Clinical Assessment of a Biofilm-disrupting Agent for the Management of Chronic Wounds Compared With Standard of Care: A Therapeutic Approach

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ABSTRACT

Objective. The authors study the use of a biofilm-disrupting wound gel designed for wound management to determine if disrupting chronic wound biofilm would be therapeutically efficacious. **Materials and Methods.** This prospective, randomized, open-label clinical trial was performed from September 2014 through March 2016. Forty-three patients (22 experimental, 21 control) with chronic, recalcitrant wounds were randomized to a 12-week treatment with a biofilm-disrupting wound gel (experimental) or a broad-spectrum antimicrobial ointment (control). The wound healing rate was assessed by measuring wound size reduction and wound closure rates. **Results.** Wound size in the experimental group decreased significantly with a 71% reduction in wound area compared with 24% for the control ($P < .001$). Wound closure was attained in more than half of the patients (14) treated with the experimental product. Fifty-two percent of these patients achieved closure by 12 weeks as opposed to 17% for the control ($P < .01$). No adverse events related to the experimental product were recorded, but 2 adverse reactions occurred with the control. **Conclusions.** The combination of the experimental product and wound debridement significantly improved wound healing rates by disrupting the biofilm, which protects multispecies bacteria within a chronic wound. Given the significant wound size reduction and closure rates observed in these long-term, nonhealing wounds, as well as the lack of related serious adverse events, the investigators believe the biofilm-disrupting wound gel to be a safe and effective treatment for recalcitrant chronic wounds.

KEY WORDS

chronic wound, biofilm, extracellular matrix, antimicrobial resistance, diabetic foot ulcer, infection

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Although the definition of a chronic wound varies, authors consider a wound chronic when it fails to proceed through an orderly reparative healing process that produces an anatomic and functional result within 1 to 3 months (as defined in surgical textbooks).[1] All wounds have the potential to become chronic, with the most common etiologies being venous insufficiency, arterial perfusion, diabetes, or unrelieved pressure.[2]

As health care costs continue to increase and conditions causing chronic wounds become more prevalent, it is necessary to find an efficacious yet cost-effective treatment for these nonhealing wounds.[3] Chronic wound care has been estimated to cost in excess of $25 billion per year, with a median cost of $3927 per wound.[4,5] Traditionally, a multidisciplinary treatment strategy is employed with the aim of correcting the underlying cause of the chronic wound; physical debridement, topical therapies, and dressings are in the armamentarium of chronic wound treatment.[6–10]

The presence of biofilms in chronic wounds presents significant obstacles to treatment, and it is estimated that biofilms are involved in more than 60% of patients with chronic wound infections.[10–12] Biofilms form when bacteria attach to a surface and aggregate to create a structure of extracellular polymeric substances (EPS) that protects bacteria and allows growth in a sheltered environment.[11,13] Biofilms can grow on a variety of surfaces, from medical devices to living tissue, and they are inherently resistant to antimicrobial agents.[14] The biofilm's resistance to antimicrobial agents occurs through multiple mechanisms, including physical protection from agents entering the biofilm, reaction of the agents with the EPS matrix (which may prevent deep

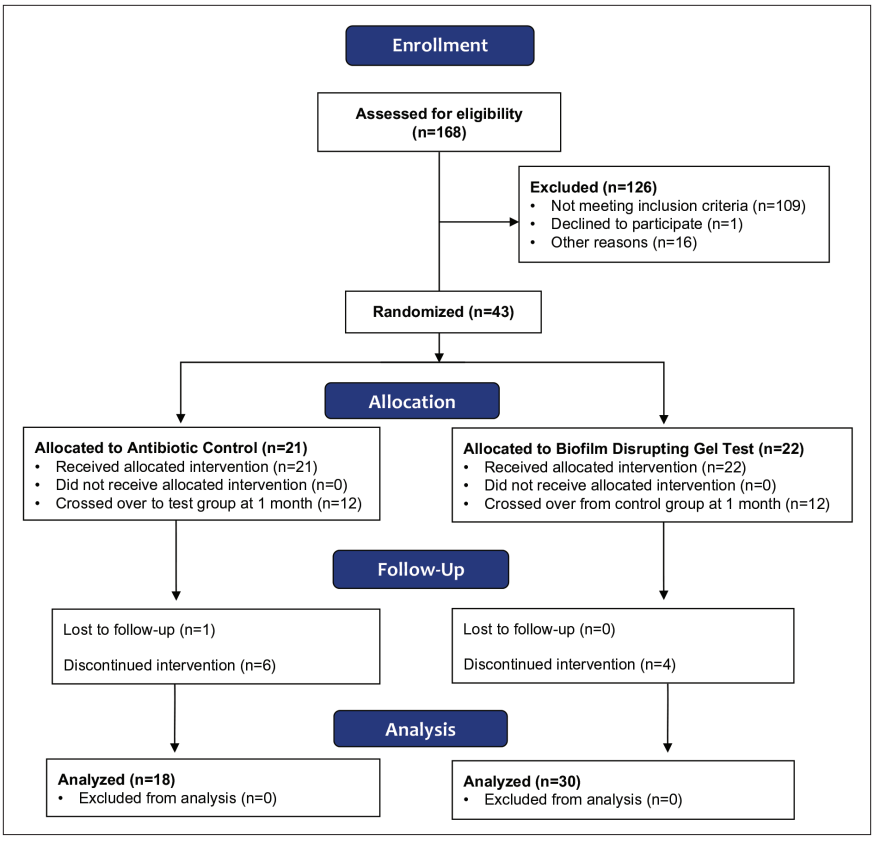

Figure 1. Consolidated Standards of Reporting Trials flow diagram.

KEYPOINTS

- The authors study the use of a biofilm-disrupting wound gel designed for wound management to determine if disrupting chronic wound biofilm would be therapeutically efficacious.
- This was a 12-week to 16-week, 2-site, prospective, randomized, open-label study of 43 patients diagnosed with a recalcitrant chronic wound.
- The treatment outcomes of standard debridement with topical application of a biofilm-disrupting wound gel were compared against a triple-antibiotic, maximum-strength ointment.

penetration), or low metabolic activity of cells within the biofilm.[15,16] This resistance persists even with biofilm bacterial cells located at the surface of the biofilm, which would be expected to behave more like single-celled planktonic bacterial cells.[11] Ammons et al[10] investigated biofilms in chronic versus acute wounds and suggested that disruption of the biofilm aids in healing of chronic wounds.

Debridement is a mainstay of treatment for wound care. Sharp debridement removes nonviable tissue along with the bacterial biofilm, making the underlying bacterial load more susceptible to targeted therapy.[2,7] Wolcott and Rhoads[11] assessed biofilm-based treatment strategies, such as application of lactoferrin and xylitol with selective biocides, in combination with standard of care treatment. They showed a 75% healing frequency of chronic wounds in patients with diabetes and critical limb ischemia.[11] Wolcott[8] further showed that biofilm-based strategies, biofilm-disrupting agents, and application of antibiotic topical gels customized to each bacterial biofilm community in a specific wound resulted in over 40% chronic wound volume reduction over 4 weeks.

Surfactants are of interest in regard to autolytic debridement of chronic wounds. Miller et al[17] found that surfactants emulsified the biofilm matrix of *Pseudomonas aeruginosa*, and they also showed an elimination of biofilm bacteria 1 day after physical wiping and application of the surfactant gel to an ex vivo model.

In this study, the biofilm-disrupting wound gel is a polyethylene glycol-based hydrogel with a pH buffer system and benzalkonium chloride surfactant, which destabilizes the biofilm matrix through the chelation of calcium and removes proteins from bacterial membranes, causing cell lysis.[17] It is a white, virtually odorless gel that helps maintain a moist wound environment conducive to healing while eradicating biofilm from the surface of the wound. This biofilm-disrupting wound gel has been approved by the US Food and Drug Administration as a medical device; with full ISO 10993 safety testing, it was proven safe in a full-thickness porcine wound, whereby it had no negative effects on healing. The efficacy of this product has been demonstrated in in vivo[17] and in vitro models (data on file). In vitro biofilm testing was performed at the Montana State University Center for Biofilm Engineering (Bozeman, MT) in a mixed-species biofilm model, whereby the gel achieved more than a 3-log reduction in *P aeruginosa* and a 5-log reduction in *Staphylococcus aureus* in 24 hours (data on file). Miller et al[17] reported the efficacy of this product in clearing biofilms from murine models with chronic infections, which

demonstrated the efficacy of this product in vivo.

Wolcott[8] conducted the first clinical study using this biofilm-disrupting gel and showed a 62% chronic wound volume reduction in 4 weeks (when applied 3x/week), while a combined application of both customized topical antibiotics and the wound gel resulted in a 72% wound volume reduction. In his 45-patient study, 80% of patients achieved at least 50% wound volume healing success within 1 month with the biofilm-disrupting wound gel alone.[8]

The present longer term clinical study is the second investigation of the efficacy of the biofilm-disrupting wound gel in the treatment of nonhealing, full-thickness chronic wounds to confirm the potential therapeutic effectiveness of this approach; this is achieved by measuring changes in the healing rate and wound closure when compared with a broad-spectrum, maximum-strength, triple antibiotic ointment over a 3-month time period.

MATERIALS AND METHODS

Study design

Study protocol was approved by both the Mayo Clinic Institutional Review Board (Rochester, MN) and The Schulman Institutional Review Board (Cincinnati, OH). This was a 12-week to 16-week, 2-site, prospective, randomized, open-label study of patients diagnosed with a recalcitrant chronic wound. This study compared the treatment outcomes of standard debridement with topical application of a biofilm-disrupting wound gel (experimental; BlastX; Next Science, Jacksonville, FL) versus a triple-antibiotic, maximum-strength ointment (control; Neosporin + Pain Relief; Johnson & Johnson, New Brunswick, NJ).

Patients who presented with a chronic wound had a medical evaluation prior to being screened against the protocol's inclusion/exclusion criteria. If criteria were met, patients were presented with the option to participate in the study and informed consent procedures were carried out in compliance with currently applicable patients' rights and safety regulations. The sample size was calculated to be 15 patients per group by power analysis (95% power at $P = .05$ comparing experimental group to control, assuming a 23% standard deviation and 32% difference), referencing the results by Wolcott.[8]

The 2 study sites were the Mayo Clinic in Jacksonville, Florida, and River City Clinical Research in Jacksonville, Florida. The study coordinators at each site enrolled and assigned participants into their respective groups in the order of enrollment per the randomization table provided by the experimental product manufacturer. Patients were randomized 1:1 to apply either the experimental or control once daily with the prescribed daily wound dressing change.

Patients randomized to the control group were required to complete 1 month of treatment comprising a screening/baseline, 2-week, and 1-month visits. After 1 month, the following occurred based on wound progression and/or the principal investigator's (PI) clinical judgment:

1. Patient continued daily control application (8-week and 12-week visits).
2. Patient crossed over to the exper-

Table 1. Inclusion and exclusion criteria

INCLUSION CRITERIA	EXCLUSION CRITERIA
• Aged ≥18 years	• Aged <18 years
• Presence of full-thickness wound > 1 month's duration	• Presence of a full-thickness wound <1 month's duration
• Wound >1cm² in area	• Wound <1cm² in area
• Not a candidate for vascular reconstructive surgery to restore blood flow to the wound area	• Candidate for vascular reconstructive surgery to restore blood flow to the wound area
• Willing to comply with study procedures and be available for the duration of the study	• Presence of bleeding dyscrasia or with conditions that would make a bleeding complication likely
• Provide signed and dated informed consent	• Known allergic reaction to the study products
	• Unable to provide signed and dated informed consent

Table 2. Demographics and characterization of study groups

	CONTROL	EXPERIMENTAL
No. of Patients	21	34
Male (%)	82	63
Female (%)	18	37
Age (y ± SD)	61±14	60±13
Wound age (mos ± SD)	17±21	22±47
Wound size (cm² ± SD)	12±17	9±22

SD: standard deviation

Table 3. Patient comorbidities

CONDITION	PREVALENCE
Diabetes mellitus	60%
Peripheral arterial disease	40%
Hypertension	35%
Obesity	26%

imental group with daily product application (4-week, 8-week, and 12-week visits).

Patients randomized to the experimental group were evaluated for 3 months, which comprised 5 visits: screening/baseline, 2-week, 4-week, 8-week, and 12-week (**Figure 1**). After the last research study visit (12-week or 16-week visits), patients could continue treatment as long as beneficial per PI judgment; no limit was set on the duration of extension. During the extension, visits were scheduled and carried out per standard clinical care. During the exit visit of the study (at the end of the treatment extension period), the wound measures were retrospectively obtained from the patients' charts.

Wound area measurements were assessed at weeks 0, 2, 4, 8, 12, and 16 using the Silhouette Star camera (ARANZ Medical, Christchurch, New Zealand). The primary endpoint was defined as a percentage reduction in wound area after 12 weeks of experimental treatment compared with the control.

There were 2 defined secondary endpoints for this study. The first secondary endpoint was defined as an improvement in the percentage of patients with closed wounds after 12 weeks of treatment compared with the control. The other secondary endpoint was to determine if there was a difference in the bacterial load and/or biodiversity in the wound when comparing treatments and treatment time.

Inclusion and treatment

Patients participated in the study if they met all of the inclusion criteria and none of the exclusion criteria listed in **Table 1**.

At the first visit, the screening/eligibility form and enrollment checklist were completed, identification numbers were assigned, and patients were randomized into either the experimental or control group. Demographics were obtained along with wound-related history and concomitant medications.

At each visit, the patients' wounds were debrided with sharp instruments, and wound measurements and images were obtained. Measurements included wound area, volume, and depth.

Biofilm samples were obtained at baseline and after 1 month of treatment. Samples were analyzed in 2 ways: (1) by quantitative real-time polymerase-chain reaction (PCR) test for bacteria and fungi (which also included a qualitative real-time PCR test for resistance factors to vancomycin and methicillin); and (2) by DecodEX Microbial Genetic Identification Sequencing (MicroGen DX, Lubbock, TX) to detect bacterial organisms and fungal pathogens that may be present in patient specimens.

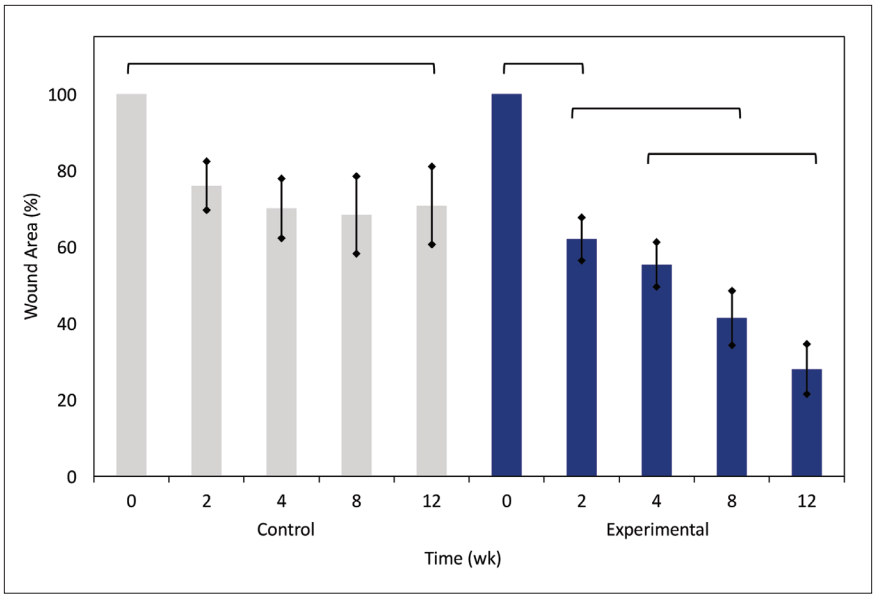

Figure 2. Comparison of normalized wound area reduction over time. After 12 weeks of treatment with the experimental product, the average wound area reduction was 72% versus 15% in the control group ($P < .01$). P value for treatment time was <.01. Histogram shows that wound areas were continuing to decrease at 12 weeks compared with the plateau in the control group. Bars depict one-standard error from the mean; grouping bars depict groups that are equivalent. Treatment bars that do not fall under the same grouping bar are statistically distinct ($P < .05$).

Statistics

Statistical analyses were performed using Minitab (Version 17.3.1; Minitab, Inc, State College, PA) on the intent-to-treat population. All patients who were enrolled and randomly allocated to treatment were included in the analysis and were analyzed in the groups to which they were randomized as well as in the crossover group where indicated. Statistical significance to the control was determined by analysis of variance (ANOVA) using a general linear model with factors as the treatment (experimental; control), time (0, 2, 4, 8, and 12 weeks), clinical research site (Mayo Clinic; River City Clinical Research), comorbidities, and patient. Tukey's pairwise comparisons were performed for grouping ($P < .05$). In the Figures, interval bars in the results section depict one-standard error and the grouping bars depict groups that are equivalent (for the healed wounds and percent of wound closure). Treatment bars that do not fall under the same grouping bar are statistically distinct ($P < .05$; for the healed wounds and percent of wound closure).

Table 4. Wound size measurements

TREATMENT	TIME (wk)	MEAN (cm²)	MEDIAN (cm²)
Control	2	76.0	83.3
	4	70.1	87.8
	8	68.4	90.2
	12	70.8	95.1
Experimental	2	62.1	63.5
	4	55.4	57.9
	8	41.4	36.5
	12	28.0	9.4

Table 5. Wound size measurements (control excluding crossover patients)

TREATMENT	TIME (wk)	N	MEAN (cm²)	MEDIAN (cm²)
Control	2	19	76.0	83.3
	4	19	70.1	87.8
	8	9	48.7	45.8
	12	9	46.8	50.0
Experimental	2	34	62.1	63.5
	4	34	55.4	57.9
	8	28	41.4	36.5
	12	27	28.0	9.4

RESULTS

Demographics

Forty-three patients were enrolled in the study with 32 completing all study visits. The study visits ended after each patient finished 12 weeks of treatment or the patient's wound was healed. The first patient visit occurred in September 2014 and the last patient study visit was in March 2016.

At the end of the enrollment period, 22 patients were randomized to the experimental group and 21 were randomized to the control group; however, 12 of the control patients crossed-over to the experimental group due to the wound worsening or failure to heal/lack of improvement, resulting in an experimental product group of 34 patients and a control group of 21 patients (**Table 2**). Patients ranged in age from 32 to 91 years (average, 62 years). The age range of the wounds in the study ranged from 1 month to 20 years (average, 21.2 months). Wound size ranged from 1 cm² to 114 cm² (average wound area, 10 cm²).

The patient population had a high number of comorbidities (**Table 3**), with all patients except 1 presenting with 1 or more. These comorbidities were not statistically significant factors affecting wound closure or healing rates when analyzed by ANOVA.

Wound size reduction

The primary endpoint was the decrease in wound area at 12 weeks (**Figure 2**). The statistical power of the wound closure percentage was greater than 99% (type I error of 0.05 comparing experimental to control), indicating that sufficient patients were evaluated to yield meaningful results.

The mean and median values for the percent wound area at each time point, with all patients included in the control group, is demonstrated in **Table 4**. The average wound area reduction in the experimental group was 72% ± 8% at 12 weeks; this was statistically significant in comparison with the control ($P < .01$) and for treatment time ($P < .01$). The wound healing reduction of the experimental group was 2.44 times greater than the control. In the case of median wounds, which removes the bias from the outliers, the difference is even greater, with median wound size for the experimental group being 90% compared with 5% for the control. The wound reduction rate increased as the treatment time progressed, indicating that those wounds that were not yet closed were progressing towards closure.

The number of patients at each time point as well as the mean and median values for the percent wound area at each time point, with the crossover patients removed from the control group, is shown in **Table 5**.

The average wound area reduction of the experimental product was 72% ± 8% at 12 weeks; this was statistically significant versus control ($P < .01$) and for treatment time ($P < .01$). Even including only those patients that were showing improvement with the control (and not including those that failed treatment with the control [ie, crossed over]), the wound healing reduction was 36% greater than the control product. In the case of median wound size reduction, which removes the bias from the outliers, the difference is even greater, with the median wound size reduction for the experimental group being 90% compared with 50% for the control. As such, median improvement with the experimental product is 1.8 times greater than the control. Moreover, the wound reduction rate in the experimental group increased as the treatment

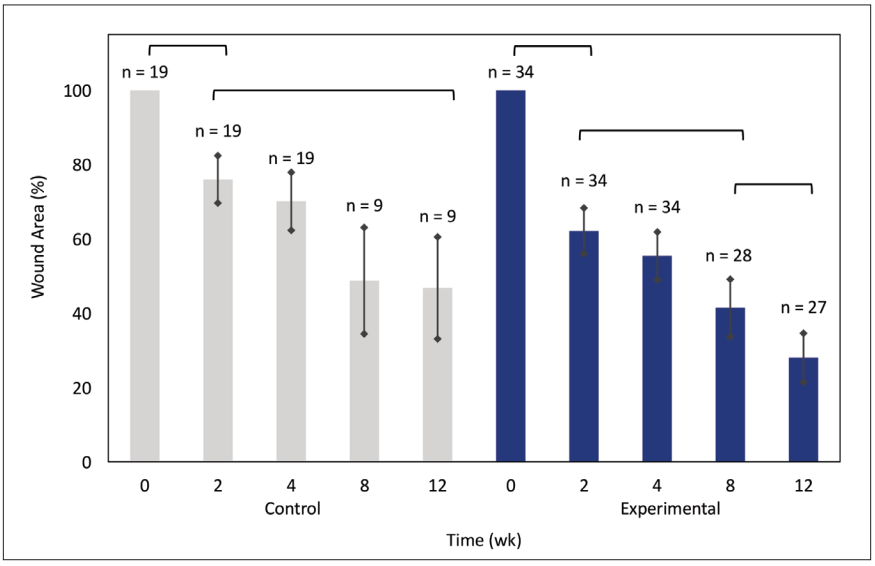

Figure 3. Comparison of normalized wound area reduction over time, including only those control patients who were not crossed over. After 12 weeks of treatment with the experimental product, the average wound area reduction was 72% versus 53% in the control group ($P < .02$). *P* value for treatment time was < .01. Histogram shows that wound areas were continuing to decrease at 12 weeks compared with the plateau in the control group. Bars depict one-standard error from the mean; grouping bars depict groups that are equivalent. Treatment bars that do not fall under the same grouping bar are statistically distinct ($P < .05$).

KEYPOINTS

- The average wound area reduction of the experimental product was 72% ± 8% at 12 weeks, which was statistically significant versus control (P < .01) and for treatment time ($P < .01$).
- The mean number of bacteria present in the wound was 2.9 species per wound (range, 0-13 bacteria); there was no statistically significant relationship between the bacteria present in the wound or number of bacterial species present and wound healing or healing rates.

time progressed, indicating that those wounds that were not yet closed were still progressing towards closure.

Wound closure

The secondary endpoint was the increase in the percentage of wounds that were closed after 12 weeks of treatment (**Figures 4, 5**). The use of the experimental agent improved the success rate for chronic wound healing. The statistical power of the wound closure percentage was 90% (type I error of 0.05 comparing experimental product to vehicle), indicating that sufficient patients were evaluated to yield meaningful results.

After 12 weeks of treatment with the experimental product, 52% of patients achieved wound closure. This was statistically significant versus the control ($P < .01$) and for treatment time ($P < .001$). The percentage of patients with healed wounds was 3.12 times greater in the experimental group than the control (17% closure). Also, it is interesting to note that 40% of the wounds treated with the experimental agent were closed within 8 weeks of once per every other day application; in fact, wound closure started to occur as soon as 2 weeks in the case of a wound that had failed to heal with the use of numerous treatments for more than 20 years. The wound size range for the group with healed wounds was 0.7 cm^2 to 7.3 cm^2 (average, 2.3 cm^2), and the age range of the wounds was 1.5 to 240 months (average, 29 months).

Biofilm analysis

There were 90 bacterial and 4 fungal species found in the wounds. Only 5% of patients had fungi in their wounds. Of the 90 bacteria, only 17 were found in at least 10% of patients. **Figure 6** shows a histogram of these bacteria. The mean number of bacteria present in the wound was 2.9 species per wound (range, 0-13 bacteria). There was no statistically significant relationship between the bacteria present in the wound or number of bacterial species present and wound healing or healing rates. An ANOVA of the bacterial load also was not statistically significant for wound closure or wound healing.

A PCR analysis for vancomycin-resistant and methicillin-resistant genes resulted in an incidence rate too low to provide enough power for statistical evaluation.

Complications

All patients enrolled in this study presented with 1 or more comorbidities and 60% had diabetes (N = 26). There were no unanticipated problems.

The root cause of all adverse events was evaluated (**Table 6**). There were 2 adverse events that were directly related to product application in the control group; one was an allergic reaction to the control product with skin desquamation, and the other was burning sensations. In both cases, the control antibiotic ointment was discontinued, and then the issues resolved and did not reoccur with the experimental product. There were no adverse events that were attributed to the experimental product, although 1 patient was discontinued in the group due to wound site pain, which was present prior to the study treatment application and

persisted with the new treatment of PolyMem (Ferris Mfg. Corp, Fort Worth, TX) dressings.

There were 11 study discontinuations: 4 patients in the experimental group and 7 in the control (**Table 6**).

DISCUSSION

This study confirms that topical applications of a biofilm-disrupting wound gel in conjunction with debridements produce clinically significant wound size reductions and wound closure versus a broad-spectrum topical antibiotic treatment control. In this study, median wound area reduction was 72% with daily use of the experimental product for 12 weeks versus 24% with the control. Chronic wound closure occurred in 52% of patients with the use of the experimental product versus 17% closure with the control.[8] These results reinforce those obtained by Wolcott,[8] who observed a wound volume reduction of 62% with the biofilm-disrupting wound gel applied 3 times per week for 4 weeks. Notably, Wolcott[8] achieved the 47% wound volume reduction with standard of care treatment versus 24% wound area reduction seen with the control treatment in this study.It is important to note that in that clinical study, Wolcott's standard of care treatment was a proprietary topical antibiotic gel customized and compounded based on each patient's identified biofilm bacterial community.[8]

The control treatment used in this study was a maximum-strength, widely available, triple-antibiotic ointment that targets common bacteria found in chronic wounds[18,19]; per 1 gram, the ointment contains 500 units of bacitracin, 3.5 mg of neomycin, 10 000 units of polymyxin B, and 10 mg of pramoxine hydrochloride. This product was chosen as the control treatment because it is a comparable antimicrobial agent with similar indication to the experimental wound gel with the broadest spectrum of activity versus bacitracin or other single-species-specific antibiotic ointments. The triple-antibiotic components aim at medically significant species implied in skin infections, such as *S aureus*, *S epidermis*, and *Streptococcus pyogenes*. This also includes polymyxin B specific to gram-negative species such as *P aeruginosa* and neomycin, which has a partial efficacy spectrum on gram-positive bacteria, *Enterobacter*

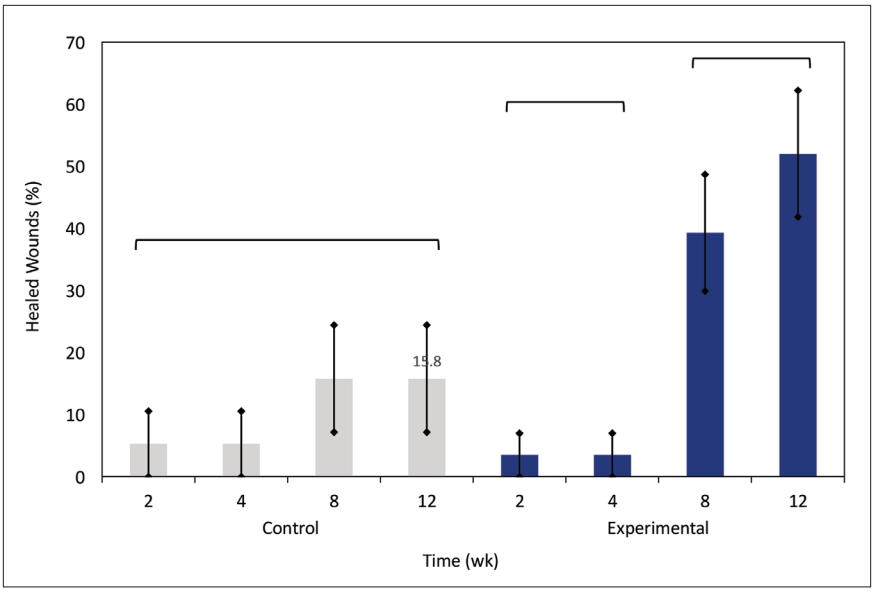

Figure 4. Comparison of percentage of healed wounds over time. After 12 weeks of treatment with the experimental product, 52% of patients achieved wound closure (*P*<.01) versus 16% in the control group. *P* value for treatment time was <.01. In the experimental group, 43% of the wounds were closed at 8 weeks versus 17% in the control group. Bars depict one-standard error from the mean; grouping bars depict groups that are equivalent. Treatment bars that do not fall under the same grouping bar are statistically distinct (*P* < .05).

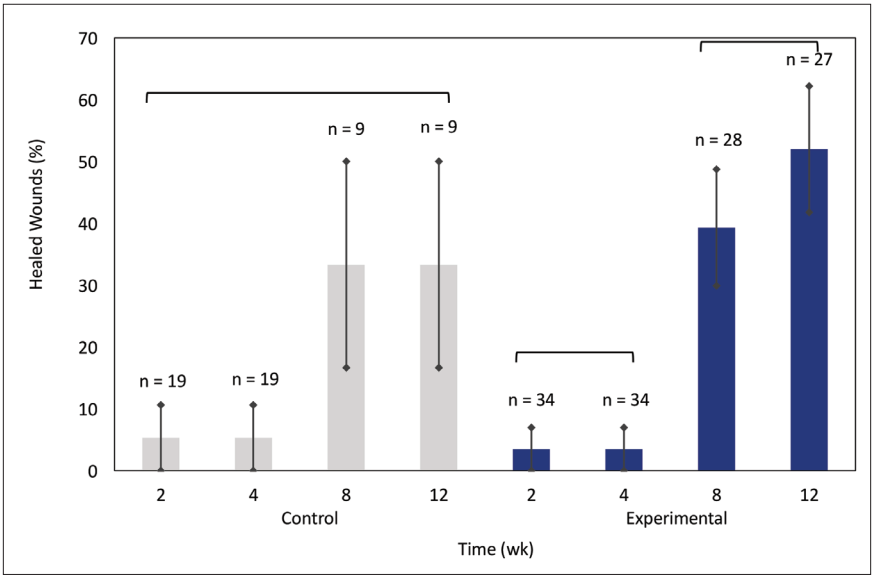

Figure 5. Comparison of percentage of healed wounds over time (excluding control patients that were not crossed over to experimental). After 12 weeks of treatment with the experimental product, 52% of patients achieved wound closure versus 33% in the control group (not statistically significant). *P* value for treatment time was < .01. In the experimental group, 43% of the wounds were closed at 8 weeks versus 33% in control group. Bars depict one-standard error from the mean; grouping bars depict groups that are equivalent. Treatment bars that do not fall under the same grouping bar are statistically distinct (*P* < .05).

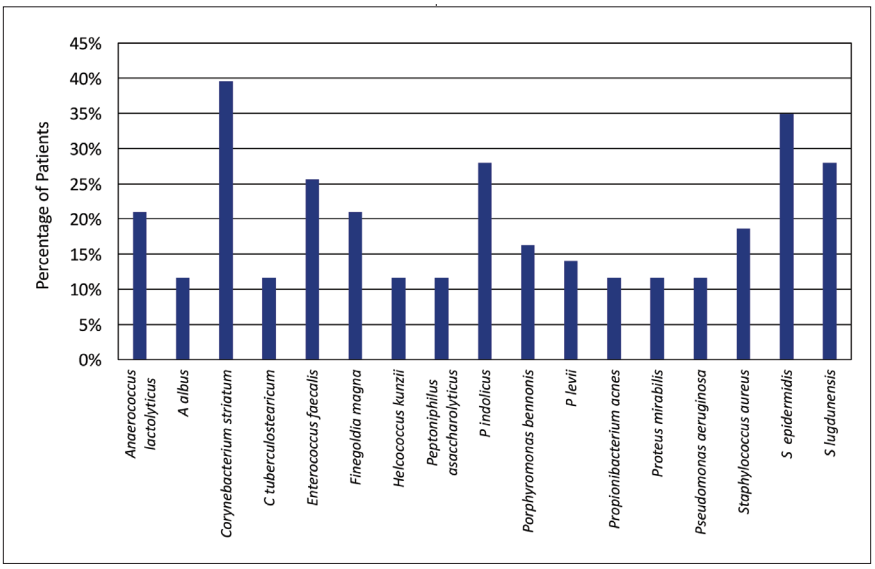

Figure 6. Histogram of the most frequently found bacteria in the study wounds. Bacterial species were detected in at least 10% of the patients' wounds.

Table 6. Study discontinuations

REASON FOR DISCONTINUATION	CONTROL	EXPERIMENTAL	TOTAL
Gangrene/osteomyelitis requiring amputation	2	1	3
Withdrawn as per physician decision	1	1	2
Patient withdrew consent	3	0	3
Lost to follow-up	1	0	1
Popliteal artery occlusion	0	1	1
Pain	0	1	1
Total	7	4	11

cloacae, *E coli*, and *Proteus vulgaris* in addition to gram-negative species activity. Furthermore, broad-spectrum topical antibiotics are used in chronic wounds to provide a high concentration of medication directly to the wound site and to avoid systemic disturbance of the normal microbiota as well as systemic allergic reactions.[18] Prior to this study, patients were treated with various therapies, including oral and topical antibiotics, antimicrobial dressings, silver alginate, and wound vacuum devices that had all failed to resolve the chronicity of their wounds.

In this study, biofilm data analysis showed that neither wound size reduction nor wound closure was significantly affected by total bacterial load or number of bacterial species. However, it has been shown that the number of individual bacteria is not considered a reliable predictor of wound healing, as numbers will change based on virulence of the bacteria, biofilm formation, and comorbidities.[20] In addition, antagonistic and synergistic interactions of bacterial species within the biofilm can result in changes in its makeup, which also was observed in this study.[21] Further study into the change in specific bacterial loads after application of the experimental wound gel may be helpful to better understand the wound gel's effects on the biofilm.

In the literature on chronic wound management, there is a lack of prospective, randomized, controlled clinical studies, which makes it challenging for practitioners to compare the efficacy of topical products currently available. Also, articles seldom provide analysis of clinically meaningful wound healing rates and closure; in most cases, the percent wound size reduction is provided as a measure of efficacy, which alone is only a partial indication of wound improvement. This leaves a desire for clinically meaningful wound healing, as the goal is for wounds to heal and remain healed. Further, biofilm-disputing technology is new and most topical treatments are antibiotic-based with narrow spectrum of efficacy.

In addition, biofilm testing methods with clinical relevance to practitioners are lacking. To the best of the authors' knowledge, there are 3 methods that could be used for product efficacy comparison, but all 3 are time-consuming to perform and reporting is too delayed for them to be used in routine practice. Montana State University has developed an in vitro biofilm model where mixed-species colonies of *P aeruginosa* and *S aureus* are grown in a *chronic wound with exudate*-like environment.[22] This drip-flow biofilm model can be used to test a product's efficacy against gram-positive and gram-negative bacteria, and it provides a measurement of log reduction of both biofilm species.[23] The University of Florida (UF; Gainesville, FL) also has developed a test method that detects and quantifies several viable biofilm microorganisms through a series of targeted specimen washes and selection by differential growth media.[24,25] This method could compare product efficacy as it provides the type and the amount of viable microorganisms present in patient specimens.[24,25] The third method available is the molecular analysis method described earlier in this clinical study, which was developed by Wolcott.[8]

All 3 of the aforementioned methods have limitations. For instance, the MSU and the UF methods are limited in the number of viable species of biofilm microorganisms that can be analyzed,

Table 7. Reported clinical study results

PRODUCT	SAMPLE SIZE	WOUNDS W/ SIZE REDUCTION (%)	HEALED WOUNDS (%)	AVG WOUND CLOSURE (%)	MEAN USE DURATION (WK)	ADVERSE REACTIONS	STUDY DESIGN
Biofilm-disrupting agent[a] (this study)	34	90	52	71	12	None	Prospective, randomized, controlled
Biofilm-disrupting agent[a] [8]	45	93	80[b]	62	4	None	Prospective, randomized, controlled
Dressing with ionic silver[c] [31]	112	65	13	NR	3.9	3	Retrospective post market surveillance
Dressing with ionic silver[c] [31]	29	90	34	62	5.4	NR	Retrospective post market surveillance
Cadexomer dressing with iodine[d] [28]	153	NR	NR	62	12	Pain; burning	Prospective, randomized, controlled multicenter
Cadexomer dressing with iodine[d] [30]	75	NR	NR	NR	24	Burning; pain; itching	Prospective, randomized, controlled
Wound cleansing solution containing PHMB and betaine[e] [32]	59	NR	60	NR	12	NR	Retrospective
Wound cleansing solution and gel containing PHMB and betaine[f] [33]	10	70	NR	NR	NR	NR	Case reports

NR: not reported; PHMB: polyhexanide
[a] BlastX (Next Science LLC, Jacksonville, FL)
[b] 50% reduction at 4 weeks
[c] Aquacel Ag Extra (ConvaTec, Deeside, Flintshire, UK)
[d] IODOSORB (Smith & Nephew, Hull, UK)
[e] Prontosan Wound Irrigation Solution (B. Braun Medical Inc, Bethlehem, PA)
[f] Prontosan Wound Irrigation Solution and Gel (B. Braun)

and these are not available as large-scale clinical diagnostic tests. The molecular analysis is available as a clinical tool that provides qualitative and quantitative results on innumerable bacterial and fungal species but does not differentiate live from dead or pathogenic from non-pathogenic microorganisms.[26] However, results from the molecular analysis are reliable, and high representation of 1 or more species in a patient specimen is unlikely to originate from nonviable microorganisms. This molecular method has been shown to be clinically relevant in improving the diagnostic and treatment outcomes of patients with chronic wounds[6] as well as actionable diagnosis where conventional diagnostic work-up is unrevealing.[23,27]

A comparison of the available antibiofilm wound products' clinical data shows that the experimental agent results in the highest percentages of healed wounds and wound size reduction available to date (**Table 7**[8,28,30,33]). Although this product was evaluated under more stringent randomized controlled study conditions compared with the retrospective record reviews of other similar products, the average wound closure rates with the experimental agent was greater than AQUACEL Ag Extra (ConvaTec, Deeside, Flintshire, UK). Compared to Pron-

tosan Wound Irrigation Solution and Gel (B. Braun Medical Inc, Bethlehem, PA) products, treatment of chronic wounds with the experimental agent in this study resulted in substantially more healed wounds and a higher percentage of improved wounds (**Table 7**[8,28,30,33]). While iodine-based topical products have been commercialized for more than 170 years,[29] efficacy data against biofilm is based on limited clinical evidence.[28,30] When comparing the results of a prospective, randomized controlled study by Hansson[28] with the experimental product in the present study at 12 weeks of treatment, IODOSORB (Smith & Nephew, Hull, UK) showed a 62% average wound closure rate and the experimental agent showed 71%.

Another important aspect for the practitioner is the patient's tolerance to wound care treatment. The use of the experimental agent did not result in any product-related pain, redness, swelling, burning/stinging, or other adverse reactions in the 34 patients in this study. This is in line with the observations of the Wolcott clinical study[8] where 30 patients were treated with the same biofilm-disrupting agent.

LIMITATIONS

The greatest challenges for patients with chronic wounds involve getting the patient to adhere to dressing changes and weekly standard of care visits for surgical wound debridements.[34,35] This was also a study limitation as patient wounds were clinically assessed at weeks 0, 2, 4, 8, 12, and 16, and patient compliance with the daily dressing and proper daily application of the wound gel was gauged by patient report alone. However, results show the wound reduction rate increased as the treatment time progressed, indicating that those wounds that were not yet closed were progressing towards closure despite the visit interval and compliance limiting factors. This may be explained by the mechanism of action and properties of the experimental product. Preclinical testing has shown biofilm bacterial load is reduced by 3.5 log to 8 log within 24 hours, and planktonic bacteria is reduced by 4 log to 7 log in 60 minutes in in vitro measurements; its residence time in the wound is measured up to 2 days following application.[8,17]

Another limitation was that the patient populations between the experimental group and the control group were not matched with regards to their comorbidities. Statistically, the experimental and control groups were equivalent for the number of patients with diabetes and hypertension. There were statistically significantly higher percentages of patients with peripheral arterial disease and classified as overweight in the experimental group than in the control, indicating that the experimental product was presented with a more challenging group to treat.

Confounding factors encountered during this study included prescribed wound treatments other than the experimental or control agent, such as becaplermin, skin grafts, and antibiotics, which rendered data invalid after that point. However, as the wound size decreases and they start to progress anew through the healing stages, using skin grafts and other debridement products is part of the multiple approaches the practitioner may use to further accelerate wound closure. Although specific concomitant treatments were confounding factors that would impair interpretation of the data analysis, previous study results[8] have shown that combining antimicrobial and the experimental agents have a synergistic effect on chronic wounds.

In the future, efficacy of the wound gel in specific types of chronic wounds, such as those caused by diabetes and venous insufficiency, should be investigated. In addition, further investigation of various standard of care protocols versus the experimental product is warranted, as well as those protocols in combination with the biofilm-disrupting agent. Finally, this experimental product's role in the prevention of chronic wounds should be investigated and could be tested in postoperative and burn treatment settings.

CONCLUSIONS

In summary, the results of this study confirm that the use of a biofilm-disrupting agent combined with debridement is more effective than the experimental antibiotic ointment combined with debridement or prior failed wound treatments. This reinforces previous results[8] obtained when combining this product with other ointments and debridement or with debridement alone. As the experimental agent specifically targets the biofilm by degrading the EPS, the results seen provide further confirmation that biofilm bacteria significantly contribute to the delay or arrest in the healing of chronic wounds. Given the significant wound healing and closure rates observed in these long-term, nonhealing wounds, as well as the lack of related serious adverse events, using the biofilm-disrupting wound gel appears to be safe and effective for the management of chronic wounds.

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REFERENCES

1. Siddiqui AR, Bernstein JM. Chronic wound infection: facts and controversies. *Clin Dermatol*. 2010;28(5):519-526.
2. Werdin F, Tenenhaus M, Rennekampff HO. Chronic wound care. *Lancet*. 2008;372(9653):1860-1862.
3. Sen CK, Gordillo GM, Roy S, et al. Human skin wounds: a major snowballing threat to public health and economy. *Wound Repair Regen*. 2009;17(6):763-771.
4. Health Quality Ontario. Management of

chronic pressure ulcers: an evidence-based analysis. *Ontario Health Technol Assess Ser*. 2009;9(3):1-203.
5. Fife CE, Carter MJ, Walker D, Thomson B. Wound care outcomes and associated cost among patients treated in US outpatient wound centers: data from the US Wound Registry. *Wounds*. 2012;24(1):10-17.
6. Dowd SE, Wolcott RD, Kennedy J, Jones C, Cox SB. Molecular diagnostics and personalised medicine in wound care: assessment of outcomes. *J Wound Care*. 2011;20(5):232, 234-239.
7. Frykberg RG, Banks J. Challenges in the treatment of chronic wounds. *Adv Wound Care* (New Rochelle). 2015;4(9):560-582.
8. Wolcott R. Disrupting the biofilm matrix improves wound healing outcomes. *J Wound Care*. 2015;24(8):366-371.
9. Diehr S, Hamp A, Jamieson B, Mendoza M. Clinical inquiries. Do topical antibiotics improve wound healing? *J Fam Pract*. 2007;56(2):140-144.
10. Ammons MC, Ward LS, James GA. Anti-biofilm efficacy of a lactoferrin/xylitol wound hydrogel used in combination with silver wound dressings [published online ahead of print April 1, 2011]. *Int Wound J*. 2011;8(3): 268-273.
11. Wolcott RD, Rhoads DD. A study of biofilm-based wound management in subjects with critical limb ischaemia. *J Wound Care*. 2008;17(4):145-148, 150-152, 154-155.
12. Høiby N, Bjarnsholt T, Moser C, et al; ESCMID Study Group for Biofilms and Consulting External Expert Werner Zimmerli. ESCMID guideline for the diagnosis and treatment of biofilm infections 2014. *Clin Microbiol Infect*. 2015;21(Suppl 1):S1-S25.
13. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science*. 1999;284(5418):1318-1322.
14. James GA, Swogger E, Wolcott R, et al. Biofilms in chronic wounds [published online ahead of print December 13, 2007]. *Wound Repair Regen*. 2007;16(1):37-44.
15. Mah TF, O'Toole GA. Mechanisms of biofilm resistance to antimicrobial agents. *Trends Microbiol*. 2001;9(1):34-39.
16. Percival SL, Hill KE, Malic S, Thomas DW, Williams DW. Antimicrobial tolerance and the significance of persister cells in recalcitrant chronic wound biofilms. *Wound Repair Regen*. 2011;19(1):1-9.
17. Miller KG, Tran PL, Haley CL, et al. Next science wound gel technology, a novel agent that inhibits biofilm development by gram-positive and gram-negative wound pathogens [published online ahead of print March 17, 2014]. *Antimicrob Agents Chemother*. 2014;58(6): 3060-3072.
18. Bowler PG, Duerden BI, Armstrong DG. Wound microbiology and associated approaches to wound management. *Clin Microbiol Rev*. 2001;14(2):244-269.
19. Hammond AA, Miller KG, Kruczek CJ, et al. An in vitro biofilm model to examine the effect of antibiotic ointments on biofilms produced by burn wound bacterial isolates [published onilne ahead of print December 3, 2010]. *Burns*. 2011;37(2):312-321.
20. Scalise A, Bianchi A, Tartaglione C, et al. Microenvironment and microbiology of skin wounds: the role of bacterial biofilms and related factors. *Semin Vasc Surg*. 2015;28 (3-4):151-159.
21. Malic S, Hill KE, Playle R, Thomas DW, Williams DW. In vitro interaction of wound bacteria in biofilms. *J Wound Care*. 2011;20(12):569-570, 572, 574-577.
22. Wolcott RD, Rumbaugh KP, James G, et al. Biofilm maturity studies indicate sharp debridement opens a time-dependent therapeutic window. *J Wound Care*. 2010;19(8):320-328.
23. Attinger C, Wolcott R. Clinically addressing biofilm in chronic wounds. *Adv Wound Care* (New Rochelle). 2012;1(3):127-132.
24. Yang C, Goss SG, Alcantara S, Schultz G, Lantis II JC. Effect of negative pressure wound therapy with instillation on bioburden in chronically infected wounds [published online ahead of print May 25, 2017]. *Wounds*. 2017;29(8):240-246.
25. Snyder R, Bohn G, Hanft J, et al. Wound biofilm: current perspectives and strategies on biofilm disruption and treatments. *Wounds*. 2017;29(6 Suppl):S1-S17.
26. Lavigne JP, Sotto A, Dunyach-Remy C, Lipsky BA. New molecular techniques to study the skin microbiota of diabetic foot ulcers. *Adv Wound Care* (New Rochelle). 2015;4(1):38-49.
27. Wilson MR, Naccache SN, Samayoa E, et al. Actionable diagnosis of neuroleptospirosis by next-generation sequencing [published online ahead of print June 4, 2014]. *N Engl J Med*. 2014;370(25):2408-2417.
28. Hansson C. The effects of cadexomer iodine paste in the treatment of venous leg ulcers compared with hydrocolloid dressing and paraffin gauze dressing. Cadexomer Iodine Study Group. *Int J Dermatol*. 1998;37(5):390-396.
29. Sibbald RG, Leaper DJ, Queen D. Iodine made easy. *Wounds Int*. 2011;2(2):s1-s6.
30. Holloway GA Jr, Johansen KH, Barnes RW, Pierce GE. Multicenter trial of cadexomer iodine to treat venous stasis ulcers. *West J Med*. 1989;151(1):35-38.
31. Metcalf D, Parsons D, Bowler IP. Development of next-generation antimicrobial wound dressing. *Acta Med Croatica*. 2016;70(1):49-56.
32. Andriessen AE, Eberlein T. Assessment of a wound cleansing solution in the treatment of problem wounds. *Wounds*. 2008;20(6):171-175.
33. Horrocks A. Prontosan wound irrigation and gel: managment of chronic wounds. *Br J Nurs*. 2006;15(22):1222, 1224-1228.
34. Erickson CA, Lanza DJ, Karp DL, et al. Healing of venous ulcers in an ambulatory care program: the roles of chronic venous insufficiency and patient compliance. *J Vasc Surg*. 1995;22(5):629-636.
35. Abner A. Rewarding compliance in wound care patients advance. *Adv Phys Ther Rehab Med*. 2002;1(14).