A double-blind trial comparing an antimicrobial combination to standard care in hard-to-heal wounds

Objective: Excessive numbers of bacteria in hard-to-heal wounds impede wound healing. Numerous topical antiseptics have demonstrated effectiveness in benchtop studies; however, few clinical studies have demonstrated efficacy in the target population: patients with hard-to-heal wounds. This study addressed the clinical efficacy of a novel antibiofilm cleanser and gel in reducing bacterial load and improving wound outcomes.
Method: Hard-to-heal wounds were photographed, measured and evaluated for bacterial load using fluorescence imaging weekly for four weeks. The target ulcers were randomised to be cleaned and treated with either a synergistic antibiofilm cleanser and antibiofilm gel with standard of care (AMC-AMG + SoC) or normal saline wash and an amorphous gel with standard of care (NSS-HG + SoC).
Results: A Chi-squared test of independence determined that the relationship between the treatment and the patient reaching 40% percentage area reduction (PAR) in four weeks was not significant ($\chi^2(1, n=54)=0.73$; $p=0.39$ at a significance level of 0.05); however, there was a strong trend favouring the antibiofilm cleanser and gel. A significant reduction ($p<0.05$) in bacterial load was observed in the antibiofilm group.
Conclusion: This randomised controlled double-blind proof-of-concept study suggests that the performance of antibiofilm agents in vivo is comparable to that in vitro studies.
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antibiofilm treatment • antiseptics • biofilm • chronic wound • clinical trial • hard-to-heal wound • wound • wound care • wound dressing • wound cleanser • wound healing

Hard-to-heal wounds, a silent epidemic, affect millions worldwide.[1] These patients suffer emotional and physical distress, reduced mobility, social isolation and financial hardship. In addition, wound healing constitutes a major economic burden, with annual costs estimated at $98 billion USD in the US alone.[2] The practice of advanced wound care, an emerging specialty, has developed to address the needs of the rapidly rising population of patients with hard-to-heal wounds. Typical of a new specialty, there is a lack of clinical evidence for much of everyday practice. In particular, treating clinically significant levels of bacteria in hard-to-heal wounds.[3]

The routine use of topical antiseptic cleansers and therapies is commonplace in wound centres across the US and globally. The term 'wound hygiene' is often used to promote the use of antiseptics;[4] however, the evidence for this practice is based largely on in vitro studies. One of the reasons for the lack of clinical evidence was the difficulty in conducting clinical trials on antiseptics. The advent of point-of-care fluorescence imaging (MolecuLight, Canada) has simplified antiseptic clinical trials. In a 350-patient clinical trial, fluorescence imaging was shown to accurately detect clinically significant bacterial load in a wide variety of hard-to-heal wounds.[5] The use of this validated technology permitted the comparison of antibiofilm therapies to saline and an amorphous gel.

It is estimated that >70% of hard-to-heal wounds contain biofilm-based bacteria.[6] Most topical antiseptics cannot disrupt biofilm due to the extracellular polymeric substance (EPS) that protects the bacteria within the biofilm. To kill the biofilm bacteria, the antiseptic therapy must first disrupt the EPS.[7] A novel antibiofilm agent, PHMB-1 (Sanara MedTech, US) is formulated to disrupt the biofilm and kill bacteria. It is composed of 0.1% polyhexamethylene biguanide (PHMB), purified water, poloxamer 407, sodium chloride, ethylhexylglycerin, hypromellose, octane-1,2-diol, and edetate disodium and edetate trisodium chelating agents. The reasoning behind the formulation is to use the poloxamer 407 surfactant and the EDTA agents to disrupt the biofilm, allowing the PHMB to kill the bacteria. The additional ingredients are designed to reduce the reformation of biofilms. Laboratory studies demonstrated that the PHMB-1 wound cleanser and gel formulations were effective in treating immature and mature biofilms produced by meticillin-resistant

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Staphylococcus aureus (MRSA), *Pseudomonas aeruginosa* and *Candida albicans*.[8]

The primary objective of this study was to compare the wound percentage area reduction (PAR) between the standard of care (SoC) and an amorphous gel with the treatment of interest: a combination of a synergistic antimicrobial cleanser and a gel.

Methods

Ethical approval and patient consent

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (IRB) of WCG Clinical (protocol code: IRB Pr #: 20215697, 8 November 2021).

Written informed consent was obtained from all subjects involved in the study, which included for the publication of photographs.

Subject characteristics

Patients with acute or hard-to-heal wounds were recruited for this study from participating wound clinics. Once patients had agreed to adhere to the study schedule (weekly visits and follow-up regimen), and had read and signed the IRB-approved informed consent form, screening was conducted to determine whether the inclusion criteria were met:

- The patient must be at least 18 years old, and if female and of childbearing potential, must be willing to use acceptable methods of contraception (birth control pills, barriers or abstinence)
- The presence of a diabetic foot ulcer (DFU) extending through the dermis or subcutaneous tissue or the presence of a full-thickness venous leg ulcer (VLU)
- To assess the primary endpoint of the trial, the index ulcer must have been present for >4 weeks before screening
- A DFU size 0.75–5.0cm^2 and a VLU size 2.0–20.0cm^2 at first treatment visit
- Circulation to the affected extremity is adequate as demonstrated by a transcutaneous oxygen measurement (TCOM) or skin perfusion pressure (SPP) measurement of ≥3mmHg, or an ankle–brachial index between 0.7–1.29 or a toe–brachial index >0.5 within 3 months of the first screening visit.

Failure to meet the above criteria resulted in patients being excluded from participating in the study.

Study procedures

Enrolled patients who met the inclusion criteria and provided consent were randomised 1:1 to either have their wound cleansed with an amorphous gel (NSS-HG) or a synergistic antibiofilm cleanser and antibiofilm gel (AMC-AMG). Unblinded staff prepared labelled solutions of the two treatments in accordance with the randomisation scheme. Unblinded staff also provided the patient with a four-week supply of their assigned treatment.

After the screening visit, enrolled patients started treatment visit 1. At this treatment visit, initial procedures were conducted:

1. Confirmation of inclusion criteria and randomisation to treatment
2. Photography of the wound and digital surface area measurement
3. MolecuLight procedure
4. Wound cleansing with randomly assigned treatment
5. Second MolecuLight procedure
6. Swab of wound for protease activity
7. Application of wound dressing at the discretion of principal investigator
8. Four-week supply of assigned treatment provided to the patient
9. If applicable, wound debridement, measurement, off-loading walker fitting (DFU applicable), or application of multilayer compression (VLU).

At the subsequent treatment visits (visits 2–4), all steps were repeated except for step eight. At the end of the study visit (treatment visit five), final assessments were made to review potential adverse events, concomitant medications, clinical signs and symptoms of infection, and pain status. If applicable, wound debridement, measurement and photography were performed. The study exit form, that documented the current status of their wound, was provided to patients and additional placement of SoC dressings were provided if the wound had not healed.

Statistical methods

Summary statistics, including mean, median, interquartile range, counts and percentages were used to produce Tables 1 and 2 based on demographic and treatment data collected from patients.

A Chi-squared test of independence was conducted to assess the primary endpoint, which was the proportion of wounds reaching 40% PAR in four weeks. The assumptions of the Chi-squared test of independence were met. These included randomly selected patients, independent treatment groups, and the use of two categorical frequency variables (treatment group and the patients reaching the threshold of 40% PAR in four weeks). The expected frequency for each group was >5. A significance level of 0.05 was used for this hypothesis test. For the primary endpoint, the null hypothesis states that the proportion of wounds reaching 40% PAR in four weeks is equal for both treatments, while the alternative hypothesis states that they are not equal for both treatments. A TI-84 graphing calculator (Texas Instruments, US) was used to calculate the Chi-squared test statistic and p-value.

A t-test was used to evaluate the secondary endpoint, which was the PAR reduction at four weeks. The assumptions of the t-test were met. These included randomly selected, independent, normal variables that have similar variance. A significance level of 0.05 was used for this hypothesis test. For the secondary endpoint, the null hypothesis states the mean difference in area (cm^2) between baseline and the end of study/fifth treatment visit is equal for both treatments, while the alternative hypothesis states that they are not equal

for both treatments. A TI-84 graphing calculator was used to calculate the test statistic and p-value.

Another separate Chi-squared test was used to evaluate the secondary endpoint, the proportion of wounds with a bacterial load of $<10^4$ colony forming units (CFU)/g. The assumptions of the Chi-squared test of independence were met. These include randomly selected patients, independent treatment groups, and the use of two categorical frequency variables (treatment group and the reduction of the bacterial load between the first treatment visit and end of service/fifth treatment visit). The expected frequency for each group was >5. A significance level of 0.05 was used for this hypothesis test. For the secondary endpoint, the null hypothesis states that the proportion of wounds with a reduction in bacterial load by the fifth treatment visit/end of service is equal for both treatments, while the alternative hypothesis states that it is not equal for both treatments. A TI-84 graphing calculator was used to calculate the Chi-squared test statistic and p-value.

Results

A total of 54 patients from multiple sites were included in this study with 21 DFUs and 33 VLUs. Each treatment group had 27 patients. Overall, there were 32 female patients (59.26%) and 22 male patients (40.74%) enrolled, with an age range of 36–96 years. Table 1 provides demographic summary statistics for the two treatment groups, with PHMB-1 synergistic antimicrobial cleanser and antimicrobial gel plus SoC (AMC-AMG + SoC) as the treatment of interest.

A Chi-squared test of independence was used to determine the relationship between the treatment and the patient reaching a threshold of 40% PAR in four weeks. The relationship of these variables was not significant (α=0.05 or α=0.10; $\chi^2(1, n=54)=0.73$; p=0.39).

The 27 patients who received AMC-AMG+SoC ($\bar{x}$_A=2.12; s=2.68) compared to the control group of 27 patients who received NSS–HG+SoC ($\bar{x}$_B=3.01; s=5.24) did not demonstrate a significantly different PAR at four weeks ($t(52)=-1.65$; p=0.053; α=0.05). However, at α=0.10, there was significant evidence to reject the null hypothesis that the mean difference in area (cm^2) between baseline and the end of study/fifth treatment visit was equal for both treatments.

A Chi-squared test of independence was used to determine the relationship between the treatment and the reduction of bacterial load to $<10^4$ CFU/g (by fluorescence using the MolecuLight device) by the fifth treatment visit. The relationship of these variables was significant ($\chi^2(1, n=40)=3.872$; p=0.049; α=0.05). However, at α=0.10, there was significant evidence to reject the null hypothesis, that the proportion of wounds with a reduction in bacterial load by the fifth treatment visit/end of service was equal for both treatments.

Fig 1 shows the reduction in bacterial fluorescence before and after treatment with the active treatment (AMC-AMG+SoC).

Table 1. Demographic summary statistics for this (Biakos Lower Extremity Ulcers (BLEU)) trial by treatment group

Variable	AMC-AMG + SOC	NSS-HG + SOC
Age, years, mean±SD	64.85±13.17	59.41±13.60
Race, n (%)		
White	21 (77.78)	23 (85.19)
African-American	5 (18.52)	3 (11.11)
Hispanic	1 (3.70)	1 (3.70)
Sex, n (%)		
Male	7 (25.93)	15 (55.56)
Female	20 (74.07)	12 (44.44)

AMC-AMG—synergistic antibiofilm cleanser and antibiofilm gel; NSS-HG—normal saline wash and an amorphous gel; SD—standard deviation; SOC—standard of care

Table 2. Wound summary statistics

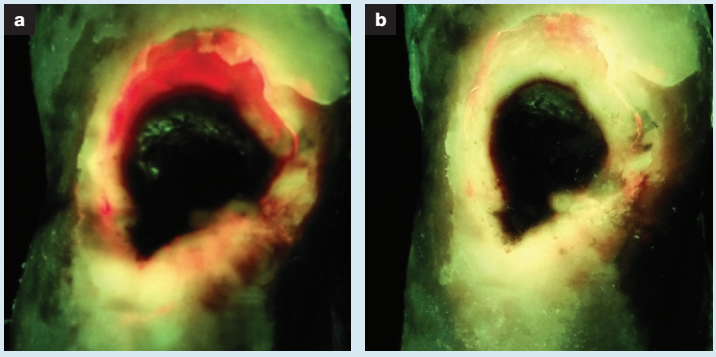
Variable	AMC-AMG + SOC	NSS-HG + SOC
Wound area, cm^2		
Mean±SD	4.44±4.37	4.62±5.31
Median (IQR)	2.52 (1.51, 6.33)	2.63 (1.40, 6.40)
Wound age, weeks		
Mean±SD	24.82±42.88	19.11±22.50
Median (IQR)	8 (4.5, 26.5)	8 (4.5, 21.5)
Wound type, n (%)		
DFU	10 (37.04)	11 (40.74)
VLU	17 (62.96)	16 (59.26)

AMC-AMG—synergistic antibiofilm cleanser and antibiofilm gel; DFU—diabetic foot ulcer; IQR—interquartile range; NSS-HG—normal saline wash and an amorphous gel; SD—standard deviation; SOC—standard of care; VLU—venous leg ulcer

Discussion

Bacterial reduction is a hallmark of wound bed preparation. This includes addressing biofilm in the

Fig 1. A diabetic foot ulcer before **(a)** and after **(b)** debridement and cleansing with the polyhexamethylene biguanide (PHMB)-1 active agent. There is complete resolution of bacterial fluorescence, indicating a reduction in bacterial load below 10^4 colony-forming units (CFU)/g



wound bed through debridement and the use of antibiofilm agents.[9,10] Treatment algorithms drafted by several wound healing societies recommend the use of antiseptics to reduce bacterial levels in hard-to-heal wounds.[11] There are many topical antiseptics commercially available; however, the evidence supporting these agents is based primarily on in vitro testing. The hard-to-heal wound bed is a hostile and complex environment. Bacteria can exist in a planktonic phenotype or protected within a biofilm. As a result, the performance of an antiseptic in the laboratory may not correlate with its antimicrobial action in a hard-to-heal wound. The goal of this proof-of-concept study was to examine a novel antibiofilm formulation in vivo: in patients with hard-to-heal wounds. This trial design may be a replicable model to evaluate topical antiseptics in hard-to-heal wounds.

The recently introduced concept of biofilm-based wound care focuses on treatment regimens that reduce biofilm in hard-to-heal wounds.[12] Debridement disrupts the biofilm, permitting killing by topical antiseptics; however, debridement does not remove all bacteria or biofilm from the wound.[13,14] It has been theorised that the addition of antiseptics may facilitate the reduction of bacteria post-debridement. A new category of topical antiseptics specifically targets the structure of the biofilm. When combined with debridement, these antiseptics may promote healing by more effectively reducing bacterial burden. In this clinical study, there was a trend toward more rapid healing in the patients receiving PHMB-1, a formulation designed to break down biofilm and kill bacteria. In addition, fluorescence imaging demonstrated that the combination of debridement, cleansing with an antibiofilm antiseptic and subsequent treatment with an antibiofilm gel significantly reduced the wound bacterial burden compared to saline and an amorphous gel. As shown in Fig 1, there were isolated cases in which debridement and the PHMB-1 cleanser alone cleared the bacterial fluorescence in a single treatment.

Limitations

The primary limitation of this study was the small sample size. The trial was designed as a proof-of-concept study to evaluate the PHMB-1 cleanser and gel in patients with hard-to-heal wounds and to test the practicality of this new trial design. Investigators should consider powering future randomised trials to demonstrate the difference between groups. This study also included two wound types, which added heterogeneity to the trial. There are differences in bacterial loads, types of bacteria, ease of debridement and SoC between VLUs and DFUs. While typical for proof-of-concept studies, in larger randomised clinical trials it may be best to study a single wound type and dressing materials.

The study demonstrated the effectiveness of the trial design. There are few double-blind wound healing clinical trials. The use of a normal saline and over-the-counter carboxymethyl cellulose gel control reduced bias in this study. The blind was not broken during the trial. Most of the therapies currently used in the wound clinic were studied in unblinded trials, which decreases confidence in the results. The introduction of point-of-care fluorescence imaging to measure bacterial burden obviated the need for expensive and invasive quantitative tissue culture biopsies. Fluorescence imaging saved time and money: the trial was completed in <1 year, and fluorescence imaging was far less expensive than quantitative tissue biopsies. The trial used the well-recognised surrogate endpoint of PAR at 4 weeks.[15] This is a more appropriate endpoint for a trial examining bacterial reduction than complete wound closure. In addition, it shortened the length of the trial and enhanced patient recruitment.

Conclusion

In this proof-of-concept study, the combination of debridement, cleansing with an antibiofilm antiseptic, and treatment with an antibiofilm gel reduced the bacterial burden in hard-to-heal wounds. **JWC**

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Reflective questions

- Does the addition of antibiofilm agents promote healing in hard-to-heal diabetic foot ulcers (DFUs)? If so, how?
- What is the efficacy of the standard of care between DFUs and venous leg ulcers?
- Does wound age affect the development of biofilm on hard-to-heal DFUs?