



LUND UNIVERSITY

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

Saleh, Karim; Sonesson, Andreas; Persson, Kerstin; Riesbeck, Kristian; Schmidtchen, Artur

Published in:

Journal of the American Academy of Dermatology

DOI:

[10.1016/j.jaad.2016.07.020](https://doi.org/10.1016/j.jaad.2016.07.020)

2016

Document Version:

Peer reviewed version (aka post-print)

[Link to publication](#)

Citation for published version (APA):

Saleh, K., Sonesson, A., Persson, K., Riesbeck, K., & Schmidtchen, A. (2016). Can dressings soaked with polyhexanide reduce bacterial loads in full-thickness skin grafting? A randomized controlled trial. *Journal of the American Academy of Dermatology*, 75(6), 1221-1228.e4. <https://doi.org/10.1016/j.jaad.2016.07.020>

Total number of authors:

5

General rights

Unless other specific re-use rights are stated the following general rights apply:

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

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

3 Karim Saleh, MD^{1*}, Andreas Sonesson, MD, PhD¹, Kerstin Persson, BS¹, Kristian
4 Riesbeck, MD, PhD², Artur Schmidtchen, MD, PhD^{1,3}

5

6 ¹Division of Dermatology and Venereology, Department of Clinical Sciences, Lund
7 University, Skane University Hospital, Lund, Sweden

8 ²Clinical Microbiology, Department of Translational Medicine, Lund University,
9 Malmö, Sweden

10 ³LKC Medicine, Nanyang Technological University, Singapore

11

12 *Correspondence: Division of Dermatology, Department of Clinical Sciences,
13 Biomedical Center B14, Lund University, Tornavägen 10, SE-221 84 Lund, Sweden.
14 Tel: +46 46 222 33 15. Fax: +46 46 15 77 56. Email: Karim.Saleh@med.lu.se

15

16 Word counts

17 Abstract: 240

18 Capsule summary: 65

19 Text: 2447

20 Number of references: 32

21 Tables: 1

22 Figures: 2

23 Supplementary tables and figures: 3

24

25 Ethical approval was granted by the ethical committee in Malmö/Lund, registration
26 number (2013/762).

27 Registered at www.clinicaltrials.gov. **ClinicalTrials.gov Identifier:** NCT02253069

28

29 This article was funded by the program "Innovation mot infektion" (IMI), financed by
30 the VINNOVA- Swedish governmental agency for innovation systems, the Swedish
31 Government Funds for Clinical Research (ALF), and The Swedish Research Council
32 (2012-1883).

33

34 Dr. Schmidtchen has received consulting support from Mölnlycke Health Care AB.

35

36

37

38

39

40

41

42

43

44

45

46

47

48

49 **ABSTRACT**

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

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

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

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

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

68 **Conclusion:** Soaking tie-over dressings with PHMB-solution in FTSG had no effect
69 on postoperative bacterial loads and increased the risk of SSI development. The
70 presence of *S. aureus* intranasally and in wounds preoperatively and postoperatively
71 increased postoperative bacterial loads, which in turn resulted in significantly more
72 SSIs.

73

74 Key words: Surgical site infections; dermatologic surgery; pathogenesis; prevention;

75 wound infection; bacteria; *S. aureus*

76

77 Classifications:

78 212: Bacterial infections

79 790: Evidence-based medicine

80 1239: Infection

81 1660: Microbiology

82 2170: Prevention

83 2520: Surgery

84 2780: Wounds & wound healing

85

86

87

88

89

90

91

92

93

94

95

96

97

98

99 Capsule summary:

- 100 • PHMB as an antiseptic has gained popularity in different clinical settings but
101 hasn't yet been studied in full-thickness skin grafting (FTSG).
- 102 • This trial showed that adding PHMB to tie-over dressings had no effect on
103 reducing bacterial loads in wounds and resulted in more surgical site
104 infections.
- 105 • Use of PHMB in FTSG as a method to prevent SSIs is questionable, and
106 further clinical studies are warranted.

107

108

109

110

111

112

113

114

115

116

117

118

119

120

121

122

123

124 INTRODUCTION

125 Polyhexamethylene biguanide (PHMB) is a polymer used as a disinfectant and
126 antiseptic.¹⁻⁶ In recent years, it has gained popularity and has been used safely in
127 different clinical settings such as in intraoperative irrigation during nail surgery¹,
128 treatment of burns⁵, orthopedic surgery antiseptics⁶, wound dressings³, prevention of
129 infections in peritoneal catheters⁴, and in combination with negative-pressure wound
130 therapy (NPWT) where it has been shown to be better than NPWT alone in treating
131 infected wounds.⁷

132

133 The advantages of PHMB include broad antibacterial activity, good cell and tissue
134 tolerability, low risk of contact sensitization, promotion of wound healing, and no
135 development of bacterial resistance.² In addition to having an effect on Gram-
136 negative bacteria⁸, it also has effects against methicillin-resistant *Staphylococcus*
137 *aureus* (MRSA).⁹ The microbicidal effect of PHMB is comparable to that of
138 chlorhexidine¹⁰, but does not contain the toxic substituents found in chlorhexidine.¹¹

139

140 In this study we investigated whether a PHMB-based antiseptic solution added to tie-
141 over dressings used in full-thickness skin grafting (FTSG) could reduce bacterial load
142 of wounds. This is a factor believed to have a role in the development of surgical site
143 infections (SSIs) as previously published by our group.¹² We hypothesized that a
144 reduction in the bacterial load would lower the risk of SSIs. We were also interested
145 in examining the presence of *S. aureus* intranasally and wanted to study its relevance
146 for SSIs. Recent studies have indicated that nasal colonization with *S. aureus* is an
147 important risk factor for development of SSIs.¹³⁻¹⁵ By analyzing bacterial quantities

148 and species at different stages of surgery, we sought to improve our understanding of
149 the development of SSIs and its complex pathogenesis.

150

151 **METHODS**

152 **Study Design**

153 We conducted this prospective, double-blinded, randomized, placebo-controlled trial
154 between September 2014 and September 2015 at Lund University Hospital, Sweden.
155 This single-center study was approved by the ethical committee in Malmö/Lund,
156 registration number (2013/762) and registered with ClinicalTrials.gov
157 (NCT02253069). All patients over age 18 planned for facial FTSG were allowed to
158 participate in the trial. We limited inclusion to surgery localized to the face because
159 bacterial loads are known to vary from one anatomical site to another.¹⁶ All grafts
160 were harvested from the neck region. Exclusion criteria were diabetes, treatment with
161 antibiotics within the last four weeks prior to surgery, and planned antibiotic therapy.
162 Written informed consent was obtained from all patients before enrollment. The same
163 nurse prepared all patients for surgery, which included using a 0.5% chlorhexidine
164 solution for preoperative skin preparation. Four dermatologists performed surgery
165 under routine sterile conditions. One principal investigator was in charge of collecting
166 bacterial samples and assessing wounds postoperatively.

167

168 **Power analysis and randomization**

169 In a previous *in vitro* study, a reduction of $>5 \log_{10}$ was achieved with a concentration
170 of 0.02% PHMB against *S. aureus*.¹⁰ We hypothesized that application of 0.1%
171 PHMB as found in the commercially available Prontosan[®] Wound irrigation solution
172 (B. Braun Medical, Switzerland) would at least reduce bacterial load in wounds by

173 half versus placebo. To get 80% power with an α -value of 0.05, it was calculated
174 that 16 patients were required in each group. By including 20 patients in each group
175 in this trial to allow for dropouts, noticeable differences in bacterial reduction would
176 be detected. Patients were randomized according to a list generated using QuickCalcs
177 (www.graphpad.com/quickcalcs).

178

179 ***In vitro* antibacterial assay**

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

182

183 **Intervention**

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

188

189 **Follow up**

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

195

196 **Bacterial load analysis**

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

205 Each swab was analyzed quantitatively by counting CFU per cm² of area swabbed as
206 well as the type of bacteria present. Bacterial quantification was done by serially
207 diluting each swab to 3 different concentrations plating each concentrate onto a Todd-
208 Hewitt agar plate using sterile glass beads and incubating all plates in 5% CO₂ at
209 37°C for 24 h. The CFU were then counted and were usually between 30 and 300
210 CFUs. The CFU was divided with the swab area to measure bacterial loads in
211 CFU/cm². Bacterial species were determined via matrix-assisted laser
212 desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry.

213

214 **Intranasal swabs**

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

218

219 **Statistics**

220 Statistical analyses were performed with SPSS v.22 software (SPSS Inc., Chicago,
221 IL). Bacterial load reduction was determined by using the following formulas:

222 CFU(1W)-CFU(BS), CFU(1W)-CFU(ES), CFU(1W)/CFU(BS), and
223 CFU(1W)/CFU(ES). All median values obtained were compared using a Mann-
224 Whitney U test to examine if differences existed between the groups. Differences in
225 categorical variables were determined using the chi-square test. Differences in
226 continuous variables were estimated using Student's *t* test. Statistical significance was
227 set at $P < .05$.

228

229 **Outcome measures**

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

234

235 **RESULTS**

236 Our *in vitro* trials showed that only dressings soaked with PHMB inhibited growth of
237 both *S. aureus* and *S. epidermidis* (Supplementary Figure 1). This was in accordance
238 with previously published studies demonstrating antibacterial properties of PHMB
239 against various skin bacteria.¹⁷⁻²⁰ As for this trial, there were no significant differences
240 in patient characteristics in each group in terms of age, sex, wound location, and
241 tumor excised (Supplementary table 1). Most wounds were located on the nose, which
242 is known to be the most common site of skin malignancies.²¹ No significant
243 differences were noted among the groups in bacterial load levels measured before
244 surgery, at end of surgery, and after one week. (Supplementary Table 2). No
245 significant differences were detected between the groups in terms of bacterial
246 reduction via the four calculations described in Methods (Supplementary Table 2).

247

248 A total of 10 wounds were assessed as infected to give an overall SSI rate of 25% in
249 this study. Eight of these wounds belonged to the intervention group, which had a
250 statistically higher rate of infection (chi-square 4.8, $P=.028$). Statistical analyses
251 showed that patient characteristics such as gender, age, and wound location did not
252 correlate to SSI rates in this study. All patients with SSIs had a significantly higher
253 bacterial load measured postoperatively after one week as illustrated in Figure 1A.
254 When *S. aureus* was isolated from wounds postoperatively after one week, patients
255 had a significantly higher bacterial load (Figure 1B). The presence of *S. aureus*
256 intranasally before surgery was also associated with a higher postoperative bacterial
257 load (Figure 1C). Whether coagulase-negative staphylococci (CoNS) were isolated
258 from wounds postoperatively or not had no effect on postoperative bacterial loads,
259 although a higher spread in the total CFUs was observed (Figure 2A). The presence of
260 *S. aureus* at the end of surgery in patients resulted in significantly higher
261 postoperative bacterial loads (Figure 2B).

262

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

268

269 **DISCUSSION**

270 SSIs in dermatologic surgery result in unnecessary health costs as well as added pain,
271 discomfort, and dissatisfactory cosmetic outcomes for patients.^{22,23} Furthermore, the

272 use of preventative measures such as antibiotic prophylaxis, although sometimes
273 warranted, can contribute to the emergence of resistant bacterial strains and give
274 unwanted side effects, such as allergic reactions in patients.²⁴ Effective evidence-
275 based measures are therefore highly needed—especially in FTSG surgery, which is
276 normally associated with a higher rate of SSI.²⁵

277

278 In this randomized controlled trial, we tested the efficacy of PHMB in preventing
279 SSIs. Our results show that PHMB had no effect on reducing postoperative bacterial
280 loads. Surprisingly, adding PHMB to tie-over dressings resulted in a significantly
281 higher risk of SSI. Previous studies have shown that applying a certain antibacterial
282 agent locally to wounds can suppress the growth of certain bacterial species, which
283 can cause an overgrowth of other species that might be harmful.²⁶ Although
284 speculative, it is possible that PHMB, by reducing the commensal flora, *i.e.* the
285 microbiome, could give rise to an increased colonization of *S. aureus* or other
286 pathogens. Indeed, there appeared to be a higher spread in the bacterial levels when *S.*
287 *epidermidis* was absent postoperatively (Fig. 2A), and Gram-negative bacterial
288 species were particularly detected in the PHMB-treated group one week after surgery
289 (Table 1), findings suggestive of possible microbiome changes induced by PHMB.
290 Clearly, the limited number of patients enrolled in this study makes it impossible to
291 draw any firm conclusions on the protective role of commensals and the role of
292 PHMB. However, it is worth noting that the microbiome has recently been attributed
293 with important roles in protection against infections. For example, *Staphylococcus*
294 *epidermidis* can produce antimicrobials, which can keep potential pathogens at bay.²⁷
295 *S. epidermidis* can also activate toll-like-receptor-2 (TLR2) signaling and induce

296 antimicrobial peptide expression, thus enabling the skin to mount an enhanced
297 response to pathogens.^{28,29}

298

299 We found 27 different bacterial species before surgery making it impossible to
300 analyze which particular species could be responsible for increasing the risk of SSIs
301 from a statistical point of view. A quantification of each particular species would be
302 necessary to investigate this further. Here, only the total quantity of all bacteria in a
303 swab was measured. Nevertheless, it was interesting to note that the variation of
304 bacterial species was highest prior to surgery and lowest postoperatively in both
305 groups. Yet in 24 out of 40 patients, bacterial loads were higher postoperatively than
306 preoperatively. It appears that certain species exhibits a stronger tendency to grow
307 directly after surgery. Further studies in larger patient groups are needed to verify this
308 observation. Another result was that the bacterial species observed here agreed well
309 with previously published studies showing that most frequently isolated species from
310 wounds are *S. aureus* and CoNS.³⁰

311

312 In this trial, we established two different starting bacterial loads due to the nature of
313 FTSG surgery where skin is moved from one anatomical site to another. Comparing
314 postoperative bacterial loads present on a graft to the presurgical swab taken on
315 anatomically different skin would be unfair. We therefore compared the postoperative
316 bacterial loads levels with the levels observed before and at end of surgery. Our
317 analyses showed that the PHMB-based dressing had no effect on reducing
318 postoperative bacterial loads. Indeed, there was actually a tendency towards higher
319 loads one week after surgery in the intervention group compared to the control group.
320 The extensive variety of bacterial species found preoperatively (27 different species)

321 is yet another interesting finding. We could only compare these data to the variety
322 present postoperatively (8 different species). Thus, this difference could again be
323 attributed to the anatomical skin flora variations *per se* at the donor sites or to the
324 microbiome and host defense changes as mentioned above. Another theory in line
325 with a recent publication³¹ is that the presence of a neoplasm in the swab taken
326 preoperatively is somehow related to a high bacterial variety.

327

328 We validated our previously published findings¹² and showed that a total
329 postoperative bacterial load correlates positively to wound infection. Furthermore,
330 postoperative bacterial loads were shown to be significantly higher when *S. aureus*
331 was present in wounds intra- and postoperatively as well as in patients who had a
332 nasal colonization with *S. aureus* detected prior to surgery. However, there was no
333 direct relationship between presence of *S. aureus* in wounds, or intranasally, and SSIs.
334 Still, *S. aureus* appears to continue to be one of the key pathogens involved in the
335 development of SSIs. The presence of CoNS in wounds on the other hand seems to
336 reduce the tendency towards developing an SSI by a reduced postoperative bacterial
337 load. However, this observation was not statistically significant ($P=.08$) as shown in
338 Figure 2a. Although speculative, it is thus possible that an expanded preoperative
339 screening of bacteria present preoperatively—not only in the nares, but also at the
340 surgical site—could aid in the prediction of SSIs. It is also possible that boosting of
341 the "healthy" microbiome—including *S. epidermidis*—could be beneficial for wound
342 healing outcomes and in ongoing *in vitro* based experiments. Thus, we therefore are
343 currently evaluating the effects of both commensal and pathogenic bacteria in skin
344 models.

345

346 A limitation of our study is that one of our outcomes (diagnosis of SSIs) was
347 dependent on a subjective assessment of a single investigator. Studies have shown
348 both inter- and intra-observer variations when diagnosing SSIs ³². These show the
349 importance of finding a more objective method of diagnosing SSIs in the future.
350 Nevertheless, the SSI scoring was performed in a blinded fashion to avoid potential
351 bias between the groups. Other limitations were that this was a single-center study
352 and that the total number of participants in the study was 40.

353

354 **CONCLUSION**

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

361

362 **Acknowledgments**

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

367

368

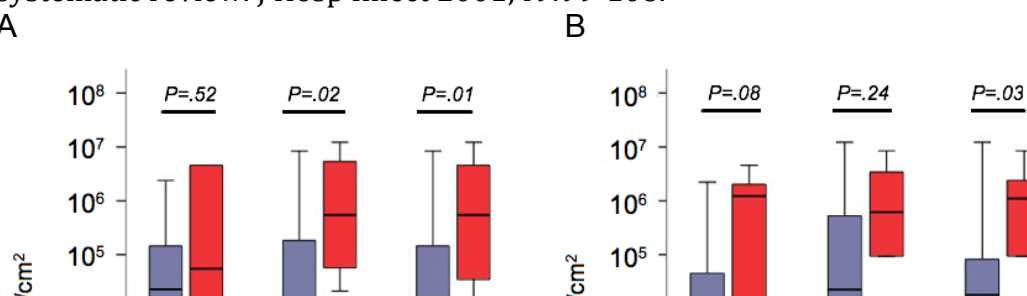
369

370

- 371 Abbreviations used:
- 372 SSI: Surgical site infection
- 373 FTSG: Full-thickness skin grafting
- 374 PHMB: Polyhexamethylene biguanide
- 375 NPWT: Negative-pressure wound therapy
- 376 MRSA: Methicillin-resistant *Staphylococcus aureus*
- 377 CFU: Colony-forming-unit
- 378 MALDI-TOF: Matrix-assisted laser desorption/ionization time-of-flight
- 379 TLR2: Toll-like-receptor-2
- 380 CoNS: Coagulase-negative staphylococcus
- 381
- 382
- 383
- 384
- 385
- 386
- 387
- 388
- 389
- 390
- 391
- 392
- 393
- 394
- 395
- 396

- 397 1. Becerro de Bengoa Vallejo R, Losa Iglesias ME, Cervera LA, Fernandez DS,
398 Prieto JP. Efficacy of intraoperative surgical irrigation with polihexanide and
399 nitrofurazone in reducing bacterial load after nail removal surgery. *J Am Acad*
400 *Dermatol* 2011;64:328-35.
- 401 2. Eberlein T, Assadian O. Clinical use of polihexanide on acute and chronic
402 wounds for antisepsis and decontamination. *Skin Pharmacol Physiol* 2010;23
403 *Suppl*:45-51.
- 404 3. Eberlein T, Haemmerle G, Signer M, et al. Comparison of PHMB-containing
405 dressing and silver dressings in patients with critically colonised or locally
406 infected wounds. *J Wound Care* 2012;21:12, 4-6, 8-20.
- 407 4. Nunez-Moral M, Sanchez-Alvarez E, Gonzalez-Diaz I, et al. Exit-site
408 infection of peritoneal catheter is reduced by the use of polyhexanide. results of a
409 prospective randomized trial. *Perit Dial Int* 2014;34:271-7.
- 410 5. Piatkowski A, Drummer N, Andriessen A, Ulrich D, Pallua N. Randomized
411 controlled single center study comparing a polyhexanide containing bio-cellulose
412 dressing with silver sulfadiazine cream in partial-thickness dermal burns. *Burns*
413 2011;37:800-4.
- 414 6. Rohner E, Seeger JB, Hoff P, et al. Preferred use of polyhexanide in
415 orthopedic surgery. *Orthopedics* 2011;34:e664-8.
- 416 7. Kim PJ, Attinger CE, Steinberg JS, et al. The impact of negative-pressure
417 wound therapy with instillation compared with standard negative-pressure
418 wound therapy: a retrospective, historical, cohort, controlled study. *Plast*
419 *Reconstr Surg* 2014;133:709-16.
- 420 8. Fabry WH, Kock HJ, Vahlensieck W. Activity of the antiseptic polyhexanide
421 against gram-negative bacteria. *Microb Drug Resist* 2014;20:138-43.
- 422 9. Rietkotter J, Korber A, Grabbe S, Dissemond J. Eradication of methicillin-
423 resistant *Staphylococcus aureus* in a chronic wound by a new polyhexanide
424 hydrogel. *J Eur Acad Dermatol Venereol* 2007;21:1416-7.
- 425 10. Muller G, Kramer A. Biocompatibility index of antiseptic agents by parallel
426 assessment of antimicrobial activity and cellular cytotoxicity. *J Antimicrob*
427 *Chemother* 2008;61:1281-7.
- 428 11. Hubner NO, Matthes R, Koban I, et al. Efficacy of chlorhexidine,
429 polihexanide and tissue-tolerable plasma against *Pseudomonas aeruginosa*
430 biofilms grown on polystyrene and silicone materials. *Skin Pharmacol Physiol*
431 2010;23 *Suppl*:28-34.
- 432 12. Saleh K, Sonesson A, Persson B, Riesbeck K, Schmidtchen A. A descriptive
433 study of bacterial load of full-thickness surgical wounds in dermatologic surgery.
434 *Dermatol Surg* 2011;37:1014-22.
- 435 13. Cordova KB, Grenier N, Chang KH, Dufresne R, Jr. Preoperative
436 methicillin-resistant *Staphylococcus aureus* screening in Mohs surgery appears
437 to decrease postoperative infections. *Dermatol Surg* 2010;36:1537-40.
- 438 14. Tai YJ, Borchard KL, Gunson TH, Smith HR, Vinciullo C. Nasal carriage of
439 *Staphylococcus aureus* in patients undergoing Mohs micrographic surgery is an
440 important risk factor for postoperative surgical site infection: a prospective
441 randomised study. *Australas J Dermatol* 2013;54:109-14.
- 442 15. Cherian P, Gunson T, Borchard K, et al. Oral antibiotics versus topical
443 decolonization to prevent surgical site infection after mohs micrographic
444 surgery--a randomized, controlled trial. *Dermatol Surg* 2013;39:1486-93.

- 445 16. Grice EA, Kong HH, Conlan S, et al. Topographical and temporal diversity
446 of the human skin microbiome. *Science* 2009;324:1190-2.
- 447 17. Kirker KR, Fisher ST, James GA, McGhee D, Shah CB. Efficacy of
448 Polyhexamethylene Biguanide-containing Antimicrobial Foam Dressing Against
449 MRSA Relative to Standard Foam Dressing. *Wounds* 2009;21:229-33.
- 450 18. Minnich KE, Stolarick R, Wilkins RG, et al. The effect of a wound care
451 solution containing polyhexanide and betaine on bacterial counts: results of an in
452 vitro study. *Ostomy Wound Manage* 2012;58:32-6.
- 453 19. Kamaruzzaman NF, Firdessa R, Good L. Bactericidal effects of
454 polyhexamethylene biguanide against intracellular *Staphylococcus aureus*
455 EMRSA-15 and USA 300. *J Antimicrob Chemother* 2016;71:1252-9.
- 456 20. Rembe JD, Fromm-Dornieden C, Schafer N, Bohm JK, Stuermer EK.
457 Comparing two polymeric biguanides: Chemical distinction, antiseptic efficacy
458 and cytotoxicity of Polyaminopropyl biguanide (PAPB) and Polyhexamethylene
459 biguanide (PHMB). *J Med Microbiol* 2016.
- 460 21. Janjua OS, Qureshi SM. Basal cell carcinoma of the head and neck region:
461 an analysis of 171 cases. *J Skin Cancer* 2012;2012:943472.
- 462 22. Zhan C, Miller MR. Excess length of stay, charges, and mortality
463 attributable to medical injuries during hospitalization. *JAMA* 2003;290:1868-74.
- 464 23. Nestor MS. Prophylaxis for and treatment of uncomplicated skin and skin
465 structure infections in laser and cosmetic surgery. *J Drugs Dermatol* 2005;4:s20-
466 5.
- 467 24. Rossi AM, Mariwalla K. Prophylactic and empiric use of antibiotics in
468 dermatologic surgery: a review of the literature and practical considerations.
469 *Dermatol Surg* 2012;38:1898-921.
- 470 25. Dixon AJ, Dixon MP, Askew DA, Wilkinson D. Prospective study of wound
471 infections in dermatologic surgery in the absence of prophylactic antibiotics.
472 *Dermatol Surg* 2006;32:819-26; discussion 26-7.
- 473 26. Smack DP, Harrington AC, Dunn C, et al. Infection and allergy incidence in
474 ambulatory surgery patients using white petrolatum vs bacitracin ointment. A
475 randomized controlled trial. *JAMA* 1996;276:972-7.
- 476 27. Christensen GJ, Bruggemann H. Bacterial skin commensals and their role
477 as host guardians. *Benef Microbes* 2014;5:201-15.
- 478 28. Lai Y, Cogen AL, Radek KA, et al. Activation of TLR2 by a small molecule
479 produced by *Staphylococcus epidermidis* increases antimicrobial defense against
480 bacterial skin infections. *J Invest Dermatol* 2010;130:2211-21.
- 481 29. Gallo RL, Nakatsuji T. Microbial symbiosis with the innate immune
482 defense system of the skin. *J Invest Dermatol* 2011;131:1974-80.
- 483 30. Saleh K, Schmidtchen A. Surgical site infections in dermatologic surgery:
484 etiology, pathogenesis, and current preventative measures. *Dermatol Surg*
485 2015;41:537-49.
- 486 31. Hoste E, Arwert EN, Lal R, et al. Innate sensing of microbial products
487 promotes wound-induced skin cancer. *Nat Commun* 2015;6:5932.
- 488 32. Bruce J, Russell EM, Mollison J, Krukowski ZH. The quality of
489 measurement of surgical wound infection as the basis for monitoring: a
490 systematic review. *J Hosp Infect* 2001;49:99-108.



493

494

495

496

497

498

499

500

501

502 C

503

504

505

506

507

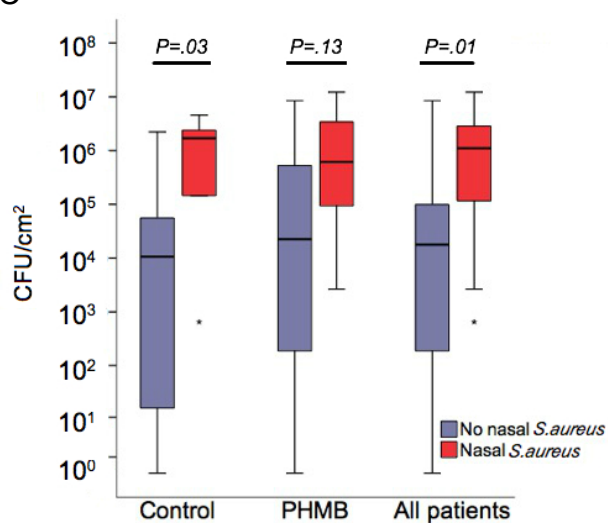
508

509

510

511

512



513

Figure 1. Postoperative bacterial loads after one week shown for each patient

514

group (controls and PHMB) or all patients combined. (A) Differences between

515

wounds classified as infected and non-infected. (B) Differences in regard to

516

presence of *S. aureus* in wounds at one week after surgery. (C) Levels

517

correlated to presence of *S. aureus* intranasally. Outliers in all plots are

518

indicated by an asterisk (*). Solid bars depict interquartile range and the hash

519

marks show the total range. A difference in median CFU/cm² (calculated

520

using Mann-Whitney's test) with a *P* value of <.05 is regarded as statistically

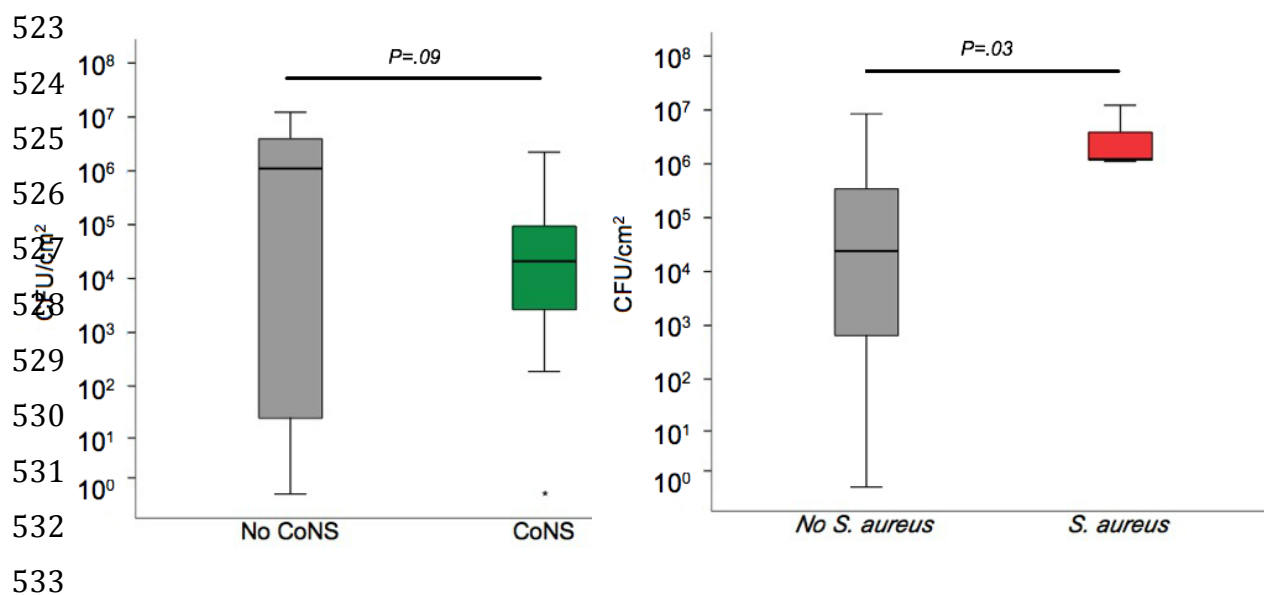
521

significant.

522

A

B



534 **Figure 2.** Bacterial loads at one week after surgery measured in all patients
 535 whether (A) CoNS were isolated postoperatively and whether (B) *S. aureus*
 536 was isolated at end of surgery. The outliers were expressed with an asterisk
 537 (*). Solid bars depict interquartile range and the hash marks show the total
 538 range. Calculations of median CFU/cm² values using a Mann-Whitney test
 539 with a *P* value of <.05 were regarded as statistically significant.

540

577 SUPPLEMENTARY DATA

578

579 METHODS

580

581 ***In vitro* antibacterial assay**

582 Todd-Hewitt (TH) agar plates were streaked with *S. aureus* ATCC 29213 and *S.*

583 *epidermidis* ATCC 14909. Each plate contained 1×10^5 colony-forming units (CFU).

584 Eight mm polyurethane dressings (Mepilex[®], Mölnlycke Healthcare, Göteborg,

585 Sweden) soaked with Prontosan[®] solution or sterile water were applied on top to

586 simulate an *in vivo* situation where the dressing is applied onto a wound.

587 The dressings were soaked with 70% of the solution, where 100% was considered as

588 the maximum wetting capacity of the dressing. 70% wetting was also to be used in

589 this patient trial. The zone of inhibition around the discs was measured.

590

591 **Preparation of Mepilex[®] dressings**

592 Prior to surgery, seven circular dressing templates with varying diameters ranging

593 from 10 mm to 34 mm were cut from Mepilex[®]. Necessary liquid volume to achieve

594 70% wetting was calculated by subtracting each template's fully saturated weight

595 from its dry weight and multiplying the result by 0.7. For each dressing template, 20

596 test tubes were prepared containing sterile water and 20 test tubes contained

597 Prontosan[®] solution. These were marked with either A or B by an external

598 investigator not involved in this trial and blinded to the nurse, surgeon, and principal

599 investigator. Prontosan[®] solution is like water both colorless and odor-free. The

600 dressing templates were used for proper determination of the volume of Prontosan[®] or

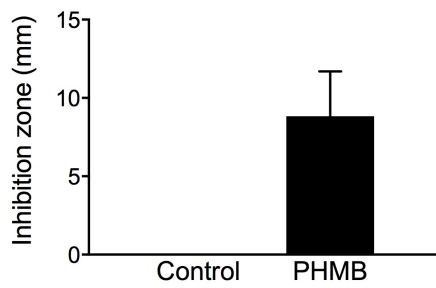
601 sterile water required for wetting tie-over dressings used during surgery.

602

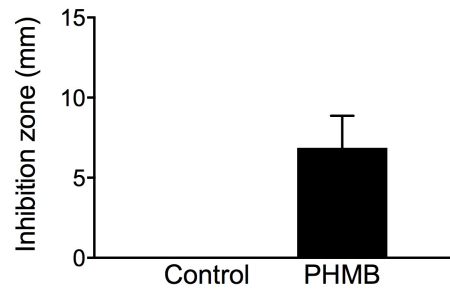
603 **FIGURES**

604 A

605



B



606

607

608

609 **Figure 1.** *In vitro* antibacterial assays illustrating measured inhibition zones of

610 dressings soaked with water (control) or PHMB on agar plates coated with

611 1×10^5 CFU of (A) *S. aureus*, and (B) *S. epidermidis* (n=3, bar indicates S

612

Item	Intervention group	Control group	<i>P</i> value
Age			.351
Range	47-92	45-91	
Mean \pm SD	74.45 \pm 12.05	78.20 \pm 13.05	
Median	74	85	
Sex, n (%)			.204
Male	11	7	
Female	9	13	
Wound location			.216
Nose	13	10	
Cheek	1	5	
Temple	3	1	
Forehead	2	2	
Ear	0	2	
Scalp	1	0	
Tumor excised			.435
BCC	15	15	
SCC	3	1	
Other	2	4	

613 BCC: Basal cell carcinoma. SCC: Squamous cell carcinoma.

614

615 **Table 1.** Patient characteristics and selected baseline values.

616

617

618

619

620

621

622

	Intervention Group	Control Group	<i>P</i> value
Median BS (CFU/cm ²)	10640.50	12180.50	.752
Median ES (CFU/cm ²)	13	13	.751
Median 1W (CFU/cm ²)	64132.50	23425.50	.752
Change (ES-1W)	5668.15	779	.608
Change (BS-1W)	2.7	1.1	.150
Difference 1W minus ES	64105.50	23415.50	.752
Difference 1W minus BS	28903.50	204.50	.343

623

624 **Table 2.** Bacterial quantification of all swabs taken before surgery (BS), at
625 end of surgery (ES), and after one week (1W).