Original Article

The International Journal of Lower Extremity Wounds
1–9

DOI: 10.1177/15347346211015656
journals.sagepub.com/home/ijl
SAGE

The Safety and Antimicrobial Properties of Stabilized Hypochlorous Acid in Acetic Acid Buffer for the Treatment of Acute Wounds—a Human Pilot Study and In Vitro Data

Ewa A. Burian, MD[1], Lubna Sabah, MD[1], Klaus Kirketerp-Møller, MD, PhD[1], Elin Ibstedt, MSc[2], Magnus M. Fazli, PhD[3,4], and Glenn Gundersen, PhD[4]

Abstract

Acute wounds may require cleansing to reduce the risk of infection. Stabilized hypochlorous acid in acetic buffer (HOCl + buffer) is a novel wound irrigation solution with antimicrobial properties. We performed a first-in-man, prospective, open-label pilot study to document preliminary safety and performance in the treatment of acute wounds. The study enrolled 12 subjects scheduled for a split-skin graft transplantation, where the donor site was used as a model of an acute wound. The treatment time was 75 s, given on 6 occasions. A total of 7 adverse events were regarded as related to the treatment; all registered as pain during the procedure for 2 subjects. One subject had a wound infection at the donor site. The mean colony-forming unit (CFU) decreased by 41% after the treatment, and the mean epithelialization was 96% on both days 14 (standard deviation [SD] 8%) and 21 (SD 10%). The study provides preliminary support for the safety, well-tolerance, and efficacy of HOCl + buffer for acute wounds. The pain was frequent although resolved quickly. Excellent wound healing and satisfying antimicrobial properties were observed. A subsequent in vitro biofilm study also indicated good antimicrobial activity against *Pseudomonas aeruginosa* with a 96% mean reduction of CFU, when used for a treatment duration of 15 min ($P < .0001$), and a 50% decrease for *Staphylococcus aureus* ($P = .1010$). Future larger studies are needed to evaluate the safety and performance of HOCl + buffer in acute wounds, including the promising antimicrobial effect by prolonged treatment on bacterial biofilms.

Keywords

antiinfective agents, hypochlorous acid, infection control, wound and injuries

Introduction

Acute wounds, such as burns, cuts, or abrasions, are likely to be contaminated by microbes. Infection rates of acute wounds have been reported to be between 5% and 10%, highly depending on the degree of contamination, localization, depth, patients' age, and definition of infection.[1-4] One of the actions taken to avoid infections is to clean the wound by mechanical irrigation, for example with tap water. It involves a steady flow of fluid to remove foreign material.[3] Today, there is a need to develop novel non-antibiotic irrigation solutions that are isotonic, nonhemolytic, nontoxic, transparent, easy to sterilize, inexpensive, stable, and that do not promote the development of antimicrobial resistance. Stabilized hypochlorous acid in acetic buffer (HOCl + buffer) is a novel combination of 2 historically well-known antibacterial substances, hypochlorous acid[5] and acetic acid,[6] and is developed to cleanse acute wounds.

The antimicrobial properties of hypochlorous acid were recognized even before the widespread use of aqueous chlorine as an antiseptic for traumatic wounds in World War I,[7]

[1]Department of Dermato-Venereology & Wound Healing Center, Bispebjerg Hospital, Copenhagen, Denmark
[2]Devicia AB, Mölndal, Sweden
[3]Department of Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark
[4]SoftOx Solutions AS, Oslo, Norway

Corresponding Author:
Ewa Anna Burian, Department of Dermato-Venereology & Wound Healing Center, Bispebjerg Hospital, Bispebjerg Bakke 23, 2400, Copenhagen, Denmark.
Email: ewaburian@gmail.com

and its antimicrobial properties have been demonstrated against a wide range of bacteria, virus, and fungi.[5] Hypochlorous acid has historically been difficult to manufacture as a stable product,[8] but the stability issue has been solved with acetic acid buffer and thus may act as an ancillary antimicrobial substance. Although used in a low concentration as a buffering agent to stabilize the pH, acetic acid (also present in vinegar) is also a well-known antimicrobial agent for the treatment of wound infections.[6] The combination of the 2 substances is new and requires a safety evaluation and validation of efficacy in humans.

The primary objective of this pilot study was to document the preliminary safety of HOCl + buffer when used on split-skin graft donor sites, serving as a model of a superficial acute wound. The primary objective of our in vitro study was to investigate the effect of treatment with HOCl + buffer against *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms.

Methods

Clinical Trial

Study Design. This was a first-in-man, prospective, open-label, exploratory pilot study to document the preliminary safety and performance of HOCl + buffer for topical use when used on acute wounds. This sponsor-initiated study (Softox Solutions AS, Oslo, Norway) was conducted at the Department of Dermato-Venereology and Wound Healing Center, Bispebjerg Hospital, Copenhagen, Denmark, between December 2018 and September 2019. Informed consent was obtained from each subject prior to inclusion. The study was approved by the Research Ethics Board (H-18034044) and the Danish Medicines Agency. The investigation was performed in accordance with the national regulations and the Declaration of Helsinki. The investigational product tested was hypochlorous acid 0.2 g/kg stabilized in acetic buffer (SoftOx Wound Irrigation Solution [SWIS], SoftOx Solutions AS, Oslo, Norway), a non-CE marked medical device class III. The study was registered on ClinicalTrials.gov prior to inclusion of any patients (identifier: NCT03742284). Due to the explorative and safety design of the study, no control was used. The study was part of a premarket development program and therefore some of the study outcomes are not presented in this manuscript as we consider the respective data less interesting from a scientific point of view (such as wound fluid capacity, user satisfaction, and odor evaluation), and the data do not withhold important safety or performance information.

Study Population. Patients scheduled for a split-skin graft transplantation for treatment of chronic leg ulcers were eligible if they met all the inclusion criteria and none of the exclusion criteria. The donor site has previously been justified as an appropriate human model for studying acute wounds.[9] The main inclusion criteria were age >18 years, with nonhealing leg ulcers, scheduled for an operation with excision of the chronic leg ulcer and split-skin transplantation, and voluntarily agreed to participate. Individuals were excluded if they were on systemic immunomodulating drugs or systemic steroid treatment (exception: prednisolone ≤10 mg/day), were pregnant, had uncontrolled pain prior to surgery, or had dementia. Participants were also excluded if they had severe neuropathy, dysesthesia on the donor site, or had any known allergies to any remedies/materials used in the trial.

Study Interventions. The total study period for each subject was 22 to 23 days, including 9 visits. The eligible subjects went through the split-thickness skin grafting with skin harvested from the thigh on day 0. The donor site was thereafter used as a model for an acute wound and was treated topically with HOCl + buffer on 6 occasions: on days 0 (immediately after split-skin harvesting, during local and/or general anesthesia), 2, 3, 4, 5, and 7. One bottle containing 250 ml HOCl + buffer (18°C-25°C) was used on each occasion. The donor site was rinsed and soaked with HOCl + buffer by pouring the solution on the donor site for at least 15 s, holding the bottle ∼20 cm above the donor site to create a light gravity irrigation pressure. The donor site was then covered with sterile gauze thoroughly soaked with HOCl + buffer for 60 s, with no mechanical rubbing. Each subject was followed up on day 14 and day 21 (final study visit). Nonadhesive neutral dressings with no antibacterial properties were used during the entire trial.

Outcomes. The primary outcome was the incidence and severity of any adverse events (AEs) associated with HOCl + buffer. The secondary outcome was the performance of HOCl + buffer, and included the measurement of bacterial presence, percentage epithelialization, inflammation evaluation, wound fluid measure (exudate), pain and discomfort scoring, and subject satisfaction.

Variables. All AEs were registered from the time point of informed consent until the final study visit. The presence of bacteria was assessed at the donor site on days 2 and 5, prior to and after the treatment procedure, after a few minutes of air-drying of the donor site. A sterile ESwab (Coban) was gently stroked in a zigzag manner across the donor site and was used for culturing and subsequent counting of the colony-forming units (CFUs). The analysis was performed at an external laboratory, Costerton Biofilm Center, University of Copenhagen (see below).

Inflammation was evaluated on days 2 and 5 through thermography and clinical evaluation. The temperature difference between the max point at the donor site and the same

point at the contralateral leg was measured with a thermal camera (FLIR Systems, Inc., E53). A difference of 2°C or more between the donor site and the contralateral site was considered to be a sign of inflammation. A subjective assessment by the study staff was made through registration of any abnormal inflammatory signs (erythema, swelling, pus, exudate, and others) and graded as none, mild, moderate, or severe. Expected baseline inflammation, which is a normal step in the wound healing process, was not recorded. Exudate levels were estimated by weighing the dressings used on the donor site between days 5 and 7.

Epithelialization was subjectively evaluated by the investigator/study nurse by filling out a scale between 0% and 100% (0%: no epithelialization and 100%: fully epithelialized) on days 7, 14, and 21.

Pain was measured using a visual analog scale (VAS) ranging between 0 and 10 cm: 0 equaled no pain and 10 the worst imaginable pain. VAS was assessed 4 times on each treatment day (except on day 0); prior to dressing change, after dressing change and 5 min of rest, directly after treatment with HOCl + buffer, and after the dressing has been applied and 5 min of rest. Subjects were also asked for their experience of the procedure as either perfectly acceptable, acceptable, neutral, or unacceptable on each occasion, with a possibility of free comments. Subject discomfort was captured similarly. An overall subject satisfaction questionnaire was performed after the last treatment day, on day 7.

Microbiological Analysis. Upon arrival to Costerton Biofilm Center, each swab sample (4 per patient) was vortexed vigorously and sonicated in an ultrasound bath before 10-fold dilution series were performed in 0.9% (w/v) NaCl. From each dilution, 100 µl was plated onto 5% blood agar plates and blue agar plates (a modified Conradi–Drigalski medium) (Statens Serum Institute), and the plates were incubated at 37°C for 24 h. The CFUs were counted to estimate the bacterial load (CFU/ml) and isolated for confirmation of species with matrix-assisted laser desorption ionization time-of-flight mass spectrometry using a Microflex LT instrument (Bruker Daltonik GmbH). The protein profiles were obtained by the software FlexControl 3.3 (Bruker Daltonik GmbH) and analyzed by FlexAnalysis 3.3 (Bruker Daltonik GmbH). The database that was used to match spectra was Bruker Taxonomy (7311 mass spectra profiles).

Statistical Analysis. No formal sample size calculation was performed as this was an exploratory pilot study. A total of 12 subjects including a drop-out rate of 20% were considered appropriate based on the number normally participating in a pilot study. Frequencies, percentages, median, mean, standard deviation (SD), and range were used as appropriate. SAS Version 9.4 (SAS Institute) was used for statistical analysis. Due to the small sample size, no significance testing was attempted.

In Vitro Study (Biofilm Model)

Biofilm Growth. The wild-type *P aeruginosa* PAO1 and *S aureus* RN4220 were grown in Lysogeny broth (LB) medium at 37 °C overnight. The overnight culture was 10^5-fold diluted in 0.9% (w/v) NaCl and 10 µl was spot inoculated on nitrocellulose membrane filters (Whatman, 0.20 µm pore size, 13 mm diameter) that were placed on LB agar plates (LB solidified with 2% [w/v] agar). The plates were incubated at 37 °C for 24 h. The 2 bacterial species were chosen because they are some of the most commonly isolated and problematic bacterial species in wounds.[10] A biofilm model was chosen as bacterial biofilms in wounds are notorious for being difficult to eradicate and can delay wound healing.[10]

Biofilm Treatment. Membrane filters with biofilms were transferred to agar plates (2% [w/v] bacteriological agar dissolved in sterile water and solidified), and a new membrane filter was placed on each biofilm so that the biofilm was sandwiched between 2 membrane filters. The biofilms were treated with HOCl + buffer. Eight-to-10 layers of gauze dressing were soaked with ~1 ml of HOCl + buffer and placed on top of the biofilms growing on membrane filters in triplicate. The treatment was carried out at room temperature for 1 or 15 min. Three independent experiments were carried out on different days. Thereby, a total of 9 biofilm interventions were made for each bacterial species and each time point. The same number of biofilms was also treated with saline (0.9% [w/v] NaCl) for 1 or 15 min, which was later used to determine the baseline CFU at time$_0$ (T_0).

Evaluation of the Treatment. The gauze layers were discarded. The membrane filters were transferred into 15 ml tubes containing 5 ml of 0.9% (w/v) NaCl, and the tubes vortexed for 20 s and sonicated in an ultrasound bath (Branson 2519 DTH ultrasonic cleaner) for 5 min to disrupt and release the biofilms from the membrane filters. Ten-fold serial dilution of the cell suspensions was made, and 10 µl of each dilution was spotted on LB agar plates for CFU counting. The CFUs were counted after overnight incubation at 37 °C. To evaluate the effect of HOCl + buffer, the saline-treated biofilms were used to assess the baseline CFU for each biofilm model, representing the CFU at T_0. The data represent the difference in mean in CFU after treatment with HOCl + buffer in comparison with the baseline saline control.

Statistical Analysis. Prism v9.0 (GraphPad Software) was used for the statistical analysis in the in vitro study. No

power analysis was made in this proof-of-concept study, but a sample size of 9 controls and 9 treatments per bacteria (1 and 15 min, respectively) was expected to reveal a significant difference. The results were logarithm transformed due to a non-Gaussian distribution. An unpaired one-tailed *t*-test was chosen as appropriate (expecting that the treatment could only result in a CFU reduction and not increase). No Bonferroni correction was performed, as we regarded each comparison as an individual experiment. A significance level of 0.05 was chosen as appropriate.

Results

Clinical study

Study Population. A total of 12 subjects were screened for eligibility, and 12 of them met the selection criteria and were included. The study population consisted of 7 males and 5 females with a mean age of 66 years (range 47-89 years) that were all admitted to the in-patient clinic until day 7 or further. Comorbidities were frequent, and 2 of the patients had diabetes. Eleven subjects completed the study according to the protocol, while 1 withdrew participation due to withdrawn consent, prior to any study-related procedures on day 2. No particular reason for withdrawal was given by the subject. At screening, no participants received immunomodulating drugs except 1 that received prednisolone ≤10 mg/day, and 4 received strong pain medication, defined as opioids. Donor sites had a mean length of 10.6 cm (SD 6.4 cm), width 4.8 cm (SD 2.4 cm), and thickness of 0.15 mm (SD 0.05 mm).

Safety. No serious AEs (SAEs) or serious adverse device effects were reported. In total, 21 AEs were registered, consisting of 12 types of AEs (Table 1). Seven (33%) of the AEs were assessed as adverse device effects (ADE) and were assessed as moderate in severity and possibly or causally related to HOCl + buffer. All of these concerned pain during the treatment. It occurred for 2 subjects during the study at several time points. For one of them, pain occurred during the whole treatment (ie, both during pouring and when adding a HOCl + buffer-soaked gauze), and for the other subject, the pain only occurred during the pouring of the solution. Both subjects had to be treated with painkillers once. The events were considered as resolved after the HOCl + buffer treatment, as the pain declined quickly. No subjects had AE as a reason for premature discontinuation.

One other event, infection donor site, was also reported as possibly related to HOCl + buffer but was not considered to be an ADE. This subject had a paraclinical infection of *P aeruginosa* on day 5 as indicated by the high CFU count, probably self-contaminated from the chronic leg ulcer. Although treatment with HOCl + buffer was able to lower the CFU count, this subject had a clinically documented infection on day 9, 2 days after the last treatment with HOCl + buffer.

Table 1. AEs Occurred in the Study.

AEs	Number of events	Percent of subjects (%)	Event type
Pain during HOCl + buffer procedure	7	33.3	ADE
Hypersensibility reaction around the donor site	3	14.3	AE
Infection skin graft	2	9.5	AE
Diarrhea	1	4.8	AE
Dizziness	1	4.8	AE
Dyspnea	1	4.8	AE
Dysuria	1	4.8	AE
Folliculitis around transplant site	1	4.8	AE
Infection donor site	1	4.8	AE
Pruritus head and neck	1	4.8	AE
Skin graft rejection	1	4.8	AE
Worsening of anemia	1	4.8	AE
Total	21	100	

Abbreviations: AE, adverse event; HOCl + buffer, hypochlorous acid in acetic buffer; ADE, adverse device effect.

Hypersensibility reaction around donor site was seen in 3 subjects, but it was evaluated as probably/possibly related to the dressing (eg, taking the outlines of the dressing) rather than HOCl + buffer itself.

Bacterial Presence. The mean CFU value decreased after the HOCl + buffer procedure on both days 2 and 5 (Table 2). The combined mean CFU/ml on both days prior to and after the HOCl + buffer procedure was 20,951 and 12,294, respectively, with an approximate decrease of 41% (58% reduction on day 2 and 36% reduction on day 5). Out of 22 paired measurements prior to and after the HOCl + buffer procedure, a CFU reduction was observed on 9 treatment occasions after the HOCl + buffer procedure, 10 treatment occasions had no bacteria prior to or after the HOCl + buffer procedure, and a slight increase in CFU was noted in 3 treatment occasions (in patients with very low CFU counts) (Table 3).

Epithelization. The mean epithelialization was estimated to be 26% (SD 30%) on day 7, 96% (SD 8%) on day 14, and 96% (SD 10%) on day 21. No subjects were fully healed (100% epithelialization) on day 7; however, 7 patients were fully healed on day 14 with an additional increase to 9 patients on day 21. The reason for the increased SD on day 21 compared to day 14 is that while several subjects had increased epithelialization, 2 subjects showed a slight decrease in epithelialization. The study personnel argued that this might have been due to the dressing

Table 2. Overall Microbiology Results Prior and After the Treatment (75 s treatment). $N = 11$.

Day	Time point of swabbing	Number of Subjects (n)	Mean, CFU/ml	SD	Median, CFU/ml	Minimum, CFU/ml	Maximum, CFU/ml	Percentage CFU decrease after treatment (%)
2	Prior treatment	11	4926	9690	0	0	30,890	—
	After treatment	11	2081	4463	0	0	14,410	58
5	Prior treatment	11	16,025	38,524	0	0	121,000	—
	After treatment	11	10,213	28,307	0	0	94,000	36

Abbreviations: CFU, colony-forming units; SD, standard deviation.

Table 3. Individual Microbiology Results, Prior and After Treatment with HOCl + buffer (75 s Treatment).

Subject	Day	Time	CFU/ml	CFU reduction?	Bacterial species
1	2	Prior treatment	7800		*Micrococcus luteus*
		After treatment	6020	Yes	*M luteus, Micrococcus flavus*
	5	Prior treatment	0		
		After treatment	0	Unchanged	
2	2	Prior treatment	0		
		After treatment	0	Unchanged	
	5	Prior treatment	0		
		After treatment	0	Unchanged	
3	2	Prior treatment	13,700		*Enterococcus faecalis* and *Pseudomonas aeruginosa*
		After treatment	1260	Yes	*E faecalis* and *P aeruginosa*
	5	Prior treatment	121,000		*P aeruginosa*
		After treatment	94,000	Yes	*P aeruginosa*
4	2	Prior treatment	0		
		After treatment	0	Unchanged	
	5	Prior treatment	0		
		After treatment	30	No	*Staphylococcus epidermis*
5	2	Prior treatment	1770		*Acinetobacter pittii* and *Staphylococcus haemolyticus*
		After treatment	60	Yes	*A pittii* and *S haemolyticus*
	5	Prior treatment	0		
		After treatment	0	Unchanged	
6	2	Prior treatment	0		
		After treatment	1140	No	*S haemolyticus*
	5	Prior treatment	55,000		*Staphylococcus aureus*
		After treatment	18,000	Yes	*S aureus*
7	2	Prior treatment	30,890		*Bacillus cereus* group and *Corynebacterium striatum*
		After treatment	14,410	Yes	*B cereus* group and *C striatum*
	5	Prior treatment	50		*C striatum*
		After treatment	290	No	*C striatum*
8	2	Prior treatment	0		
		After treatment	0	Unchanged	
	5	Prior treatment	150		*S haemolyticus*
		After treatment	0	Yes	
9	2	Prior treatment	30		*P aeruginosa*
		After treatment	0	Yes	
	5	Prior treatment	80		*P aeruginosa*
		After treatment	20	Yes	*P aeruginosa*
10	2	Prior treatment	0		
		After treatment	0	Unchanged	
	5	Prior treatment	0		
		After treatment	0	Unchanged	
11	2	Prior treatment	0		
		After treatment	0	Unchanged	
	5	Prior treatment	0		
		After treatment	0	Unchanged	

Abbreviations: HOCl + buffer, hypochlorous acid in acetic buffer; CFU, colony-forming units.

Table 4. Subject Discomfort Question. $N = 11$.

	How did the subject experience the use of the device on the donor site? N = 11			
Day	Unacceptable	Neutral	Acceptable	Perfectly acceptable
2	0 (0.0%)	2 (18.2%)	6 (54.5%)	3 (27.3%)
3	1 (9.1%)[a]	1 (9.1%)	8 (72.7%)	1 (9.1%)
4	1 (9.1%)[a]	4 (36.4%)	5 (45.5%)	1 (9.1%)
5	1 (9.1%)[a]	2 (18.2%)	7 (63.6%)	1 (9.1%)
7	0 (0.0%)	4 (36.4%)	5 (45.5%)	2 (18.2%)

[a]Same subject that replied unacceptable on days 3, 4, and 5.

change, which may have accidentally pulled some of the new epithelium off (this was not regarded as an AE).

Inflammation and Exudate Levels. On day 2, all subjects had a temperature difference of ≥2 °C between the donor site and the contralateral site, predefined in our protocol as inflammation. This was still the case for 9 subjects on day 5. On day 2, the average max temperature on the donor site was 36.1 °C (SD 1.3 °C) versus 31.7 °C (SD 1.7 °C) on the contralateral site. On day 5, the average max temperature on the donor site was 36.0 °C (SD 0.5 °C) versus 32.4 °C (SD 2.1 °C) on the contralateral site. The thermography findings were only occasionally associated with clinical abnormal inflammatory signs. Abnormal inflammatory signs were recorded for 3 subjects, 1 subject on day 2 (odor) and 3 subjects on day 5 (2 with erythema and 1 with green-colored exudate), all assessed as mild. The mean difference in exudate levels between days 5 and 7 was 14.8 g (SD 10.3 g).

Pain. The highest mean values were received on day 2; prior to removal of dressing VAS 2.1 cm (SD 2.1 cm), after removal of the dressing and 5 min of rest VAS 4.2 cm (SD 2.8 cm), directly after the HOCl + buffer procedure VAS 5.1 cm (SD 3.7 cm), and after new dressing had been applied and 5 min of rest VAS 2.4 cm (SD 2.6 cm). The highest mean VAS value on each day was received directly after the HOCl + buffer procedure, with VAS 5.1 cm (SD 3.7 cm) on day 2 and then decreasing to VAS 3.1 cm (SD 3.4 cm) on day 7. It was noted that primarily the pouring phase of the HOCl + buffer procedure was painful, and quickly declined within seconds to minutes in the majority of the cases. Pain was usually considered as an AE if the subject or study personal judged the treatment as unacceptable on the specific treatment occasion. Eight subjects administrated fast-acting pain medication <4 h prior to the HOCl + buffer procedure on one or several occasions.

Discomfort Scoring. More than 90% of the subjects experienced that the usage of HOCl + buffer on the donor site was neutral, acceptable, or perfectly acceptable on all occasions (Table 4). One subject replied that the experience was unacceptable on days 3, 4, and 5. On almost all treatment occasions, the subjects reported feeling something when HOCl + buffer was applied on the donor site. The most common words they used were coldness, sting, and pain.

Subject Satisfaction. On day 7, the patients were asked to assess the whole study experience until that time point. Three out of 11 subjects (27%) thought that the cleansing procedure was very uncomfortable, 3 (27%) thought that it was a little uncomfortable, 2 (18%) deemed it as neutral, 2 (18%) were satisfied, and 1 (9%) was very satisfied from a pain perspective. Most subjects (46%) were neutral when it comes to satisfaction with respect to other discomforts during the wound cleansing procedure and 3 subjects (27%) were satisfied or very satisfied. Three subjects (27%) reported that it was a little uncomfortable or very uncomfortable. More than 91% of the subjects were satisfied or very satisfied with the overall wound cleansing procedure in the study, and 1 subject replied that he/she was neutral. No subjects replied that they were unsatisfied.

In Vitro Study (24 h Biofilm Model)

After 1 or 15 min of treatment, HOCl + buffer killed 50% (0.3 $\log^{10}$, $P = .1595$) and 96% (1.4 $\log^{10}$, $P < .0001$) of *P aeruginosa*, respectively. Regarding *S aureus*, after 1 or 15 min of treatment, HOCl + buffer killed 7% (0.04 $\log^{10}$, $P = .3937$) and 50% (0.3 $\log^{10}$, $P = .1010$) of the bacteria, respectively. The study results are presented in Figure 1 and Table 5.

Discussion

In this clinical pilot study, treatment with HOCl + buffer was overall found to be safe and well tolerated, in a surgically inflicted acute wound model (split-skin graft donor sites). A satisfying bacterial reduction was seen both in the pilot study and in the in vitro biofilm model.

Bacterial presence was overall low and well controlled in the clinical study. In the cases where bacteria were present, they were usually reduced after the treatment with HOCl + buffer, with a mean 41% reduction in bacterial load after the treatment. Although this may not seem impressive at first glance, we should consider the short contact time being only 75 s per treatment. This may be seen as clinically useful in the preventive setting. The methodological limitations with swabs should also be mentioned due to the random location of bacteria in a wound.[11] This could be the reason for the small increase in CFUs seen after the treatment in a few number of cases (Table 3).

In order to further investigate the effect of treatment on the eradication of microbes, we performed an in vitro biofilm study, as eradication of bacterial biofilms is a challenge and a known factor for delayed wound healing.[10] Promising antimicrobial results were seen by HOCl + buffer, for both species tested. Although statistically nonsignificant results were seen after 1 min of treatment, there was a trend toward a bacterial reduction. However, the data indicates that increasing the duration of the treatment from 1 to 15 min may increase the number of bacteria eradicated, demonstrating the importance of extended duration of treatment for eradication of bacteria in biofilms. This would have to be validated clinically, so as the safety measures of prolonged treatment. The results are satisfying, as acute wounds would primarily be expected to be contaminated by planktonic bacteria in the initial phase. Interestingly, *P aeruginosa* was also more susceptible to killing by HOCl + buffer compared to *S aureus*.

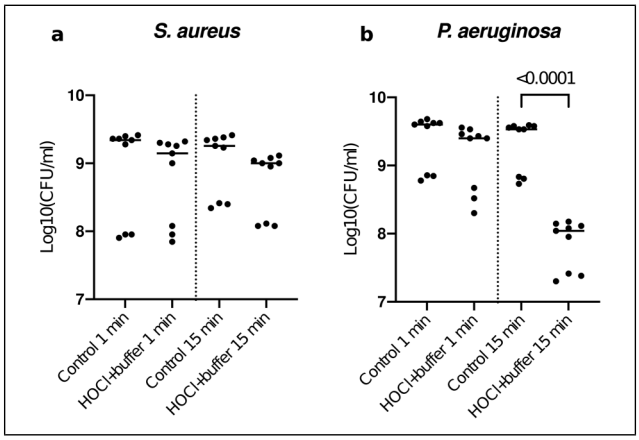

Figure 1. In vitro study. Graphical overview of the biofilm eradication efficacy of viable bacterial cells in mature surface biofilms of *Staphylococcus aureus* (a) and *Pseudomonnas aeruginosa* (b) after treatment with HOCl + buffer or saline control for 1 or 15 min. The horizontal line represents the mean.
Abbreviations: HOCl + buffer, hypochlorous acid in acetic acid buffer; CFU, colony-forming units.

No SAEs or SADEs were reported in our pilot study. Of 21 mild to moderate AEs, 7 events were assessed as ADEs, all registered as pain during the HOCl + buffer procedure for 2 subjects. All ADEs were considered resolved directly after the HOCl + buffer procedure since the pain declined quickly. Pain was an expected reaction since the donor sites can be sensitive and painful wounds, with the exposure of numerous pain receptors (the pain being proportional to the size of the donor site).[12] The physical action of pouring HOCl + buffer onto the donor site seemed to induce the most pain and was often described as a sting. However, the pain quickly declined within seconds or minutes. The following application of HOCl + buffer-soaked gauze onto the donor site was less painful, indicating that HOCl + buffer itself was well tolerated. Also, the frequent dressing changes may have had an impact on the pain scoring. Nevertheless, the adverse effect of pain may in some cases reduce the patients' compliance. Planned future investigations may also confirm or negate the pain observation in comparison with standard treatment.

One other event "infection at donor site" with a possible relation to HOCl + buffer was reported; however, it was not considered to be an ADE. Nevertheless, this may be an indication of the potential ADE "infection due to loss of effect" as the infection that occurred in the study may have been a result of the early termination of the HOCl + buffer

Table 5. In Vitro Study. Biofilm eradication efficacy measured as reduction in the number of viable bacterial cells in mature surface biofilms of *Pseudomonas aeruginosa* or *Staphylococcus aureus* after treatment with HOCl + buffer or saline control, for 1 or 15 min.

		Median CFU	Mean CFU	SD	CFU reduction (mean) %	*P* value
P aeruginosa	Control 1 min	4.0×10^9	3.0×10^9	1.8×10^9	32.2	0.1595
	Treatment 1 min	2.5×10^9	2.1×10^9	1.4×10^9		
	Control 15 min	3.4×10^9	2.6×10^9	1.5×10^9	96.6	<.0001
	Treatment 15 min	1.1×10^8	9.0×10^7	5.3×10^7		
S aureus	Control 1 min	2.2×10^9	1.6×10^9	1.1×10^9	25.5	.3937
	Treatment 1 min	1.4×10^9	1.2×10^9	8.7×10^8		
	Control 15 min	1.8×10^9	1.5×10^9	1.0×10^9	50.0	.101
	Treatment 15 min	1.0×10^9	7.6×10^8	4.9×10^8		

Abbreviations: HOCl + buffer, hypochlorous acid in acetic acid buffer; CFU, colony-forming units; SD, standard deviation.

treatment. This subject had a paraclinical infection (high CFU count) of *P aeruginosa* on day 5, probably self-contaminated from a chronic leg ulcer. Although HOCl + buffer was able to lower the CFU count, this subject had a clinically documented infection on day 9, 2 days after the last HOCl + buffer procedure.

Most importantly, very good healing rates were seen. Almost all subjects had 100% epithelialization after 3 weeks. Time to wound healing at donor sites (means) has previously been reported between 16 and 33 days.[13,14] Although no control group was included in this pilot study, HOCl + buffer did not seem to have any adverse effects on the epithelialization process. This promising finding is of importance, as topical antimicrobials can cause cytotoxicity of cells involved in the wound healing process, for example keratinocytes and fibroblasts.[15] One should also keep in mind that several of the included subjects were elderly and had concomitant diseases that could delay the wound healing process.

Limitations to our study need to be noted. Although the donor site served as a very good standardized model of an acute wound, the frequent dressing changes may be impractical from a clinical point of view, especially in large donor sites that are painful. Being an exploratory pilot investigation, the sample size was also limited to 12 eligible subjects. This small number does not allow us to draw significant conclusions, especially with no comparator and blinded observers. The promising antimicrobial effect on bacterial biofilms by prolonged treatment duration (observed in the in vitro experiments) would need larger and more specific investigations, including a safety evaluation by prolonged treatment, for example regarding cytotoxicity and pain. However, the clinical and in vitro data that have been generated give valuable primary insights into the safety and performance of the treatment.

In conclusion, the outcome of this first-in-man pilot study, exploring the preliminary safety and efficacy of HOCl + buffer in acute wounds, indicates that HOCl + buffer is a safe and well-tolerated wound irrigation solution for acute skin trauma and is not associated with any major risks. Temporary pain was frequent, especially in the pouring phase, but quickly dissolved within seconds or minutes. Excellent wound healing was observed with normal epithelization, and satisfying antimicrobial properties were observed with a mean CFU reduction of ~41% after only 75 s of treatment time. The collected data will serve as a valuable basis for larger controlled human studies. Our subsequent in vitro biofilm study indicated that prolonged treatment time (to 15 min) may result in higher bacterial killing. The promising effect against bacterial biofilms needs further investigations, including a safety evaluation by the prolonged treatment duration.

Acknowledgments

We are grateful to all the patients in this study, and the excellent study nurses at the site: Stine Ingvertsen, Stine Ørst-Pedersen, Lisbeth Vorbeck, Susan Bermark, and Britta Østergaard. We also thank Lasse Andersson Kvich at Costerton Biofilm Center for the microbiological analyses in the clinical study. EAB and LS were investigators at the study site. EAB wrote the first manuscript, with help from LS. EI, KKM, and GG designed the study. GG and EI were project managing the study. MMF designed and conducted the in vitro study. All authors approved the manuscript.

Declaration of Conflicting Interests

The investigators EB and LS have no previous affiliation with SoftOx solutions A/S, and no conflicts of interest to declare that might impact the interpretation or validity of this study. Grants were given to the institution, for the conduction of this study. KKM is a co-inventor of acetic acid against biofilm and a minor shareholder of SoftOx and did not participate in the execution of the studies. EI monitored the study together with other colleagues from Devicia AB. GG is a director of medical affairs and MMF is the head of science and research, both at SoftOx Solutions A/S.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the SoftOx Solutions AS, Oslo, Norway.

ORCID iD

Ewa A. Burian https://orcid.org/0000-0003-0630-2694

References

1. Weiss EA, Oldham G, Lin M, Foster T, Quinn JV. Water is a safe and effective alternative to sterile normal saline for wound irrigation prior to suturing: a prospective, double-blind, randomised, controlled clinical trial. *BMJ Open*. 2013; 3(1):e001504.
2. Angerås MH, Brandberg A, Falk A, Seeman T. Comparison between sterile saline and tap water for the cleaning of acute traumatic soft tissue wounds. *Eur J Surg*. 1992;158(6–7):347-350.
3. Nicks BA, Ayello EA, Woo K, Nitzki-George D, Sibbald RG. Acute wound management: revisiting the approach to assessment, irrigation, and closure considerations. *Int J Emerg Med*. 2010;3(4):399-407.
4. Lammers RL, Hudson DL, Seaman ME. Prediction of traumatic wound infection with a neural network-derived decision model. *Am J Emerg Med*. 2003;21:1-7.
5. Ono T, Yamashita K, Murayama T, Sato T. Microbicidal effect of weak acid hypochlorous solution on various microorganisms. *Biocontrol Sci*. 2012;17(3):129-133.
6. Bjarnsholt T, Alhede M, Jensen PO, et al. Antibiofilm properties of acetic acid. *Adv Wound Care*. 2015;4(7):363-372.
7. Smith JL, Drennan AM, Rettie T, Campbell W. Experimental observations on the antiseptic action of hypochlorous acid and its application to wound treatment. *Br Med J*. 1915;2(2847):129-136.
8. Ishihara M, Murakami K, Fukuda K, et al. Stability of weakly acidic hypochlorous acid solution with microbicidal activity. *Biocontrol Sci*. 2017;22(4):223-227.

9. Weingart D, Stoll P. The epithelialization of split skin graft donor sites—a test model for the efficacy of topical wound therapeutic agents. *Eur J Plast Surg*. 1993;16(1):22-25.
10. Gottrup F, Apelqvist J, Bjarnsholt T, et al. EWMA document: Antimicrobials and non-healing wounds. Evidence, controversies and suggestions. *J Wound Care*. 2013;22(5 Suppl): S1-S89.
11. Fazli M, Bjarnsholt T, Kirketerp-Moller K, et al. Nonrandom distribution of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in chronic wounds. *J Clin Microbiol*. 2009;47(12): 4084-4089.
12. Sinha S, Schreiner AJ, Biernaskie J, Nickerson D, Gabriel VA. Treating pain on skin graft donor sites: review and clinical recommendations. *J Trauma Acute Care Surg*. 2017; 83(5):954-964.
13. Brölmann FE, Eskes AM, Goslings JC, et al. Randomized clinical trial of donor-site wound dressings after split-skin grafting. *Br J Surg*. 2013;100(5):619-627.
14. Werdin F, Tenenhaus M, Becker M, Rennekampff HO. Healing time correlates with the quality of scaring: results from a prospective randomized control donor site trial. *Dermatol Surg*. 2018;44(4):521-527.
15. Rani SA, Hoon R, Najafi RR, Khosrovi B, Wang L, Debabov D. The in vitro antimicrobial activity of wound and skin cleansers at nontoxic concentrations. *Adv Skin Wound Care*. 2014;27(2):65-69.