colony-forming units (CFU).

Eight mm polyurethane dressings (Mepilex®, Mölnlycke Healthcare, Göteborg, Sweden) soaked with Prontosan® solution or sterile water were applied on top to simulate an *in vivo* situation where the dressing is applied onto a wound.

The dressings were soaked with 70% of the solution, where 100% was considered as the maximum wetting capacity of the dressing. 70% wetting was also to be used in this patient trial. The zone of inhibition around the discs was measured.

Preparation of Mepilex® dressings

Prior to surgery, seven circular dressing templates with varying diameters ranging from 10 mm to 34 mm were cut from Mepilex®. Necessary liquid volume to achieve 70% wetting was calculated by subtracting each template’s fully saturated weight from its dry weight and multiplying the result by 0.7. For each dressing template, 20 test tubes were prepared containing sterile water and 20 test tubes contained Prontosan® solution. These were marked with either A or B by an external investigator not involved in this trial and blinded to the nurse, surgeon, and principal investigator. Prontosan® solution is like water both colorless and odor-free. The dressing templates were used for proper determination of the volume of Prontosan® or sterile water required for wetting tie-over dressings used during surgery.
Figure 1. *In vitro* antibacterial assays illustrating measured inhibition zones of dressings soaked with water (control) or PHMB on agar plates coated with 1x10^5 CFU of (A) *S. aureus*, and (B) *S. epidermidis* (n=3, bar indicates S...
<table>
<thead>
<tr>
<th>Item</th>
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<th>Control group</th>
<th>$P$ value</th>
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<tr>
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<tr>
<td>Range</td>
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<tr>
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<td>78.20 ± 13.05</td>
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<tr>
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</tr>
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BCC: Basal cell carcinoma. SCC: Squamous cell carcinoma.

**Table 1.** Patient characteristics and selected baseline values.
<table>
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<th>Control Group</th>
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Table 2. Bacterial quantification of all swabs taken before surgery (BS), at end of surgery (ES), and after one week (1W).