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ORIGINAL ARTICLE

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Antimicrobial effects of a multimodal wound matrix against methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* in an in vitro and an in vivo porcine wound model

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Abstract

Chronic non-healing wounds pose significant challenges due to an elevated inflammatory response caused in part by bacterial contamination (*Physiol Rev*. 2019;99:665). These wounds lead to billions being spent in the health care system worldwide (*N Engl J Med*. 2017;376:2367, *Int J Pharm*. 2014;463:119). We studied the *in-vitro* and *in-vivo* antimicrobial effects of a multimodal wound matrix (MWM) against two common wound pathogens, Methicillin-Resistant *Staphylococcus aureus* (MRSA USA300) and *Pseudomonas aeruginosa* ATCC 27312 (PA27312) (*Int Wound J*. 2019;16:634). The *in-vitro* study conducted was a zone of inhibition test with the two microbes at 104 Log CFU/mL inoculated on Tryptic soy agar with 5% sheep blood (TSAII) plates. Treatments used were MWM, Mupirocin (Positive control for MRSA), Silver Sulfadiazine (Positive Control for PA), Petrolatum and Sterile Saline (both serving as Negative Controls). Treatments were allowed to diffuse into the agar for 3 h and then were incubated for 24 h at 37°C. The *in-vivo* study utilized a deep dermal porcine wound model (22 × 22 × 3 mm) created on six animals. Three animals were inoculated with MRSA USA300 and the other three with PA27312 with each allowing a 72-h biofilm formation. After 72 h, baseline wounds were assessed for bacterial concentration and all remaining wounds were treated with either MWM alone, Silver Treatment or Untreated Control. Wounds were assessed on days 4, 8 and 12 after treatment application for microbiological analysis. *In-vitro*, MWM exhibited significant inhibition of MRSA USA300 and PA27312 growth when compared to

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negative controls ($p \leq 0.05$). Likewise, *in-vivo*, the MWM-treated wounds exhibited a significant ($p \leq 0.05$) bacterial reduction compared to all other treatment groups, especially on days 8 and 12 for both pathogens. MWM demonstrated promise in addressing colonized wounds with biofilms. Additional studies on MWM's benefits and comparisons with existing treatments are warranted to optimize wound care strategies (*Adv Wound Care*. 2021;10:281).

KEYWORDS

antimicrobial, biofilms, fish peptides, microbiology, porcine model

Key Messages

- Bacterial contamination can result in detrimental healing and reduction in bioburden in wounds using new technologies is one of the most critical challenges for the scientific community.
- The aim of our study was to evaluate the antimicrobial effects of a novel multimodal wound matrix (MWM) against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* in vitro and in vivo using a porcine wound model.
- MWM demonstrated promise in addressing colonized wounds with biofilms due to the significantly higher reduction in all assessment days in both pathogenic strains tested.
- MWM exhibited significant inhibition of MRSA USA300 and PA27312 growth in vitro, compared to negative controls ($p \leq 0.05$). Wounds treated with MWM for the in vivo study, were more effective reducing the proliferation of both MRSA USA300 and PA27312 not only to baseline before and after debridement, but all treatment groups as well.

1 | INTRODUCTION

Chronic wounds resulting from aging, diabetes, and vascular disease can become challenging to heal and may lead to the development of diabetic foot ulcers, venous leg ulcers and pressure ulcers.[1–7] Under homeostatic conditions, intact skin protects the underlying tissues from the external environment, but when the barrier gets disrupted, opportunistic microbes become a threat by causing persistent infections, thus prolonging the inflammatory response and obstructing the overall healing process.[3,8] The normal healing process for wounds includes haemostasis, coagulation, inflammation, proliferation and remodelling.[9,10] In chronic wounds, stagnation is usually incited by anticoagulation, infection and debris tissue, all of which contribute to bacterial growth and thereby lead to a delay in healing time.[11,12] Likewise, various comorbidities contribute to a delay in cutaneous wound closure, including depression, multitrauma, and isolation.[13] Moreover, chronic wounds are commonly colonized by multiple bacterial species including *Staphylococcus aureus* (SA) and *Pseudomonas aeruginosa* (PA).[14–16]

One of the primary culprits in hindering the healing process is the presence of biofilms, as approximately 60% of chronic wounds are estimated to be infected by this microbial community.[17] Biofilms are a complex community of microorganisms encased in a matrix of extracellular polymeric substances (EPS) which are often associated with increased pathogenicity and can contribute to nosocomial infections.[18] Development of the biofilm starts with microbial cells that attach to the surface, followed by a self-produced EPS[19] Biofilm has been found in various environmental settings, demonstrating its resilience across different locations.[20] Despite the recognized threat of biofilms, there remains a significant gap in our understanding when it comes to their role in the pathophysiology of chronic wounds.

Dealing with chronic wounds is a significant challenge and can worsen if an infection occurs. In addition to debridement, products that exhibit antimicrobial efficacy are needed.[21–23] Various collagen extracts have been available, initially sourced from porcine or bovine tissue. Recently, novel marine extracts from fish skins, scales, and bones have been developed as an alternative source for collagen to evade disease transmission.[24,25] Fish

collagen that is further hydrolysed into 3–6 kDa peptides has shown promising antimicrobial assets and is found in various health-related industries, such as food, medicine, pharmaceutics and cosmetics.[26,27] In wound healing, previous studies have demonstrated that these peptides can accelerate re-epithelialization, thus leading to faster healing times.[28] However, currently, it is not well understood whether such an approach could also target biofilm-associated infections and, as such, present novel therapeutic strategies to address biofilm-associated challenges in chronic wounds.

To this end, a multimodal wound matrix (MWM) was designed with specific chemical and physical properties to treat chronic wounds.[29] Containing cold–water fish peptides to reduce inflammation and increase perfusion and blood flow, the formulation combines with other ingredients known generally to have antimicrobial activity (e.g., monolaurin, caprylic acid and medium-chain triglycerides [MCTs]) to target the impediments seen in chronic wounds for each stage of the healing process.[29] MWM also includes marine-sourced ω-3 fatty acids, which have previously demonstrated to promote synthesis and activation of immune cells, as well as exhibit antimicrobial activities.[29–31] Therefore, the objective of this study was to evaluate, for the first time, the antimicrobial effects of MWM against methicillin-resistant *Staphylococcus aureus* (MRSA) USA300 and PA27312 using in vitro and in vivo models. Initially, we used a zone of inhibition test to determine potential antimicrobial activity and then performed an in vivo porcine infection model to assess antimicrobial activity.

2 | METHODS

2.1 | MWM formulation

The matrix is a 510(k) cleared drug/device and is indicated for the management of wounds, including partial and full-thickness wounds, pressure ulcers, ulcers and trauma wounds which include superficial partial thickness burns, lacerations and draining wounds. Cod liver oil is the drug component, and the device component of the formulation is the cold water fish collagen peptides which are between 3 and 6 kDa in size. Other ingredients include MCTs, coconut oil, hemp seed oil, triethyl citrate, ethyl linoleate, caprylic acid, monolaurin, cetyl esters and ascorbyl palmitate. The formulation is applied directly to the wound from a vial which holds 1.6 g of sterilized MWM. The formulation is spread across the wound to cover the entire wound bed. Physical properties allow the formulation to melt at the interface of matrix and exposed tissue and allow the polarized release of molecules at the wound surface.

2.2 | In vitro experiment

MRSA USA300 and PA27312 bacterial strains (chosen for their biofilm-forming properties) were used to prepare inoculum suspensions of 10^4 colony forming unit (CFU)/mL. Test treatments included the MWM (OCM™, Omeza®, Sarasota, FL, USA), Mupirocin ([TARO, Ontario, Canada] positive control for MRSA), silver sulphadiazine (SSD) ([ASCEND Laboratories, Parsippany, NJ] positive control for PA) and petrolatum and sterile phosphate-buffered saline (PBS) as negative controls. A modified Kirby Bauer method was used to test these products and determine the zone of inhibition for each strain.[31,32] For both MRSA and PA, three plates of Tryptic soy agar with 5% sheep blood (TSAII) were challenged with 100 μL of each inoculum (10^4 CFU/mL) per treatment. Three punch biopsies (10 mm) were created on each inoculated plate (3 plates). Approximately 150 μL of each treatment was placed in 10-mm wells per plate ($n = 9$). Plates were set at room temperature for 3 h to allow the treatment to diffuse and then incubated at 37°C for 24 h to allow for bacteria growth, after which the zone of inhibition was measured using ImageJ, as previously described and using the following formula.[33]

$$\text{Area of Inhibition}\,(\text{cm}^2) = \text{Area of the halo}\,(\text{cm}^2) - \text{Area of the}\,10-\text{mm well}\,(\text{cm}^2).$$

2.3 | In-vivo study

Due to the morphological similarities between swine and human skin, a porcine model was used.[34–36] Twenty-one deep reticular wounds (22 × 22 × 3 mm) were made across the paravertebral and thoracic areas on each of 6 specific pathogen-free pigs (Looper Farms, NC, USA). To create the wounds, we used a specialized electrokeratome fitted with a 22-mm blade. The wounds will be separated from one another by 5–7 cm of unwounded skin. Three animals were infected with MRSA USA300 and the other three animals with PA27312 to avoid competitive inhibition between bacteria.[37] The inoculum suspensions (10^6 CFU/mL) for each bacteria were used to inoculate all wounds within 20 min after wounding by pipetting a 25 μL aliquot into the centre of the wound site and scrubbing it for 10 s with a sterile spatula. Inoculated wounds were covered with polyurethane dressing (Tegaderm, 3M, St. Paul, MN, USA) for 72 h to allow for biofilm

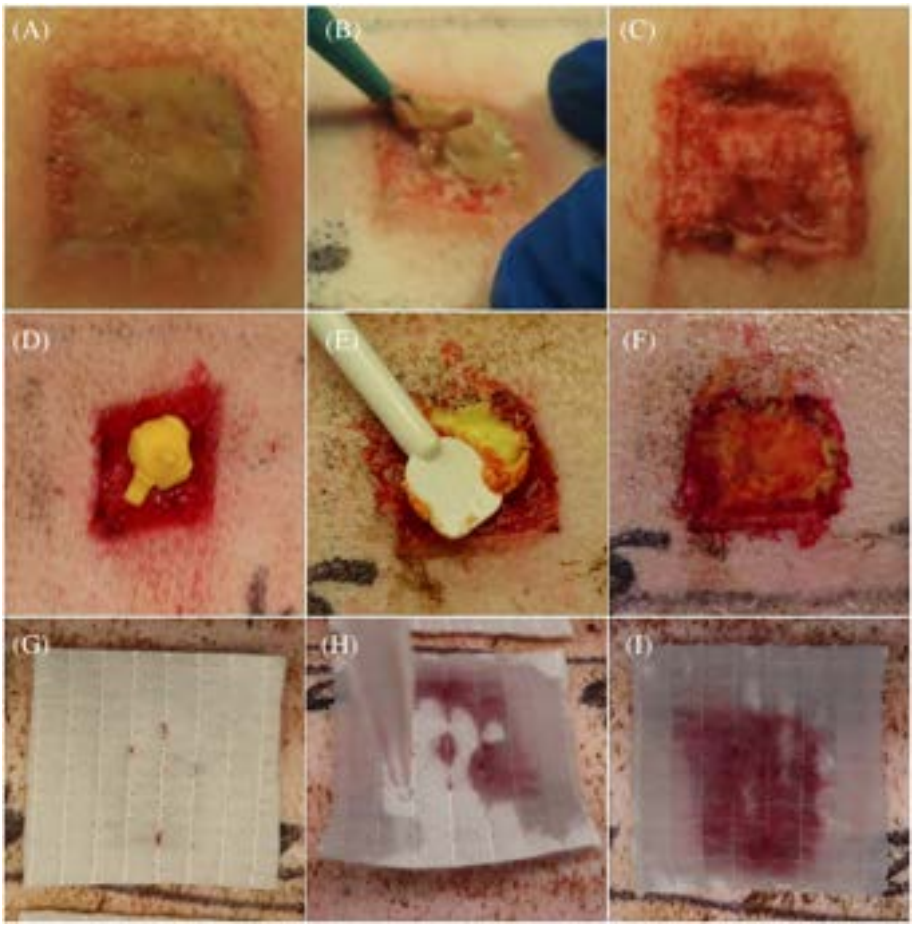

FIGURE 1 Debridement and treatment application. (A) Wounds after 72 h biofilm formation, (B) mechanical debridement using a 4-mm curette, (C) wound after debridement, (D–F) multimodal wound matrix application and (G–I) Silver treatment application.

formation.[36] After 72 h (day 0 for treatment), 3 wounds per animal were recovered as a baseline for before and after debridement bacterial counts. All remaining wounds were surgically debrided before applying treatment using a sterile 4-mm curette (Figure 1A–C). Eight wounds per treatment were treated with either MWM alone, silver treatment (positive control) or untreated control (negative control). MWM (Omeza, Sarasota, FL, USA) treated wounds each received ~200 μL of agent which was spread around the wound with a sterile spatula (Figure 1D–F). Silver treatment (AQUACEL® Ag Advantage, 2″ × 2″ dressing; Convatec, UK) was carefully placed with sterile forceps and then saturated with 4 mL of sterile saline via pipette (Figure 1G–I). All wounds, including the untreated control, were covered with a Tegaderm dressing after treatment application, and all treatments and Tegaderm dressings were reapplied on days 4 and 8. Two to three wounds were biopsied with a 6-mm punch biopsy for microbiological assessment on days 4, 8 and 12 after treatment. These biopsies were weighed and immediately placed in 1 mL of a prepared all-purpose neutralizing solution (containing tween 80, lecithin, sodium oleate, sodium thiosulphate, protease peptone and tryptone), used to neutralize the antimicrobial activity of active agent during the culturing, without any reported inhibition in bacterial growth. The sample was combined with an additional 4 mL of neutralizing solution and homogenized using a sterile homogenization tube (Wheaton, Tissue Grinder, Millville, NJ, USA). Serial dilutions were made from all culture samples, and the extent of microbiological colonization was assessed using the spiral plater system (Spiral Biotech, Norwood, MA, USA). All plates were incubated aerobically overnight (24–48 h) at 37°C, after which the number of viable colonies was counted.

2.4 | Statistics

The mean of the $\log_{10}$(CFU) and standard deviations were calculated for significance using IBM SPSS statistics 27 for both studies and was analysed using one-way analysis of variance (ANOVA) Tukey's post hoc analysis (comparison between treatments and assessment days or inhibition zones).

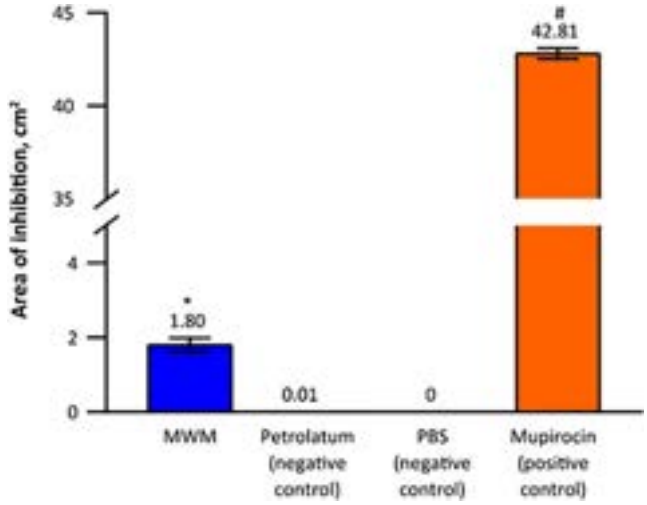

FIGURE 2 Area of inhibition with bacterial strain methicillin-resistant *Staphylococcus aureus* USA300. *$p \leq 0.05$ compared to negative controls (petrolatum and sterile phosphate-buffered saline [PBS]), #$p \leq 0.05$ compared to all treatments. MWM, multimodal wound matrix.

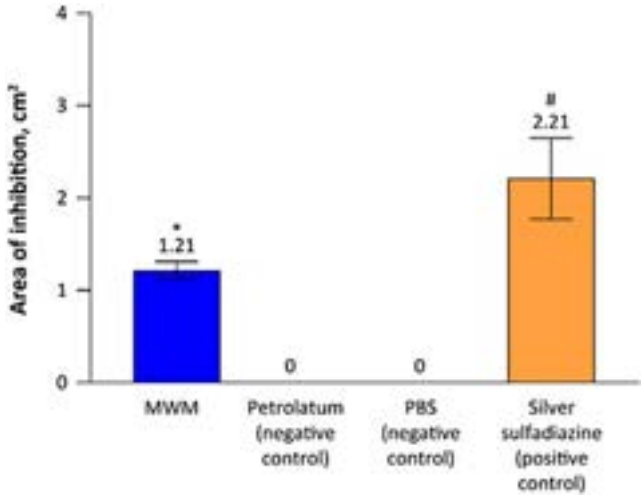

FIGURE 3 Area of inhibition with bacterial strain PA27312. *$p \leq 0.05$ compared to negative controls (petrolatum and sterile phosphate-buffered saline [PBS]), #$p \leq 0.05$ compared to all treatments. MWM, multimodal wound matrix.

3 | RESULTS

3.1 | In vitro study

For MRSA USA300, MWM exhibited a statistically significant inhibition zone of 1.80 ± 0.19 cm^2 ($p \leq 0.05$) compared to both negative controls (petrolatum and sterile PBS), whereas Mupirocin exhibited a statistically significant zone of inhibition that was 41.01 ± 0.10 cm^2 larger than MWM () ($p \leq 0.05$) (Figure 2).

Similarly, for PA27312, MWM active treatment presented significantly ($p \leq 0.05$) larger zones of inhibition (1.21 cm^2) than both petrolatum and sterile PBS (negative controls), whereas positive control SSD exhibited a statistically significant increase in the zone of inhibition of 1 cm^2 ($p \leq 0.05$) inhibition zones when compared to MWM (Figure 3).

3.2 | In vivo study

3.2.1 | MRSA USA300

The three animals infected with MRSA USA300 were recovered for baseline enumeration after a 72-h biofilm formation, with an average bacterial count of 7.12 ± 0.30 $\log_{10}$(CFU/g) prior to debridement. After debridement, the same baseline wounds were assessed and exhibited significantly lower counts by 1.59 ± 0.13 $\log_{10}$(CFU/g) when compared to the before debridement count ($p \leq 0.05$).

On day 4, wounds treated with MWM exhibited the lowest bacterial counts (4.71 ± 0.21 $\log_{10}$[CFU/g]) (Figure 4). Bacterial counts for MWM-treated wounds were significantly ($p \leq 0.05$) lower than those of both silver treatment and untreated control treatment groups. In addition, silver treatment resulted in a significant ($p \leq 0.05$) bacterial reduction of 71.67% or 0.55 ± 0.04 $\log_{10}$(CFU/g) compared against untreated control. The untreated control wounds displayed the highest MRSA counts on day 4, and there was a 0.51 ± 0.02 $\log_{10}$(CFU/g) bacterial increase when compared to baseline wounds before debridement.

On day 8, wounds treated with MWM continued to have the lowest bacterial count with significant ($p \leq 0.05$) bacterial reductions compared to all treatment groups and both baseline groups (Figure 4). Bacterial reductions of 99.99% and 99.52% (3.91 ± 0.10 and 2.31 ± 0.23 $\log_{10}$[CFU/g], respectively) were observed when comparing MWM to both baseline before and after debridement, respectively. MWM-treated wounds showed significant ($p \leq 0.05$) differences of 1.35 ± 0.17 and 2.41 ± 0.24 $\log_{10}$(CFU/g) for silver treatment and untreated control, respectively. When comparing silver treatment against untreated control on day 8, silver treatment yielded a significant 91.33% bacterial reduction ($p \leq 0.05$).

The same trend continued for day-12 samples, as all groups decreased from previous days, with MWM again exhibiting the lowest bacterial counts and untreated control the highest (Figure 4). When comparing MWM against both baseline before and after debridement, there was a significant ($p < 0.05$) bacterial decrease of 5.17 ± 0.24 and 3.58 ± 0.37 $\log_{10}$(CFU/g) (at least 99.97%

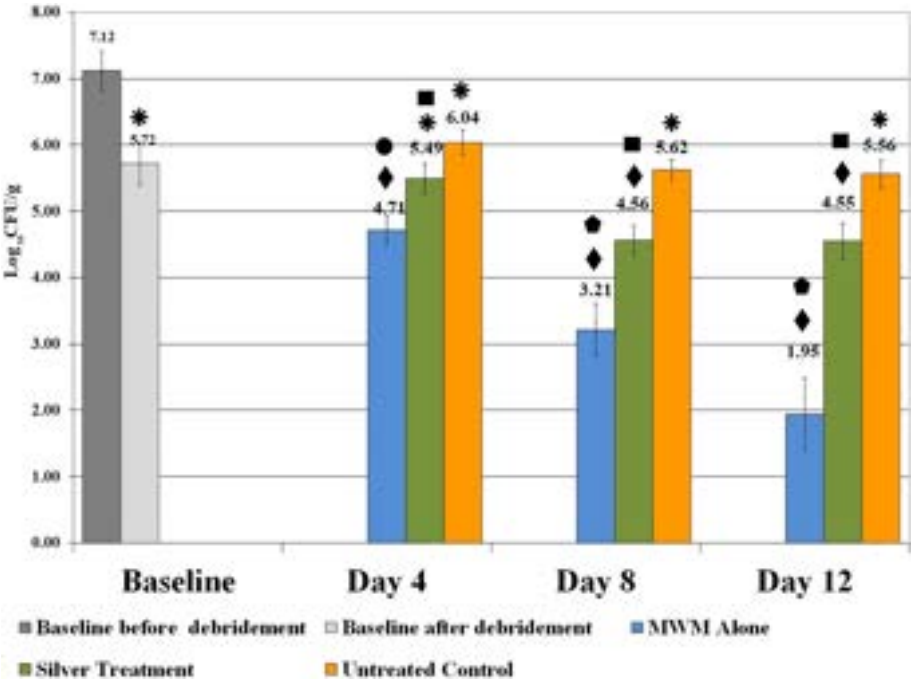

FIGURE 4 Bacterial counts in wounds inoculated with methicillin-resistant *Staphylococcus aureus* USA300 by treatment per assessment day. * $p \leq 0.05$ compared to baseline before debridement, ♦ $p \leq 0.05$ compared to baseline before/after debridement, ● $p \leq 0.05$ compared to silver treatment and untreated control, ⬟ $p \leq 0.05$ compared to all treatments and ■ $p \leq 0.05$ compared to untreated control.

reduction), respectively. MWM significantly lowered ($p \leq 0.05$) bacterial counts by 2.60 ± 0.00 $\log_{10}$(CFU/g) and 3.61 ± 0.33 $\log_{10}$(CFU/g) compared to silver treatment and untreated control, respectively.

Further analysis demonstrated that all treatment groups decreased bacterial counts on each assessment day. MWM on day 12 exhibited the lowest counts and had a significant ($p \leq 0.05$) reduction compared to both says 4 and 8.

3.2.2 | PA27312

The other three animals infected with PA, after 72 h of biofilm formation, averaged bacterial counts for baseline wounds before and after debridement of 7.36 ± 0.69 $\log_{10}$(CFU/g) and 5.72 ± 0.33 $\log_{10}$(CFU/g), respectively. Baseline bacterial counts were significantly ($p \leq 0.05$) lower after debridement with a difference of 1.64 ± 0.37 $\log_{10}$(CFU/g).

Wounds treated with MWM exhibited the lowest PA27312 counts ($p \leq 0.05$) among all baseline counts and treatment groups on day 4 (Figure 5). MWM resulted in significant ($p \leq 0.05$) bacterial reductions of 99.74% (2.58 ± 0.38 $\log_{10}$[CFU/g]) and 88.71% (0.95 ± 0.01 $\log_{10}$[CFU/g]) when compared with both baseline before and after debridement, respectively. MWM-treated wounds showed significant ($p \leq 0.05$) PA reductions when compared to silver treatment and untreated control wounds representing 84.55% and 95.59% reductions, respectively.

On day 8, MWM-treated wounds continued to be significantly lower than all other treatment groups and both baseline assessments (Figure 5). A significant ($p \leq 0.05$) bacterial reduction of 96.44% was also observed for MWM compared to baseline after debridement. The MWM-treated wounds showed significant ($p \leq 0.05$) reductions compared to silver treatment and untreated control wounds (1.30 ± 0.10 $\log_{10}$ [CFU/g] and 1.46 ± 0.38 $\log_{10}$[CFU/g], respectively), with reductions of 94.97% and 96.55%, respectively.

MWM-treated wounds showed the lowest PA27312 counts on day 12 that were statistically significant ($p \leq 0.05$) when compared to every treatment group, including baseline before and after debridement counts (Figure 5). MWM resulted in significant ($p \leq 0.05$) reductions of 3.35 ± 0.29 and 1.72 ± 0.40 $\log_{10}$(CFU/g) (99.96% and 98.07%) compared to before and after debridement baseline wounds. PA27312 counts were significantly ($p \leq 0.05$) lower for MWM wounds than for silver treatment and untreated control wounds, achieving a more than 97% reduction when compared with both treatments. At all assessment times, there was no significant difference in PA counts between silver treatment and untreated control. Additional analysis compared the results at different time points within the same treatment group. All treatment groups exhibited a comparable trend by demonstrating bacterial reductions as days progressed. Bacterial counts in MWM-treated wounds were significantly lower ($p \leq 0.05$) on days 4–12. However, no significant reductions were found when comparing all days for

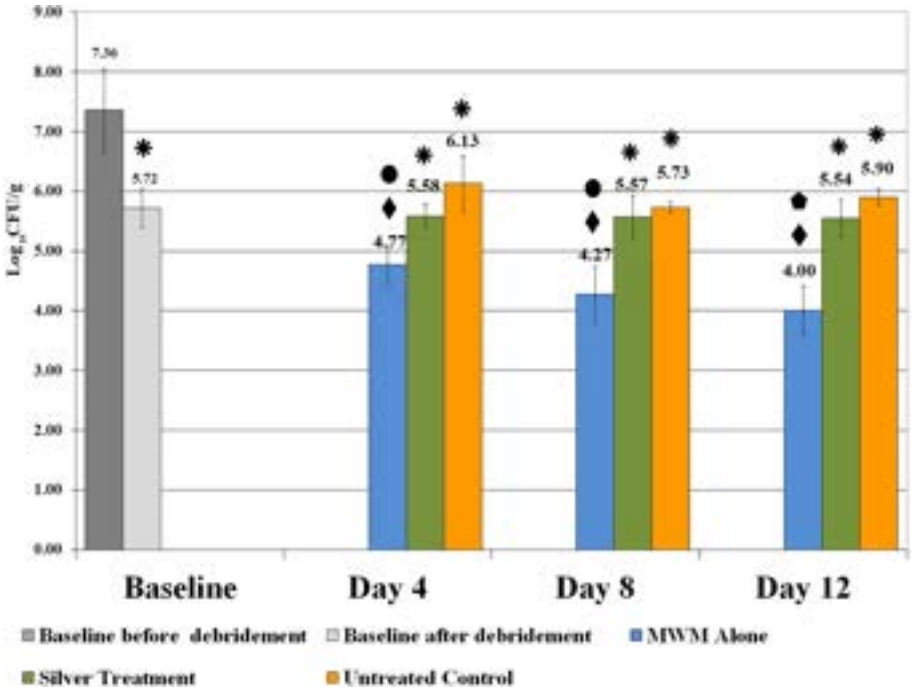

FIGURE 5 Bacterial counts in wounds inoculated with PA27312 by treatment per assessment day. * $p \leq 0.05$ compared to baseline before debridement, ♦ $p \leq 0.05$ compared to baseline before/after debridement, ● $p \leq 0.05$ compared to silver treatment and untreated control and ⬟ $p \leq 0.05$ compared to all treatments.

silver treatment and untreated control wounds because all values were similar.

4 | DISCUSSION

The present study demonstrated that MWM was able to effectively reduce the microbial burden of MRSA and *P. aeruginosa* both in vitro as well as in in vivo infection models. These results indicate that MWM is a promising novel treatment to combat infections by these organisms as the threat of multidrug-resistant strains of these pathogens continues to increase globally.[38] In vitro, MWM resulted in greater reductions of *P. aeruginosa* than the broad-spectrum antimicrobial SSD.[39] In vivo, MWM achieved a greater than 99.9% reduction in MRSA bacterial load in infected wounds despite an established biofilm. The antimicrobial properties of MWM observed in the present study may be attributed to several active compounds contained within the MWM treatment, including hydrolyzed fish collagen peptides or collagen hydrolysates, ω-3 and ω-9 polyunsaturated fatty acids, MCT oil containing caprylic acid, monolaurin, and vitamin D.

Fish collagen peptides are short amino acid sequences, typically with biological activity, obtained by hydrolysing fish skin. Numerous peptides with antimicrobial properties have been isolated from fish skin which serve protective roles by providing these marine species with innate antimicrobial defence against pathogens. Broad spectrum efficacy against Gram-positive and Gram-negative pathogens has been observed for some of these isolates.[40] In vitro studies on fish skin collagen peptides have demonstrated antimicrobial activity against *S. aureus*, which is in alignment with the reduction of this pathogen by MWM that was observed in the present study.[41,42] Abdelhedi et al. evaluated hydrolysates of a fish skin collagen gelatin in vitro with area of inhibition and observed antimicrobial efficacy against Gram-positive.

S. aureus as well as the notable Gram-negative pathogens *K. pneumoniae*, *S. enterica* and *S. typhi*.[41] ω-3 and ω-9 polyunsaturated fatty acids, abundant in fish oil and present in MWM, demonstrate considerable antimicrobial effectiveness. An in vitro study found that the ω-9 fatty acid erucic acid inhibited biofilm formation of MRSA by more than 85%.[43] Additionally, a review by Chanda et al.[44] reported that ω-3 fatty acids exhibit potent antibacterial properties against a wide array of pathogens, inhibiting biofilm formation and reducing pathogen virulence factors. These effects have been observed both in vitro and in vivo, highlighting the potential of ω-3 fatty acids as effective agents against a variety of infections caused by multidrug-resistant strains such as MRSA.[45] Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are two significant ω-3 polyunsaturated fatty acids, both of which demonstrate substantial antimicrobial properties.[46] Both of these fatty acids have been shown to demonstrate a substantial reduction in biofilm formation and the count of planktonic bacteria of patient-isolated strains.[45]

MCT oil is primarily composed of caprylic acid (C8) followed by capric acid (C10).[47] Caprylic acid has

previously been demonstrated to have antimicrobial properties that are relevant to the present study. Rosenblatt et al. evaluated caprylic acid against MRSA, *P. aeruginosa*, *Candida albicans*, *Escherichia coli* and *Salmonella enteritidis* biofilms in vitro. The authors found that caprylic acid completely eradicated *P. aeruginosa*, *S. enteritidis* and *E. coli* biofilms in 60 min and reduced MRSA count by several factors compared to control.[48] These findings are closely aligned with the results of the present study, with MWM significantly reducing *P. aeruginosa* and MRSA burdens in wounds with biofilms that were allowed to be established for 72 h after inoculation prior to debridement.

Monolaurin is a natural fatty acid monoglyceride and an active component in the MWM treatment. When monolaurin was tested against *S. aureus* and *P. aeruginosa* in vitro, Wang et al.[49] reported that the compound had the most significant inhibitory activity against Gram-positive bacteria such as *S. aureus*, but that activity was minimal against the Gram-negative organisms *P. aeruginosa* and *E. coli*. The antimicrobial efficacy against *S. aureus* by monolaurin was corroborated by a similar in vitro study against two additional strains of *S.* aureus.[50] The present study demonstrated a significant reduction in both MRSA and *P. aeruginosa* burden in wounds by MWM, but this reduction was less pronounced against the Gram-negative *P. aeruginosa* which may be attributable to the selective efficacy of monolaurin.

Finally, vitamin D is also present in MWM as an added component of the treatment. The topical administration of vitamin D was demonstrated by Weber et al.[51] to upregulate the human cathelicidin antimicrobial peptide gene (CAMP). A similar finding was reported in a porcine cell line model by Tian et al.[52] who observed increased porcine cathelicidin mRNA expression after supplementation with vitamin D.

To the best of our knowledge, no previous studies have evaluated the efficacy of the combined active compounds within MWM against clinically relevant pathogens either in vitro or in vivo. The unique combination of fish collagen peptides, ω-3 fatty acids, caprylic acid, monolaurin and vitamin D investigated in the present study significantly reduced the burden of MRSA and *P. aeruginosa* in established wound infections with biofilm. These findings indicate that MWM could be readily effective in treating multidrug-resistant skin and soft tissue infections (SSTI) infections as well as chronic wounds that originate from and persist due to infections.[53] The broad-spectrum antimicrobial efficacy of MWM against Gram-negative and Gram-positive bacteria could reduce the incidence of ineffective initial treatment and subsequent delays in patient care.

This study did not determine the degree to which each of the active compounds in MWM contributed to the antimicrobial efficacy observed. Additionally, the interactions between the compounds in the combination may affect efficacy. It is possible that the combination resulted in a synergistic effect that enhances efficacy, but this was not evaluated in the present study. Previous work has demonstrated, for instance, that monolaurin combined with an antimicrobial peptide produced synergistic antimicrobial efficacy in vitro that was not observed in vivo. The authors speculated in vivo synergy was not observed because host AMPs such as cathelicidin would be predominant over added peptides.[54] MWM, though, contains vitamin D which has been shown to upregulate cathelicidin, adding complexity to the elucidation of these interactions.[55] An additional limitation of this study was that the impact of MWM on wound healing was not assessed.

5 | CONCLUSION

The in vitro results demonstrated that both positive control treatments (Mupirocin and SSD) created the largest significant zone of inhibition against all treatment groups on both MRSA USA300 and PA27312, respectively. MWM demonstrated statistically significant larger zones when compared to both negative controls (Petrolatum and Saline). All treatments established greater zones of inhibition as compared to negative controls.

In the microbiological analysis for the in vivo study, wounds treated with MWM were more effective at halting the proliferation of both MRSA USA300 and PA27312 not only to baseline before and after debridement but also to all treatment groups.

Overall, the MWM alone was able to decrease bacterial bioburden which would have important clinical implications. Additional studies are warranted to support the use of MWM for treating various types of wounds, including burns and chronic wounds.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The data utilized in this study is not publicly available through scientific conferences. It is considered proprietary information owned by the company and plays a crucial role in maintaining our competitive advantage in the field. If any party is interested in accessing this data, it can be requested from the corresponding author. Access is subject to approval, which is based on confidentiality agreements and the nature of the request. This approach helps maintain confidentiality, safeguard intellectual property, and encourages thorough scientific evaluation, promoting transparency and collaboration within the scientific community.

ETHICS STATEMENT

All animal procedures were conducted in accordance with the protocol that was reviewed and approved by the University of Miami's Institutional Animal Care and Use Committee.

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Treatment of bacterially contaminated lower extremity ulcers with a fatty acid-containing wound matrix: a case series

Objective: The aim was to evaluate the effectiveness of a marine omega fatty acid-containing multimodal wound matrix (MWM) in reducing bacterial contamination and supporting wound area reduction (WAR) in patients with hard-to-heal wounds of varying aetiologies.
Method: A prospective, single-site, pilot case series of patients with hard-to-heal wounds. All wounds were considered non-healing prior to inclusion as they had failed to achieve at least 50% WAR after at least four weeks of standard of care (SoC) treatments. Patients were seen once weekly for wound assessments, matrix application and dressing changes. Baseline and weekly fluorescence images, standard wound images and wound measurements were obtained.
Results: A total of three patients, two with venous leg ulcers (VLUs) and one with a diabetic foot ulcer (DFU) were enrolled in this pilot study. The mean baseline wound age prior to study enrolment was 24 weeks, with a mean baseline wound size of 8.61cm^2. The two VLUs went on to complete closure. The DFU displayed a total WAR of 53% by six weeks, when the patient was lost to follow-up due to a geographical relocation. The mean percentage area reduction of all wounds combined was 82% upon study completion.
Conclusion: The use of MWM proved to be effective and safe in this patient cohort. The wounds included in this case series failed to enter a healing trajectory with SoC wound therapies. The MWM supported wound closure and reduced bacterial loads in this patient cohort.
Declaration of interest: No funding was provided for this case series. The MWM to be studied was provided by the sponsor (OCM; Omeza, US). The author has no conflicts of interest to declare.

antimicrobial • DFU • diabetic foot ulcer • fatty acids • hard-to-heal wound • VLU • venous leg ulcer • wound • wound care • wound dressing • wound healing • wound matrix

Hard-to-heal wounds attract an estimated annual Medicare spend of $28.1–96.8 billion USD.[1–3] The longer a wound remains unhealed, the more costs accumulate, as does the risk for downstream complications such as infection and amputation.[4,5] It is well-accepted that wound repair is a complex process that involves multiple interlinking phases for regeneration of healthy tissue to occur. The healing cascade is an intricate process, with platelets, neutrophils, macrophages, endothelial cells, keratinocytes and fibroblasts all playing an important part. A delay or stall in wound area reduction (WAR) and healing is often due to a variety of systemic and local factors, primary among these are a disorganised extracellular matrix (ECM), excessive devitalised tissue, unregulated inflammation and high microbial burden.[6,7] Thus, it stands to reason that wound healing would be optimised by using multimodal therapies that simultaneously control bacterial burden, decrease tissue inflammation, support the ECM and establish a balanced healing environment.

Since antiquity, animal fats and vegetable oils have been widely used in therapeutic applications and for medicinal purposes.[8] Fatty acids (FAs) are membrane phospholipids that participate in the inflammatory response, having influence on skin structure integrity, tissue regeneration and immunological status to support wound healing from inflammation to repair.[9] The effects of various FAs have been demonstrated to promote wound healing through induction of cell migration and angiogenesis, as well as through promotion of antioxidant and anti-inflammatory metabolites.[9] Animal studies evaluating the systemic effects of essential FAs are available in the literature. In one such investigation, Lania et al.[10] found that rat wounds treated with topical FA (sunflower oil) had higher levels of insulin-like growth factor (IGF)-1, a known mitogenic influence on keratinocytes, and increased interleukin (IL-6) levels, a proinflammatory cytokine that resolves inflammation, than the control animals.[10] The ability of FAs to control the invasion of microorganisms and avoid the development of infection during wound repair promotes healing and makes them attractive for therapeutic use in topical wound treatments.[11] Although, to date, there is a paucity of clinical studies investigating the effects of FA administration (topically or orally) in the treatment of hard-to-heal wounds in human subjects.

Of particular interest, omega-3, -6 and -9 FAs have been shown to play a significant role in skin repair by reducing transepidermal water loss, improving skin hydration, regenerating the damaged skin lipid barrier and stabilising skin metabolism.[12] An anhydrous conformable sheet wound matrix containing cold water fish omega FA peptides and

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other non-cytotoxic components is commercially available in the US. The matrix is designed to conform to the wound bed, achieving intimate contact with the surface, ultimately absorbing into the wound during the natural healing process. The potential biological effects of this omega FA-containing wound matrix could have wide utility in the treatment of hard-to-heal wounds of the lower extremity.

In this prospective, single-site pilot case series the author evaluated the effectiveness of this novel marine omega FA-containing multimodal wound matrix (MWM) (OCM; Omeza, US) in reducing bacterial contamination and supporting WAR in patients with hard-to-heal wounds of varying aetiologies. It was hypothesised that weekly application of MWM would result in a reduction of wound bed bacterial levels and a decrease in total wound area. A fluorescence imaging device (MolecuLight i:X; MolecuLight Inc., Canada) was used to objectively measure the bacterial contamination on the surface of the wound bed at each treatment visit, as well as to take standard wound images and digital measurements.

Fluorescence imaging is a validated tool to rapidly provide diagnostic information on the presence and location of moderate-to-heavy bacterial loads in wounds and surrounding tissues that would not otherwise be visible with the naked eye. Clinical trials have shown that endogenous, red fluorescence emitted from porphyrins in bacteria and cyan fluorescence in the presence of *Pseudomonas* bacteria enable the MolecuLight i:X to visualise and locate bacteria present at loads >10^4 colony forming units (CFU)/g on and beneath the surface of wounds up to ~1.5mm deep.[13]

Methods

Patient recruitment

A total of three patients from the author's clinical practice, >18 years old and having hard-to-heal (>4 weeks' duration) open wounds of the lower extremity, were enrolled.

Ethical approval and patient consent

Ethics Board approval from Richmond Heights Wound Center, Ohio, US was not required for this case study. The study was conducted in accordance with Health Insurance Portability and Accountability Act guidelines, adhered to the tenets of the International Conference on Harmonization E6 Good Clinical Practice (ICH GCP) and the Declaration of Helsinki. Before study enrolment, all patients provided written informed consent to publish case details and associated deidentified image assessments. This was not a blinded study. No compensation was provided for participation.

Wounds

Patients had a variety of hard-to-heal wound aetiologies, including two venous leg ulcers (VLUs) and one diabetic foot ulcer (DFU). All wounds were considered non-healing prior to inclusion as they had failed to achieve at least 50% WAR after at least four weeks of treatment with standard of care. Previous wound management had varied and included debridement, offloading, compression bandages, alginates, collagen, gelling hydrofibres and foam dressings. All patients had an ankle–brachial pressure index >0.9mmHg and ≤1.3mmHg. No patients showed any clinical signs or symptoms of wound infection based on the International Wound Infection Institute checklist.[5]

Wound measurement and fluorescence imaging

Fluorescence and standard wound photographs and measurement were obtained weekly via a fluorescence imaging device. The device also contained digital wound area measurement software that automatically detected the wound border and generated instant, accurate wound measurements (wound surface area, length and width).[14]

Wound assessment and treatment

All patients were seen once weekly in the author's clinic for wound assessment and dressing changes. Baseline and weekly fluorescence and standard wound images were obtained. All wounds were debrided to remove devitalised tissue at the clinician's discretion. The MWM was applied once weekly to each patient's wound according to the manufacturer's instructions for use and covered with a primary inert dressing material to manage exudate and promote a moist wound healing environment. The DFU was offloaded via total contact cast and multilayer compression bandages were used in the treatment of the VLUs.

Results

In this case series, two male patients and one female patient participated, and included one DFU and two VLUs. The mean baseline wound age prior to study

Table 1. Age, sex, wound type, wound age, number of treatments and negative fluorescence results of each patient

Patient number	Age, years	Sex	Wound type	Wound age, weeks	Number of treatments	Wound base negative fluorescence, weeks
1	57	M	DFU	24	6	2
2	95	F	VLU	35	4	2
3	64	M	VLU	12	3	2

DFU—diabetic foot ulcer; F—female; M—male; VLU—venous leg ulcer

enrolment was 24 weeks, with a mean baseline wound size of 8.61cm^2. Patient demographic information is presented in Table 1.

The two VLUs progressed to complete closure, Patient 3 at visit 3 (Fig 1j) and Patient 2 at visit 4 (Fig 2j). The DFU displayed a total WAR of 53% at six weeks when Patient 1 was lost to follow-up due to a geographical relocation; however, we included the patient data in this analysis (Figs 3a–j). The mean percentage area reduction of all wounds combined was 82% upon study completion. Healing trajectory results are represented in Fig 4.

Fluorescence imaging showed clearance of pathological levels of bacterial contamination in the wound base by week 2 (Table 1). However, fluorescence of the periwound skin, which was not treated with the MWM, was observed to persist. Additionally, wounds remained free of clinical evidence of infection over the entire course of the study for all patients (Figs 1–3).

Fig 1. Patient 3. Venous leg ulcer. Wound at treatment visit (TV) 1 pre-debridement (pre) **(a)**; fluorescence image at TV1 (pre) **(b)**; wound measurement at TV1 (pre) **(c)**; wound at TV1 post-debridement (post) **(d)**; fluorescence image at TV1 (post) **(e)**; wound at TV2 (pre) **(f)**; fluorescence image at TV2 (pre) **(g)**; wound measurement at TV2 (pre) **(h)**; fluorescence image at TV2 (post) **(i)**; healed wound at TV3 **(j)**

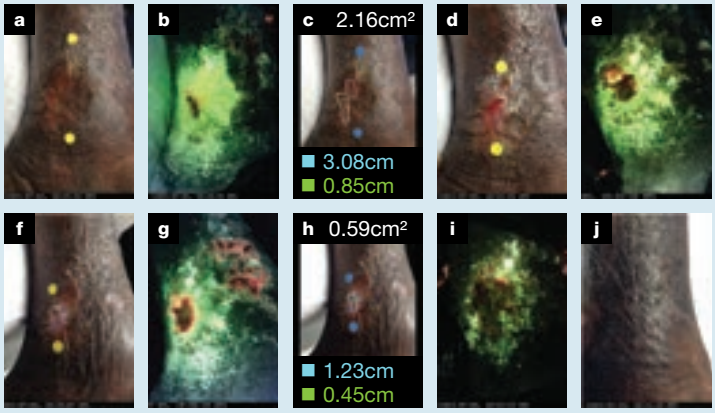
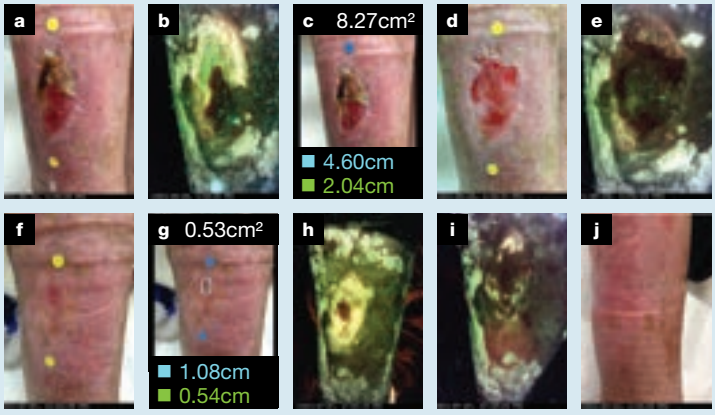


Fig 2. Patient 2. Venous leg ulcer. Wound at treatment visit (TV) 1 pre-debridement (pre) **(a)**; fluorescence image at TV1 (pre) **(b)**; wound measurement at TV1 (pre) **(c)**; wound at TV1 post-debridement (post) **(d)**; fluorescence image at TV1 (post) **(e)**; wound at TV2 (pre) **(f)**; wound measurement at TV2 (pre) **(g)**; fluorescence image at TV2 (pre) **(h)**; fluorescence image at TV2 (post) **(i)**; healed wound at TV4 **(j)**



Discussion

As far back as 2800 BCE, the Babylonians boiled animal fats with wood ashes to obtain soap-like substances. These soaps are one of the oldest known uses of FAs as surfactants.[15] In 1881, investigations by Koch illustrated that FAs had benefit beyond soaps and cleaning agents. Through his studies, Koch established the value of FAs as antibacterial agents, noting that these agents can prevent bacterial growth and directly kill bacteria by inserting directly into the bacterial membrane.[16] Subsequently, the bactericidal effects of FAs gained importance in medicine. Although FAs have been recognised for their antibacterial properties for decades, they have been underused, most likely due to the widespread use of prescription antibiotics in the practice of modern medicine. With a rise in global antimicrobial resistance, alternative antimicrobial agents with no known resistance are of increasing importance.[17] Once again, FAs for use as antimicrobial agents are of particular interest due to their effectiveness, abundance and affordability.

The utility of animal fats and oils for other medicinal purposes has been recognised for the past 50 years.[18] The rich FA concentration found in fish oils makes this source one of particular interest. Potential health benefits of fish oils have been linked to the large amounts of polyunsaturated FAs (PUFAs) found in these oils. Docosahexaenoic acid and eicosapentaenoic acid, which are long-chain omega-3 FAs, are the predominant PUFAs derived from fish oils.[19] Studies on the systemic effects of fish oils gained momentum after widespread reports on the low incidence of inflammatory and heart diseases observed in the Inuit and Yupik populations.[20] It was postulated that this finding was directly related to the diet of the Inuit and Yupik peoples, which is high in fish oils.[20] In response, fish oil FAs have proved to be advantageous in the treatment of conditions, such as heart disease, rheumatoid arthritis, diabetes, ulcerative colitis, asthma, pulmonary dysfunction and Parkinson's disease.[21]

In the last two decades, there has been increasing research studying the effects of PUFAs in the field of dermatology and in the cosmetic industry. Of note, skin barrier function is limited when a lack of PUFAs is found within the skin.[22] The end result is a disruption in the epidermis, leading to an increase in transepidermal water loss.[22] Additionally, lack of PUFAs in the skin induces an upregulation of inflammatory-related keratin (K17) in the basal cells of the epithelium.[23] Overexpression of K17 is known to lead to a variety of skin conditions including psoriasis.[23] Cytokine generation and function are also regulated by PUFAs.[24] Proinflammatory cytokines play a pivotal role in wound healing, and thus the use of

Fig 3. Patient 1. Diabetic foot ulcer. Wound at treatment visit (TV) 1 pre-debridement (pre) **(a)**; fluorescence image at TV1 (pre) **(b)**; wound measurement at TV1 (pre) **(c)**; wound at TV1 post-debridement (post) **(d)**; fluorescence image at TV1 (post) **(e)**; wound at TV4 (pre) **(f)**; fluorescence image at TV4 (pre) **(g)**; wound measurement at TV4 (pre) **(h)**; wound at TV4 (post) **(i)**; fluorescence image at TV4 (post) **(j)**

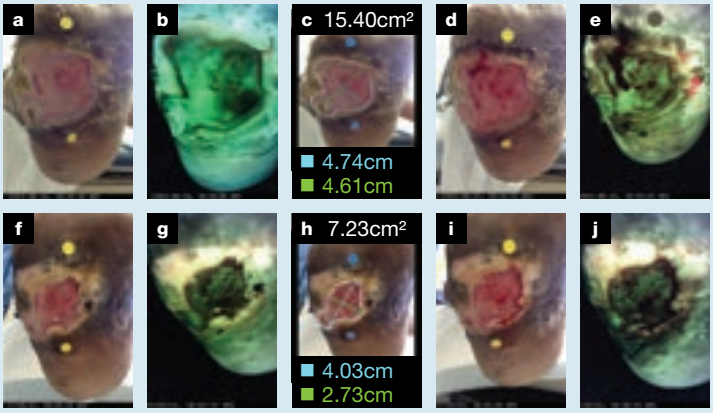
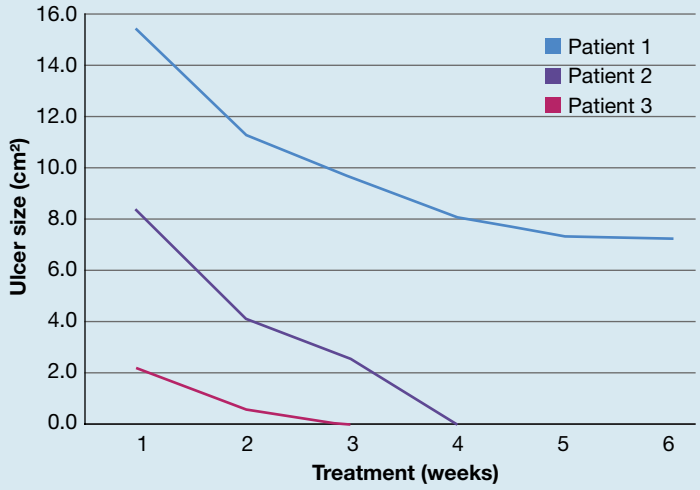


Fig 4. Wound healing trajectory



PUFA-containing products has the potential to bolster the essential cellular processes necessary for tissue repair and regeneration.

There are a wide variety of wound dressings and skin replacement products available to aid in wound healing, although there remains a need for safe and efficient topical therapies in wound management. A key to successful wound management is regulating the appropriate level of inflammation to foster cellular migration. Fish-derived FAs, such as omega-3, -6 and -9, have the potential to attenuate the extended inflammatory phase observed in hard-to-heal wounds.

Despite evidence indicating the successful application of fish oil and omega FAs on a variety of skin disorders, there has been a lack of clinical studies investigating the use of FAs in wound management. Wu and Goldman[25] published the results of a 34-patient randomised controlled trial showing that the use of a topical FA treatment (linolenic acid) after CO_2 laser treatment led to an increase in keratinocyte proliferation and epidermal tissue formation in the treatment cohort. In addition, patients receiving the FA had a decrease in oedema and itching by post-procedure day 3.[25] It is not unreasonable to extrapolate that the results of this study could also be applied to the management of hard-to-heal wounds.

The MWM investigated in this three-patient case series is an anhydrous, amorphous solid formulated for topical application to wounds. The product is a combination of cold water fish peptides, cod liver oil, plant-derived polyunsaturated FAs, medium chain triglycerides, and other plant oils and waxes. It is hypothesised that the FAs contained in this product successfully modulated the inflammatory response in the wounded tissues, thus accelerating the healing rate of otherwise stalled wounds. The impact of the MWM on wound progress is in agreement with in vitro studies on the utility of PUFAs in cosmetic and dermatological use.[26] It has been established in the literature that FAs are potent antimicrobial/microbicidal agents in vitro that can kill enveloped viruses, Gram-positive and Gram-negative bacteria, and fungi on contact.[27] Thus, the author postulates that the FAs found within the MWM used in this case series were able to disrupt the bacterial cell membrane to promote solubility or lysis, leading to the decrease in bacterial contamination levels observed on fluorescence imaging throughout the treatment phase of this study.

Limitations

The major limitations of this pilot study are that it is a single-site, single-arm case series on a very small number of patients. The study period was also limited, with one patient being lost to follow-up. However, the results of this case series lend credence to the idea that the MWM supports wound healing in hard-to-heal lower extremity wounds of varying aetiologies. Future studies should include larger recruitment and a longer duration of follow-up.

Conclusion

In this case series, the use of MWM proved to be effective and safe. The wounds included in this study were stalled in the inflammatory stage with no signs of healing. MWM supported wound closure and reduced bacterial loads in this patient cohort. Topically applied formulations of marine-derived FAs via anhydrous sheet matrices have been developed for wound management. These innovative products have the potential to impact the landscape of topical wound therapies. **JWC**

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Reflective questions

- How can fatty acid-containing products be used in wound management?
- Which hard-to-heal wound aetiologies could benefit from the use of anhydrous, amorphous solid matrices?
- How can products such as the multimodal wound matrix fit into antimicrobial stewardship programmes?

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REVIEW

A Novel Comprehensive Therapeutic Approach to the Challenges of Chronic Wounds: A Brief Review and Clinical Experience Report

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ABSTRACT

Following the clinical perspective and concept that a healthy body will not develop chronic wounds, the central approach for the treatment described here is based on an understanding of how the body transforms the wound microenvironment from a non-healing to a healing state. As part of a comprehensive treatment regimen that includes OCM™ (complete matrix), wound preparation, and skin protectant formulations, the OCM contains components for complete wound healing by attending to the individual needs required to promote the closure of each unique chronic wound. During application of the comprehensive treatment regimen in independent investigator-led trials, the total wound percentage average reduction over the first 4 weeks of treatment was 60% across multiple wound types; median time to total wound closure was 6.9 weeks. Safety testing of the OCM formulation shows no potential allergenicity, no potential sensitization, and no known product-related adverse events. Clinical trials evaluating the OCM formulation as part of the comprehensive treatment regimen of multiple wound types are underway. Results of clinical trials and real-world experiences will expand current knowledge of the wound-healing potential of this novel product.

Keywords: Chronic wound; Complete matrix; OCM; Refractory wound; Wound healing; Comprehensive wound management

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Key Summary Points

The report presents an overview of a combination therapy that includes a complete matrix (OCM™), a novel polyunsaturated fatty acid wound therapy matrix, and summarizes outcomes from real-world experiences in using it to treat various types of chronic or refractory wounds.

OCM is an anhydrous, amorphous solid that contains all natural components, including fish- and plant-derived mono- and polyunsaturated fatty acids (omega 3, 6, and 9), tissue-generating signaling molecules, and nutrients (vitamins A, C, D, and E and minerals).

Treatment with OCM resulted in closure across multiple wound types in 77% of patients with a mean healing time of 6.5 weeks and a 69% area reduction of wounds after 4 weeks of treatment. Safety testing shows no potential allergenicity, no potential sensitization, and no known product-related adverse events.

INTRODUCTION

A chronic wound is one that has failed to proceed through an orderly and timely series of events and does not heal at an expected rate [1]. Wounds that do not respond within 2–4 weeks of standard treatment are considered chronic and are characterized by an abnormal microenvironment (e.g., edema, reduced blood perfusion, inflammation, infection, and tissue degeneration) [2, 3]. Healthy individuals generally do not develop chronic wounds because of the body's natural healing ability; however, the risk of wounds becoming chronic increases with comorbidities, such as diabetes, vasculitis, immune suppression, certain health conditions that cause poor circulation, neuropathy and reduced mobility, or diseases that cause ischemia [4]. Chronic wounds are generally indicative of a far more serious, underlying condition and should be regarded in a similar manner as, e.g., a lump in breast tissue, with the patient receiving a comprehensive workup including medical history, location, duration, physical assessment, vascular status, skin integrity, and so on. As the general population ages and individuals have an increasing number of comorbid conditions, successfully managing and treating chronic wounds will become increasingly important.

The most common types of chronic wounds are diabetic foot ulcers (DFUs), venous leg ulcers (VLUs), and pressure ulcers (PrUs) [1]. The current annual incidence of DFUs worldwide is between 9.1 and 26.1 million [5]. Approximately 15–25% of patients with diabetes will develop a chronic ulcer at some point during their lifetime, and 1% of those patients will require an amputation [6]. With an aging population, numbers of patients presenting with chronic leg ulcers, including VLUs, have increased [7]. Recurrence rates of VLUs are high, 54–78%, and their chronicity can last for many years, having a devastating impact on an individual's physical and emotional well-being [8–10]. Currently, VLUs lead to an estimated annual direct cost for conservatively managed patients in developed countries as US $5527 per person per year [11]. Finally, PrUs can be a result of reduced mobility, a lack of protective sensation, and less supple skin, i.e., conditions for which risk increases during the aging process [7]. Immune suppression, emotional stress, and advanced age also adversely affect the body's natural healing ability [4].

In healthy, non-immunocompromised individuals, wound healing progresses through four stages of healing in a process that is complex and exquisitely orchestrated. First, a hemostasis stage forms a clot to seal and protect; then an inflammatory phase occurs during the first few days to further protect against infection, which is followed by a proliferative phase that lasts at least 3 weeks to build new tissue and seal the wound; and finally, a maturation and remodeling phase that can last up to 2 years [4]. Biological processes required for healing—i.e., formation of granulation tissue via cellular

Table 1 Challenges of treating chronic and refractory wounds

Type of wound	Standard treatment approaches [13]	Challenges to treating/healing
Chronic	Debridement	Excessive and painful debridement
	Moisture balance	Excess moisture
	Infection control	Inadequate control of inflammation
	Optimization of comorbidities	Heavy bioburden
		Lack of control of comorbidities
		Adherence to treatment
Refractory	Biologics	Difficult to treat
	Dermal substitutes	

proliferation, angiogenesis, and tissue remodeling—are stalled in chronic nonhealing wounds [12]. The majority of chronic wounds do not move past the inflammatory stage, characterized by bacterial burden, localized edema, poor perfusion, and persistent inflammation (Table 1) [13, 14]. While infection is most common to chronic wounds, the degree of bioburden is a determining factor in the ability of the individual's wound to heal [2].

The presence of bacteria and endotoxins within a wound can cause a prolonged elevation of proinflammatory cytokines (e.g., interleukin-1 and tumor necrosis factor-α) and a prolonged duration of the inflammatory phase, eventually leading to a chronic nonhealing state [15]. An elevation of matrix metalloproteases associated with prolonged inflammation can cause degradation to the extracellular matrix [15]. Infected wounds contain multiple species of bacteria, including *Staphylococcus aureus*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa*, and once established, they become persistent and resistant to antimicrobial treatment [16, 17]. Resistance is often conferred by bacteria secreting extracellular polymeric substances, forming a 3-dimensional matrix called a biofilm that supports the adherence of certain microorganisms, including pathogenic bacteria, within the wound [16]. The biofilm increases the resistance of bacteria to antimicrobial and antiseptic treatment, highlighting the need for effective non-antibiotic therapies with antimicrobial properties for chronic nonhealing wounds.

An effective method to remove the biofilm in a chronic wound is by sharp debridement, a procedure recommended by the US Food and Drug Administration (FDA) to not only remove bioburden but also necrotic tissue. Other recommended standard care procedures that are used when needed for nonhealing wounds include off-loading, compression therapy for venous stasis ulcers, establishment of adequate blood circulation, maintenance of a moist wound environment, management of wound infection, wound cleansing, nutritional support (including blood glucose control for individuals with DFUs), and bowel and bladder care for individuals with PrUs at risk of contamination [18].

Every chronic and refractory wound is unique, and the varying pathophysiology of each wound, which affects healing time, depends on its type (e.g., DFU, PrU, VLU), in addition to individual factors such as blood supply, blood pressure, infection, and the patient's preexisting comorbidities [1]. Products designed to manage and regulate chronic or refractory wound healing include, e.g., human- and porcine-tissue-derived skin grafts or collagen matrices (Table 2). However, some of the skin substitutes and cellular tissue products are limited in their use: some are effective when the wound is showing signs of moving out of the inflammatory phase; some can only be used only on certain types of wounds; and some can only be used after treatment with conventional therapy has failed. Products derived from mammalian tissue, primarily those of porcine origin, carry a risk of disease transfer that is addressed through an FDA-required viral inactivation process [1]. If the viral inactivation process is inadequate, then a risk of pathogen transmission and infection remains. The FDA-recommended mitigation measures are aimed at

Table 2 Description and mechanism of action of products, including skin substitutes and cellular tissue products, used for the management of chronic and/or refractory wounds

Product name	Product description	Mechanism of action	Types of wounds	Contraindicated wound types
Apligraf	Bioengineered living cellular skin graft substitute [58] Human source	Activates keratinocytes at the wound edge; corrects and regulates growth factor signaling [59] Restores fibroblast function at the wound base Downregulates fibrosis formation [60]	Chronic wounds with limitations [58]: VLUs after 4 weeks of failed conventional therapy DFUs after 3 weeks of failed conventional therapy	Clinically infected wounds [58]
PuraPly	Combined collagen matrix and PHMB antimicrobial [61] Porcine source [61]	PHMB blocks microbial attachments, helping prevent biofilm reformation [62] The native collagen matrix forms a durable biocompatible scaffold that supports healing [61]	Various wound types [61]: Partial- and full-thickness wounds PrUs Venous ulcers Diabetic ulcers Chronic vascular ulcers Tunneled/undermined wounds Surgical wounds (donor sites/grafts, post Mohs surgery, post laser surgery, podiatric, wound dehiscence) Trauma wounds (abrasions, lacerations, second-degree burns, skin tears) Draining wounds	Third-degree burn wounds [61]

Table 2 continued

Product name	Product description	Mechanism of action	Types of wounds	Contraindicated wound types
Kerecis	Intact fish skin graft for tissue regeneration Fish source	Omega-3 anti-inflammatory properties Fish skin has an appropriate surface chemistry and microstructures that facilitate cellular attachment, competent mechanical strength, and biodegradation rate without undesirable by-products [63]	Various wound types [64]: Partial- and full-thickness wounds Trauma wounds Surgical wounds (donor sites, post Mohs surgery, post laser surgery, podiatric, wound dehiscence)	
Epifix	Dehydrated human amniotic membrane allograft [65] Human source	Supports the healing cascade and protects the wound bed to aid in the development of granulation tissue [65] Provides a human-biocompatible extracellular matrix that retains numerous inflammatory and wound-healing regulatory proteins [66, 67]	Various wound types [65]: Dehisced wounds DFUs VLUs PrUs Mohs surgery	Wounds with active or latent infection [65]
Epicord	Dehydrated human umbilical cord allograft (donated from C-sections of live births) [68] Human source	Forms a reinforced matrix to support the wound-healing cascade [69] Protects the wound bed to aid in the development of granulation tissue [69] Provides a biocompatible human extracellular matrix that retains multiple regulatory proteins [69]	Various acute and chronic wounds [68]: Smaller, deeper wounds DFUs VLUs PrUs Post debridement Complex defects	

Table 2 continued

Product name	Product description	Mechanism of action	Types of wounds	Contraindicated wound types
Grafix	Cryopreserved human amniotic membrane [70] Human source	Retains the native components of the human placental membrane: extracellular matrix, growth factors, and endogenous neonatal mesenchymal stem cells, fibroblasts, and epithelial cells [70] The extracellular matrix provides structural support and acts as a growth factor reservoir; growth factors and mediators stimulate growth and repair	Various acute and chronic wounds, including but not limited to the following [70]: DFUs VLUs PrUs Dehisced surgical wounds Burns Acute surgical wounds Pyoderma gangrenosum Epidermolysis bullosa	
Integra BioFix	Tissue allograft derived from allogeneic dehydrated and decellularized amniotic membrane [71] Human source	The extracellular matrix provides a foundation for regeneration, growth factors, fibronectin, integrins, laminins, and hyaluronic acid support cell proliferation, differentiation, and adherence to the scaffold [71]	Intended for use as a wound covering for surgical sites, voids, and tissue defects [71]	
Mirragen Advanced Wound Matrix	Biodegradable, bioactive borate-based glass fiber [72]	Bioactive glass materials have the potential to heal wounds by improving angiogenesis and supporting cell proliferation and differentiation [73]	Acute and chronic soft tissue wounds and injuries [72]	

Table 2 continued

Product name	Product description	Mechanism of action	Types of wounds	Contraindicated wound types
Theraskin	Skin allograft from donated human skin [74]	The allograft contains living cells, endogenous growth factors, and a native extracellular matrix [74]	Including, but not limited to the following [74]: DFUs VLUs PrUs Dehisced surgical wounds Necrotizing fasciitis Traumatic burns Radiation burns	

DFU diabetic foot ulcer, *PHMB* polyhexamethylene biguanide, *PrU* pressure ulcer, *VLU* venous leg ulcer

addressing potential health risks associated with animal-derived materials (e.g., adverse tissue reaction, infection, immunological reaction, pathogen transmission), which may increase the cost of some wound care products [1]. In addition, some wound-healing products are not acceptable to patients who have cultural or religious barriers surrounding the use of mammalian tissue [19]. Potential limitations of some skin substitutes and cellular tissue products highlight the need for a wound-healing therapy suitable for all patients with chronic or refractory wounds of any type or of any duration.

The goals of chronic and refractory wound therapy should be to manage and regulate bioburden, edema, and perfusion [20]. The most effective wound-healing therapy will meet these goals while providing the metabolites, growth factors, and signaling peptides required for the healing of all types of chronic or refractory wounds [13]. An amorphous solid combination drug/device product called OCM^{TM} (Omeza complete Matrix, Omeza, LLC, Sarasota, FL) [21] and supporting therapeutics were developed to follow the clinical perspective and concept that a healthy body will not experience chronic wounds. When wounds are present on a healthy body, they are provided with all components needed for optimal results throughout each stage of healing. Understanding the aspects of an acute wound becoming a chronic wound and how to stimulate the body to transform the wound microenvironment from a non-healing to a healing state was a central concept in the development of this technology.

A COMBINATION THERAPY PROTOCOL FOR CHRONIC WOUND CARE

The combination therapy technology described here is composed of three investigative products: OCM [21] and two supporting formulations, a periwound prep with lidocaine (0.8%) and a skin protectant that both follow respective over-the-counter monographs [22, 23]. These products were designed to be applied in sequence (periwound prep, OCM, followed by skin protectant) and to target components of wound healing in chronic or refractory wounds (except third-degree burn wounds), regardless of pathology. All investigative products are anhydrous, with the intention of limiting water availability to provide a hostile environment for microorganism survival [24].

The first step in the treatment protocol is to apply the lidocaine formulation to the peri-wound 5 min prior to debridement, if debridement is required [25, 26]. The OCM product is applied next, directly onto the wound bed. The third step is to apply the skin protectant to the tissue surrounding the wound. The products contained in this combination therapy are noncytotoxic and safe, and OCM has been cleared by the FDA as having no potential allergenicity, no potential sensitization, and no known directly related adverse events (NCT04510376, NCT04510675, and NCT04512274). All three formulations contain no porcine or bovine products, thereby reducing the risk of virus transmission.

OCM Formulation

The OCM formulation contains all natural components, including fish- and plant-derived mono- and polyunsaturated fatty acids (omega 3, 6, and 9), tissue-generating signaling molecules, and nutrients (vitamins A, C, D, and E and minerals). These natural and sustainable components are formulated as an amorphous solid that coats the entire wound and provides a sheet to support the healing process below. The physical properties of OCM provide malleability so that it can easily conform to regular and irregular wound beds and into tunneling wounds.

OCM includes components, such as omega fatty acids, medium-chain triglyceride (MCT) oil (including caprylic acid and monolaurin), and hydrolyzed collagen peptides, that are shown to have anti-inflammatory and antimicrobial properties. Omega fatty acids are known to promote synthesis and activation of immune cells and to reduce inflammation, specifically the omega fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [27, 28]. Found only in oily fish, EPA and DHA have shown positive effects in animal models against rheumatoid arthritis, inflammatory bowel disease, and asthma [27, 29]. Studies with EPA and DHA have also shown disruption of lipid rafts for inhibition of the transcription factor nuclear factor-κB and consequently downregulation of pro-inflammatory genes [27, 30]. Results of in vitro and in vivo studies have shown that MCT oil regulates the activation of macrophages from an M1-type to M2, and increases the production of anti-inflammatory cytokines while decreasing the production of pro-inflammatory cytokines through upregulation of β-oxidation [31]. Caprylic (octanoic) acid is an eight-carbon, short-chain fatty acid that has shown to be more effective than medium-chain and long-chain fatty acids as an antimicrobial [32, 33]. Caprylic acid is used as an antimicrobial agent in the horticultural industry and in commercial food handling and healthcare facilities [34]. Monolaurin (1-lauroyl-glycerol) is a monoglyceride with antimicrobial and antiviral activity [35]. In humans, lauric acid is converted to monolaurin, which has antimicrobial properties in human breast milk [36]. A recent study incorporated monolaurin into electrospun shellac nanofibers for potential use as a medicated dressing for wound treatment, based on the antimicrobial properties of monolaurin [37]. Importantly, OCM contains hydrolyzed fish collagen peptides that have shown both antimicrobial and antioxidant properties [38]. Results of in vitro studies showed that hydrolyzed collagen peptides induce an anti-inflammatory response that activates both human fibroblast and keratinocyte proliferation [39]. In addition, hydrolyzed collagen peptides promote new collagen formation by signaling to fibroblasts to increase elastin synthesis and the inhibition of metalloproteinase (MMP)-1 and MMP-3 degradation through transforming growth factor beta (TGFβ) signaling [40]. Because of their size, collagen lysates are less susceptible to MMP collagenases, which is an advantage for wound-healing progression from proliferation to remodeling; also, because of other physical properties, they are soluble and can bind calcium ions to improve their bioavailability [38, 41–44]. The hydrolyzed collagen is sourced from North Atlantic white fish skins (Kosher and Halal certified) and contains no additives, preservatives, or sulfites; all ingredients and excipients in the formulation are naturally and sustainably sourced.

OCM also includes the vitamins A, C, and D, all of which have been seen in wound studies to support new tissue synthesis [45–49]. Ascorbyl palmitate is the fat soluble form of vitamin C and is frequently used in topical formulations because it is more stable that some aqueous forms [50]. As an amphipathic molecule, the structure not only allows incorporation into the formulation but also across cell membranes. In an in vitro study in keratinocytes, ascorbyl palmitate was shown to reduce intracellular levels reactive oxygen species at low doses [51]. In other studies, it was seen as an essential cofactor for collagen transcription and for post-translational modification of type I and type III collagen. Vitamins A and D are known to have a positive effect on the inflammatory response in chronic wounds, and vitamin D, as a pleiotropic steroid hormone, also plays a role as an antibacterial by upregulating cathelicidin, an immune cell signaling peptide [52, 53]. Vitamin A also modulates epithelial cells and fibroblasts and is known to stimulate epithelialization [54].

Depending on the individual needs of the patient and their wound(s), OCM can be used in conjunction with other wound-healing therapies, including standard care, hyperbaric oxygen therapy, and negative pressure therapy, or in sequence with skin substitutes or cellular tissue products.

Clinical Experience

Allergenicity of OCM was assessed in 25 healthy adult participants based on skin prick reactions compared with positive and negative controls (NCT04510376). Results showed no potential allergenicity of OCM (no positive response) among 23 evaluable participants. The sensitization potential of OCM was evaluated in 58 healthy participants (aged 18–65 years) in a randomized study (NCT04510675). Participants were randomly assigned to receive repetitive and continuous patch applications of OCM or 0.9% aqueous sodium chloride (negative control) to the same site over 3 weeks for a total of nine induction applications. Application sites were evaluated after each patch removal, followed by a 10- to 17-day rest period. A challenge application was applied to untreated sites for approximately 48 h. Evaluation of challenge sites were from 30 min to 72 h after patch removal. There were three cases of slight erythema during induction and one case of grade 0 irritation on day 17. During the challenge phase, one participant had slight patchy or confluent erythema 48 h after application assessment (not indicative of sensitization). No other participants experienced irritation, and OCM was determined to be safe for use. Skin protectant and anti-inflammatory properties of OCM on damaged skin were assessed in a single-blind study with 22 healthy participants (NCT04512274). Occlusive patches with 0.75% sodium lauryl sulfate (SLS) solution were applied to the forearm for approximately 24 h to induce an inflammatory response. Clinical grading of the test sites was performed before SLS application, after removal of the SLS patches on day 1, and then 10 min after the first OCM application. Clinical grading was subsequently performed before OCM application on days 2 to 4. The induction of inflammation elicited significant erythema during the study, which, on a scale of 1 to 4, was reduced to a greater extent by OCM versus untreated control: post SLS, 3.05 vs 3.09; day 1, 2.86 vs 3.20; day 2, 2.23 vs 3.34; day 3, 1.34 vs 3.09; and day 4, 0.14 vs 2.16. Data from the transepidermal water loss (TWEL) measurements showed no statistically significant differences between test sites before OCM application and after SLS application, confirming study validity. Application of OCM led to statistically significant improvements in skin barrier versus untreated sites ($P < 0.05$). No adverse events were reported during the study.

Pilot studies to assess OCM response in the clinic were conducted by 16 investigators, including wound care-certified podiatrists, surgeons, nurse practitioners, and dermatologists, in clinical settings such as physicians' offices, wound care centers, outpatient clinics, and skilled nursing facilities. This real-world assessment of OCM as part of a combination therapy protocol included patients with various types and ages of chronic and refractory wounds. Data from these independent investigator-led trialing of the products showed that of 60

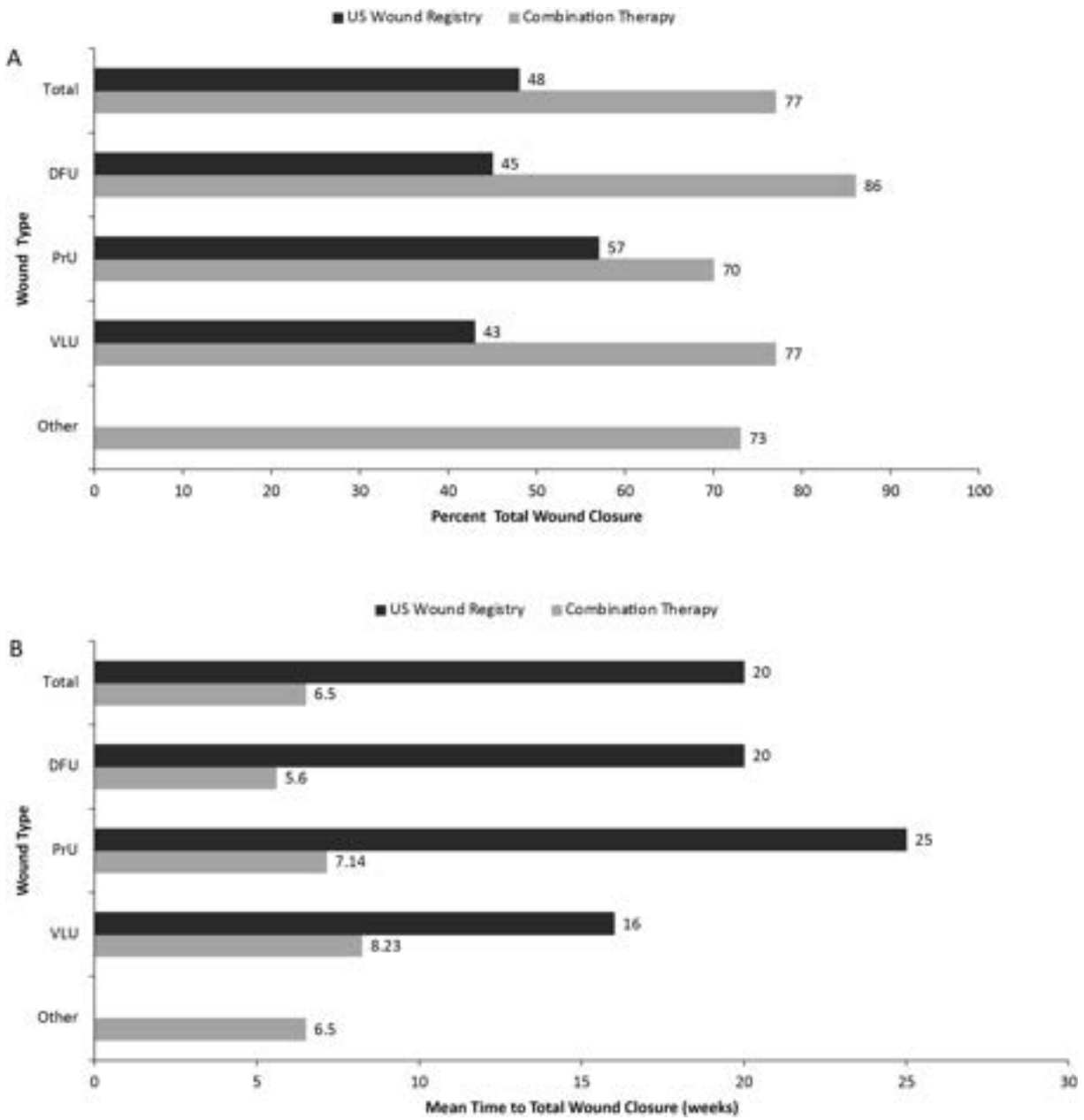

Fig. 1 **A** Total wound closure and **B** mean time to total wound closure with combination therapy and from the US Wound Registry [55]. *DFU* diabetic foot ulcer, *PrU* pressure ulcer, *VLU* venous leg ulcer

evaluable chronic wound cases, 86%, 77%, 70%, and 73% of patients with DFUs, VLUs, PrUs, and other wound types, respectively, experienced wound closure after receiving combination therapy (Fig. 1A). For the DFU wounds, the mean wound size was 3.18 cm^2 (range 0.16–15.4 cm^2), for VLUs 8.80 cm^2 (range 1.62–37.39 cm^2), 1.77 cm^2 (range 0.5–5.81 cm^2) for PrUs, and 16.86 cm^2 (range 1.1–74.42 cm^2) for other wound types. Mean time to total wound closure for the 60 cases was 5.6 weeks for DFUs, 8.2 weeks for VLUs, 7.1 weeks for PrUs, and 6.5 weeks for other wound types (Fig. 1B). Although not a direct comparison to the combination therapy outcomes, data from the US Wound Registry reported a 48% total wound

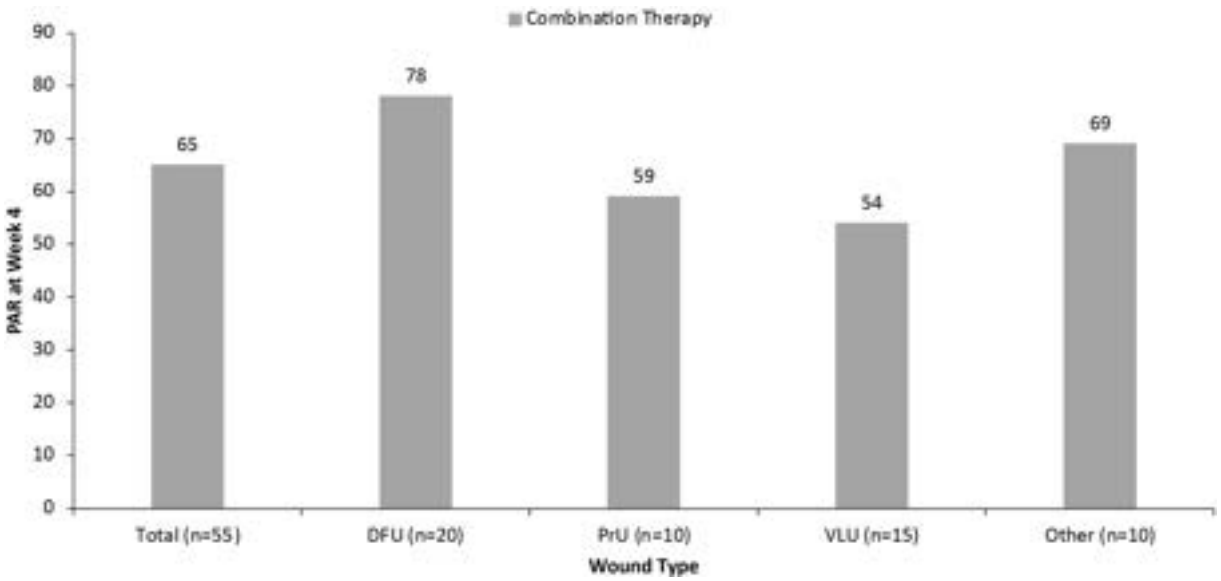

Fig. 2 PAR of the wound at week 4 of combination therapy. *DFU* diabetic foot ulcer, *PAR* percent area reduction, *PrU* pressure ulcer, *VLU* venous leg ulcer

closure rate with a mean time of 20 weeks to complete healing across patients with DFUs, VLUs, and PrUs (Fig. 1A, B) [55].

It is reported that if the area of a wound has not reduced more than 50% after 4 weeks of treatment, then the chances of the wound closing are less than 9% [56]. On the basis of this report, we evaluated the total wound percent average reduction (PAR) after the first 4 weeks of treatment with the combination therapy. In 55 evaluable cases that included DFUs (n = 20), VLUs (n = 15), PrUs (n = 10), and other wound types (n = 10), the total PAR was 69% (Fig. 2). In addition, the total average time to wound closure was 6.5 weeks (Fig. 1B). Patients in this dataset had comorbid conditions and/or lifestyle challenges that were not beneficial for their innate healing ability, but complete closure of their chronic or refractory wounds was observed. As an example, a 56-year-old male patient presented to a wound care clinic with a lower extremity venous stasis ulcer (24 mm × 22 mm) of 7-month duration. The patient had a history of hypertension, hypercholesterolemia, and venous stasis disease. His wound had not responded to previous treatment with silver alginate and amnion products. After five weekly applications of the combination therapy and a compression wrap, the patient experienced complete closure of his wound (Fig. 3).

Data collected here was conducted in accordance with ethical principles of the Declaration of Helsinki and Good Clinical Practice guidelines. Patients, who were not involved in the research other than receiving treatment for their condition, signed informed consent regarding use of their data.

DISCUSSION

When assessing wound-healing rates from clinical trials, it is important to consider enrollment criteria and the potential exclusion of patients with significant comorbidities and more severe refractory wounds [55]. In randomized trials specifically, it is necessary to minimize the variables between the cohorts so that equivalent comparisons can be made. Also, as time to closure in chronic wounds can be lengthy, patients are required to commit to regular weekly treatment visits for longer than 3 months. As a result of the comorbidities that most patients with chronic wounds endure daily, consenting trials can select for healthier, more compliant patients. In consideration of

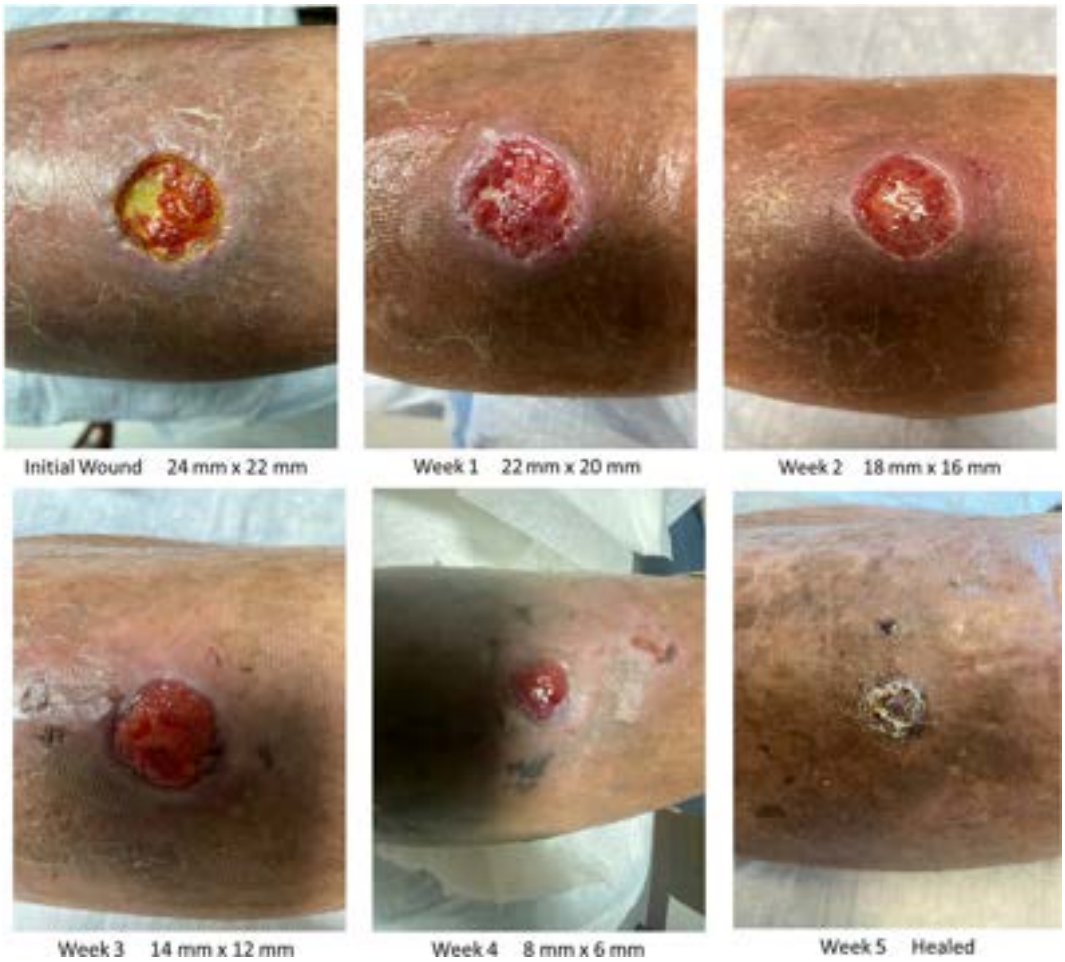

Fig. 3 Case study of total closure of a chronic venous stasis ulcer after 5 weeks of combination therapy

the cost and resources needed for potential lengthy healing rates, protocols may also limit enrollment to smaller-sized wounds so that endpoints can be met sooner. These factors can lead to trial results that translate poorly to the general population of patients with chronic wounds that wound care providers treat in the clinics.

The time range of wound-healing duration reported from the control cohorts from 48 randomized clinical trials of patients with chronic wounds treated with various products varies from a mean of 5.1 weeks in patients with DFUs to a median of 36 weeks in patients with VLUs [55]. The 12-week mean healing rates reported were 42.7% (range 12.5–88.3%) in patients with VLUs, 37.9% (range 4.0–90.6%) in patients with DFUs, and 40.0% (range 36.0–44.0%) in patients with PrUs [55]. In comparison, an analysis of real-world data gathered by the US Wound Registry show 12-week healing rates of 44.1% for VLUs, 30.5% for DFUs, and 29.6% for PrUs [55].

Acknowledging the size of the dataset presented for the combination therapy and understanding the necessity to evaluate the efficacy of OCM in a controlled setting, clinical trials are currently underway for its further evaluation. Study NCT05291169 is a randomized trial that includes patients with VLUs and compares treatment with OCM combined with standard of care to standard of care alone. This trial includes fluorescent imaging of the wounds to compare the bioburden over time between the treatment and control groups. Study NCT05921292 is a multisite trial

evaluating chronic wounds of multiple etiologies and includes patients who are usually excluded from clinical trials (e.g., current smoking status, higher body mass index, and comorbidities). Study NCT05417425 is a single-site trial of patients with DFUs to evaluate PAR at 4 weeks and then after 12 weeks of combination therapy compared to standard of care. In addition to the clinical trials, animal and preclinical studies are underway to further elucidate OCM's mechanism of action. In vitro and porcine in vivo studies are currently being conducted to assess antimicrobial properties of all components of the combination therapy; near-infrared perfusion pilot studies are scheduled to evaluate OCM's effect on perfusion and lymphedema studies are planned to identify mechanisms for reducing edema in chronic wounds.

One of the challenges for wound care professionals is that there is little evidence for the selection of an appropriate therapy [57]. To address this and other challenges inherent to managing chronic wounds, it is necessary that wound care products are evaluated for efficacy and safety in both randomized trials and in trials with broader inclusion criteria and narrower exclusion criteria. Results compiled from these various types of studies will hopefully lead to a better understanding of a product's value in closing a chronic wound, especially in the therapeutic area of wound management where patient compliance is possibly the greatest challenge for the clinicians. In addition to wound-closure rate, clinically relevant and patient-centered outcomes of wound healing should focus on reduced amputation, reduced economic burden, improved function/ambulation, and improved quality of life [55].

CONCLUSION

The novel combination wound-healing therapy provides unique formulations with the intention of meeting individual healing needs, allowing each patient's body to utilize the specific components to address the deficient aspects of their specific wound. Patients in the dataset described here presented with comorbid conditions and/or lifestyle challenges that were not beneficial for their innate healing ability; however, 77% of patients experienced complete closure of their chronic or refractory wounds after being treated with the combination therapy.

Clinical trials evaluating the combination therapy for the treatment of DFUs (NCT05417425), VLUs (NCT05291169), and wounds/ulcers of multiple etiologies (NCT05921292) are underway. Results of studies assessing mechanism of action and results of these clinical trials will expand and enhance current evidence supporting use of the combination therapy in chronic or refractory wounds in the real world.

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Declarations

Conflict of Interest. Griscom Bettle is a former employee of Omeza, LLC; Desmond Bell and Suzanne Bakewell are employees of Omeza, LLC.

Ethical Approval. Data collected here was conducted in accordance with ethical principles of the Declaration of Helsinki and Good Clinical Practice guidelines. Patients, who had no involvement in the research other than being treated for their condition, signed informed consent regarding use of their data.

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A novel approach for the treatment of diabetic foot ulcers using a multimodal wound matrix: a clinical study

Objective: Innovation in wound healing, particularly regarding diabetic foot ulcers (DFUs), is needed to reverse the number of diabetes-related amputations. This study evaluated a novel approach and performance of a multimodal wound matrix in converting stalled DFUs into a healing trajectory.
Method: Patients with either type 1 or 2 diabetes and with foot ulcers (Wagner grade 1 and 2), were screened to determine eligibility for treatment. Ulcers improving >30% in area during a two-week screening phase were not eligible for the study treatment phase. The study was an open-label trial conducted in three phases: screening, treatment and healing confirmation. Patients enrolled in the study received a treatment protocol that included application of a wound matrix to the ulcer and offloading.
Results: A total of 19 patients (15 males, four females) with a median age of 60 years, and a median ulcer duration of 36 weeks took part in the study. Patients showed an average four-week percentage area reduction (PAR) of 62%, a 12-week PAR of 94%, and a 12-week healing rate of 57% (8/14).
Conclusion: Results of this study support the viability and potential of a novel approach to treating DFUs that includes use of a multimodal wound matrix.
Declaration of interest: SB, Chief Scientific Officer, and DB, Chief Medical Officer, are employees of Omeza, LLC, US. The remaining authors have no conflicts of interest to declare.

CAMPS refractory wounds • cellular, acellular and matrix-like products • customised wound healing • diabetic foot ulcer • DFU • multimodal wound matrix • wound • wound care • wound dressing • wound healing

As the prevalence of diabetes continues to increase in the US and globally, so does its associated complications, including limb and life-threatening diabetic foot ulcers (DFUs).[1] Individuals with diabetes have a 19–34% lifetime risk of developing a DFU—one of the most serious complications associated with the disease.[2] Despite an ever-increasing understanding of the pathophysiology pertaining to the development and course of DFUs, current standard treatment for their management includes: surgical debridement; dressings to control exudate and to provide a moist environment; vascular assessment; infection control; glycaemic control; and offloading.[3] Although guidelines and other algorithms exist for the treatment of DFUs, adoption of such recommendations is inconsistent among wound care practitioners.[4]

Technological advancements designed to promote greater efficiency in DFU healing have not produced residual results that positively impact diabetes-related lower extremity amputation rates.[5–7]After a 20-year decline in lower-extremity amputations, the US may now be experiencing a reversal in the progress—from 2009–2019, the number of diabetes-related hospitalisations due to amputation doubled.[8] Innovation in wound healing, particularly in relation to DFUs, is one part of a larger strategy needed to reverse these trends, and positively impact both individuals and global healthcare systems. Adjunctive wound healing therapies used in the treatment of DFUs include cellular, acellular, and matrix-like products (CAMPs) that support tissue repair or regeneration through various mechanisms of action.[9] These products are generally used to manage wounds that have failed to respond to conservative treatment, and after the patient's risk factors and comorbidities have been addressed.[9] A retrospective Medicare database analysis showed that first administration of advanced wound healing therapy was delayed until approximately 80 days after diagnosis of a lower extremity diabetic ulcer, and that reapplication of the product did not occur regularly.[10] A delay in time to administer effective treatment for DFUs ultimately increases healthcare costs and has a negative impact on patients' morbidity and mortality.[11,12]

In this paper, we present the results of a study that examined the treatment of DFUs with a therapy that has been developed as a multimodal wound matrix (MWM) (OCM, Omeza, LLC, US).[13] This drug/device includes peptides, omega fatty acids, and anabolic metabolites in a matrix that supports comprehensive synthesis of new tissue and regeneration of the wound.[13] The central concept in the design of this technology originated from a physiological understanding of how the healing cascade becomes stalled in wounds, with a focus on stimulating the body to transform the wound

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microenvironment through the inflammatory, proliferative and regenerative phases into a healing state.[13]

This study was designed to evaluate the performance of the wound matrix in converting stalled ulcers into a healing trajectory. Moving a hard-to-heal ulcer from the inflammatory phase to the proliferative phase increases the potential for eventual healing. It has been shown that a >50 percentage area reduction (PAR) in DFUs after four weeks of treatment is a predictor of healing.[14,15] The present study was designed to assess the PARs of DFUs managed with the wound matrix application and offloading after four weeks of treatment, and to evaluate the performance of the matrix in a 12-week period.

Methods

Patients, study design and treatment

This study was an open-label trial conducted in three phases: screening, treatment and healing confirmation. The study was conducted from September 2022 to December 2023.

Patients were enrolled from the Promedica Health Care System at the Toledo Wound Care Center, Toledo, US, and the Defiance Clinical Wound Center, Defiance, US. Patients were enrolled at the clinical site and pre-screened.

After provision of informed consent, screening, including a physical examination, occurred to determine patient eligibility, diabetes status, medical history, vital signs, body mass index (BMI), pregnancy status, duration and measurement of the target ulcer.

Adult (≥21 years) patients with a diagnosis of type 1 or 2 diabetes and with a DFU (Wagner grade 1 or 2) were eligible for enrolment. Patients whose ulcers were caused by a condition other than diabetes were not eligible to participate in the study. Study DFUs could not be infected, and could not have been treated with tissue engineered material or other scaffold materials within 30 days before start of study. Patients with an unlimited BMI or who were active smokers, were currently undergoing dialysis, or had active cancer or suspicion of malignancy in the study DFU were also excluded.

Screening phase

The screening phase (1–14 days) was designed to determine whether patients' DFUs were eligible to proceed to the treatment phase. At screening visit 1, target DFUs were identified, cleaned, debrided as necessary, dressed and offloaded. If a ≥30% decrease in DFU size over the previous 14 days with standard treatment was observed, the DFUs were not eligible for inclusion in the study treatment phase.

Treatment phase

The treatment phase included 12 weekly visits. During this time the patients' DFUs were assessed, and treated with a preparation solution applied to the periwound area. This was followed by application of the wound matrix to the DFU, and a skin protectant applied to the periulcer area. Adherence to offloading was addressed at each visit, as best as could be determined. Non-adherence to offloading resulted in the patient being withdrawn from the study. Debridement of the study DFU at each treatment visit was left to the judgement of the provider treating the patient.

Healing confirmation

DFU closure confirmation occurred at any time during the study. If the study DFU was 100% re-epithelialised, as determined by the principal investigator, it was considered closed.

DFU evaluation

Photographic ulcer evaluation, measurements and ulcer progress in study patients were captured by Tissue Analytics (Net Health, US), a US Food and Drug Administration Class 1 artificial intelligence-powered digital planimetry and imaging application. The study was closed by agreement of the sponsor and study principal investigator after completion of wound matrix treatment for the final patient.

Ethical approval and patient consent

The study was approved by the ProMedica Institutional Review Board/Independent Ethics Committee (Approval #22-142) and was conducted in accordance with the principles consistent with the Declaration of Helsinki, Good Clinical Practice, applicable regulatory requirements, and the Belmont Principles of respect for persons, beneficence and justice.

All patients provided written informed consent prior to enrolment, which included consent to publish photographs of their DFUs. Photographs have been examined to eliminate patient identification.

Study outcomes

The primary objectives of this study were to evaluate the safety profile of the wound matrix, and to evaluate the impact of treatment and offloading on chronicity of DFU healing after four weeks of wound matrix treatment.

Secondary objectives were to evaluate the healing of DFUs over four weeks of wound matrix treatment, and the time to maximum or complete ulcer closure.

Primary endpoints were change in PAR at four weeks compared with baseline measurements and incidence of DFU closure at four weeks and by 12 weeks.

Statistical analysis

All analyses of data from this study were descriptive (without p-value generation) as the study was not powered for inferential analyses and no formal hypothesis testing was performed.

Results

The study included 19 patients (15 males, four females) (Fig 1) with a median age of 60 years (range: 44–85 years) (Table 1). Median DFU duration prior to treatment was 36 weeks (range: 4–72 weeks) (n=17), with five DFUs present for >1 year without closure (Fig 2).

A total of five patients included in the analysis did not complete the study (Fig 2): four patients were withdrawn by the investigator for non-adherence to offloading, and one patient died from their comorbidities.

Median DFU size was 1.7cm^2 (mean: 5.05cm^2; range: 0.37–25.0cm^2) at treatment visit (TV)1 (Table 1). At TV5, the median DFU size reduced to 0.63cm^2 (n=16; mean: 1.45cm^2; range: 1–10.06cm^2) (Table 1).

The average four-week PAR was 62%, with three patients experiencing 100% closure of their DFUs (Fig 2). At TV12, median DFU size was 0.0cm^2 (mean: 0.38cm^2; range: 0–2.59cm^2) (Table 1). The average 12-week PAR was 94%, with five additional patients experiencing 100% closure of their DFUs. In total, eight patients experienced 100% closure of their DFUs by week 12. No DFUs increased in size over the 12 weeks of the study (Fig 2).

Among patients with both 4- and 12-week PAR measurements (n=14), all DFUs treated with the wound matrix improved from treatment week 4 to treatment week 12 (Fig 2). For one patient, their DFU healed at 13 weeks (12-week PAR of 99%). Another patient's DFU continued to close with two further treatments through week 14 (PAR of DFU at 12 weeks was 73%) (Fig 1). At the time of the 12-week timepoint, the DFUs of six patients had not healed and presented with PARs of 73%, 80%, 85%, 90%, 90% and 99% (Fig 1).

Of the 14 patients who completed the study, eight experienced complete closure of their DFUs and six experienced PARs of 73–99%. Among these, three experienced complete healing of their DFUs after four applications of wound matrix and offloading.

Of the five patients in the study who had DFUs of ≥1 year's duration, three patients' DFUs completely healed by week 12 (the remaining two DFUs had 12-week PARs of 73% and 85%).

The dressings and treatments used on the DFUs prior to treatment in this study with the wound matrix included alginate with dry dressing, gentian violet, collagen, manuka honey and Dakin's solution (0.125%).

Fig 3 provides some examples of use of the MWM in patients with DFUs.

Safety

There was one serious adverse event in the study where the patient was admitted for an acute kidney injury and later died from comorbidities. The event was not related to the product or the study. No other safety events were reported in the study.

Discussion

This clinical trial evaluated DFUs managed with a wound matrix and offloading. The trial results were encouraging, with an overall PAR of 62% at week 4, which increased to a PAR of 94% by week 12.

The complex challenges that make each DFU unique demands skills from the wound practitioner in identifying the best tools to move the DFU to healing and to optimise healing rates. By treating these DFUs with a multimodal approach, such as that delivered by the wound matrix in this study, the aim is to push stalled DFUs towards healing and to reduce the need for multiple tools required to optimise healing rates, but which can, potentially, take longer.

Fig 1. Plot with ulcer outcomes and treatment duration. Start time denotes treatment visit 1

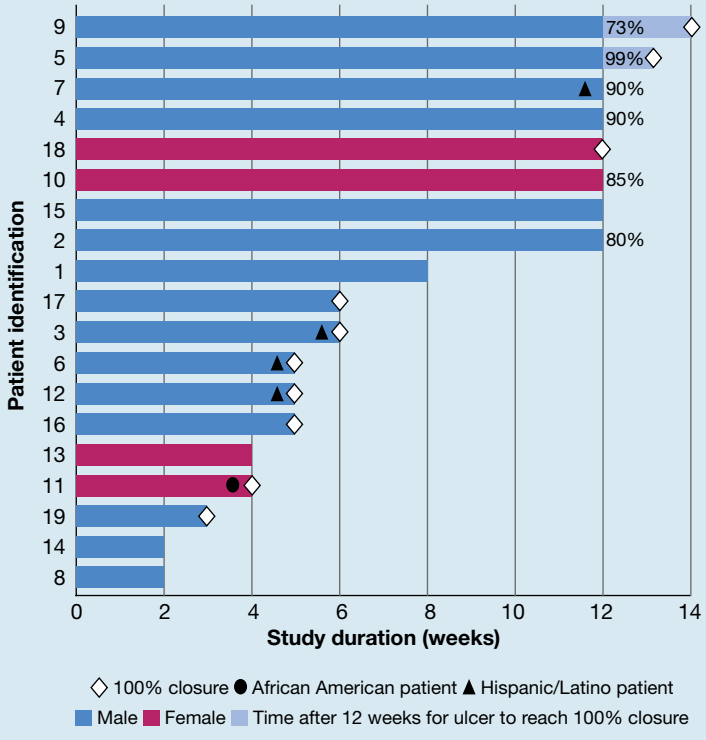
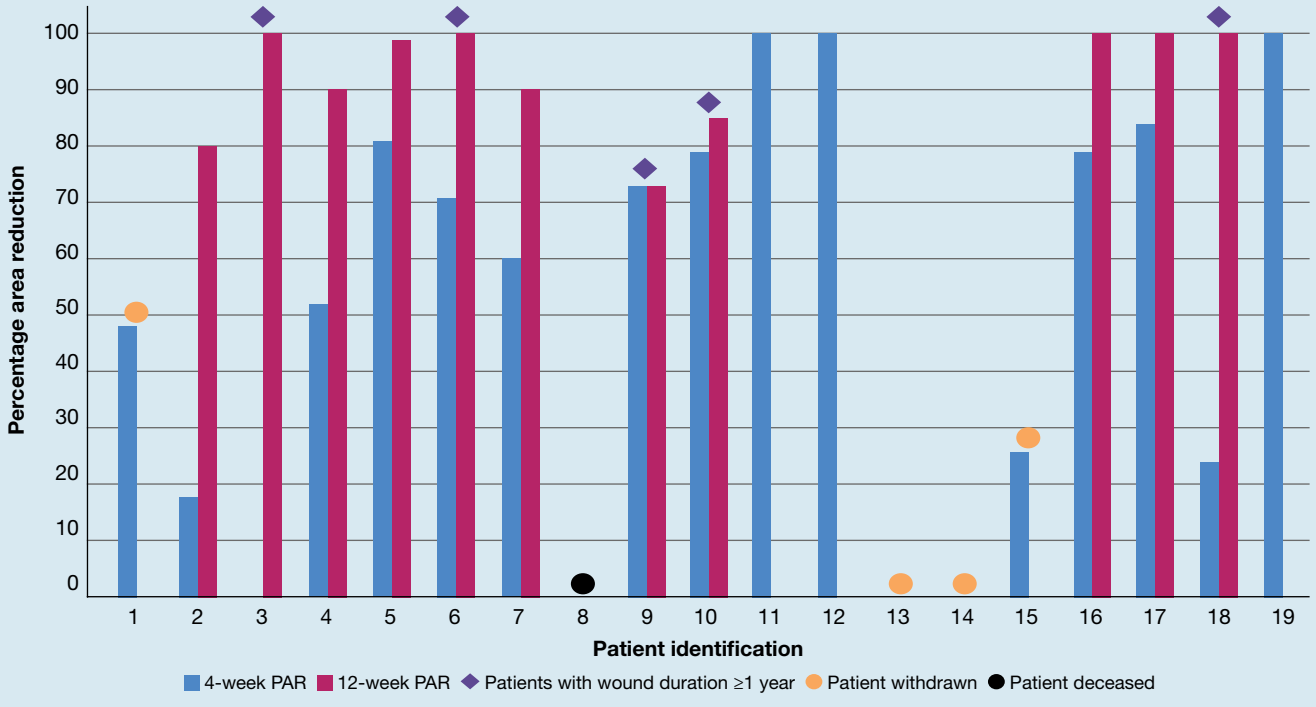


Table 1. Characteristics of patients and diabetic foot ulcers (DFU) treated with the wound matrix

Wound matrix	Patient age, years	DFU duration, weeks	DFU size, cm^2	TV5 DFU size, cm^2	TV12 DFU size, cm^2
Range	44–85	4–72	0.37–25.0	1.0–10.1	0–2.59
Median	60	36	1.7	0.63	0.0
Mean	59.75	32.50	5.05	1.43	0.38

TV5—treatment visit 5; TV12—treatment visit 12

Fig 2. Plot showing percentage area reduction (PAR) of patient ulcers at four weeks and at 12 weeks



The 12-week healing rate of DFUs managed with standard treatment in 26 clinical trials and reported in the US Wound Registry was 37.9%.[7] Results of a randomised controlled trial (RCT) that compared bioengineered skin substitute and standard treatment in patients with DFUs (n=208) without restriction on DFU duration showed 12-week healing rates of 56% and 38%, respectively.[16] Similarly, 12-week healing rates for a fish skin graft product and standard treatment were 56.9% and 31.4%, respectively, in a RCT of DFUs (n=102).[17] Mean 12-week PAR of DFUs managed with the fish skin graft product was 86.3%.[17]

Although the sample size of the current study was small (n=19), results for DFUs of any duration (including five wounds of ≥1 year) managed with the wound matrix are encouraging, with a 12-week healing rate of 57% (8/14), and a 12-week PAR of 94%.

Limitations

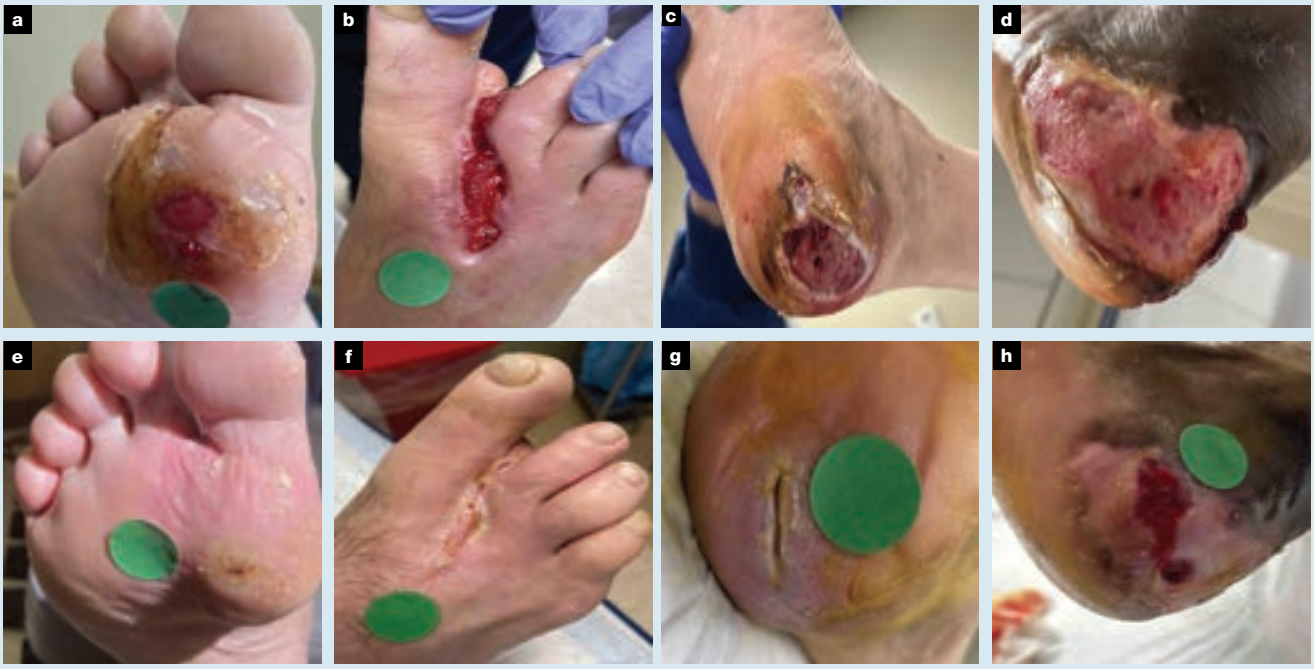
As noted, this was an open-label trial with a population of 19 patients with limited diversity. Results from clinical studies that enrol a more diverse population of patients from multiple sites are needed to validate the growing body of evidence supporting use of the wound matrix therapy in the management of DFUs. The small number did not allow statistical analysis of the efficacy outcomes. Further clinical trials and studies to increase the size and number of patients treated with the wound matrix are needed, as well as RCTs comparing the matrix to other treatments.

Table 2. Offloading types for each patient

Patient ID	Offloading	Max PAR
1	TCC	48
2	TCC	80
3	Offloading surgical boot	100
4	Surgical shoe with peg	85
5	TCC/soft cast	99
6	Diabetic show with insert	100
7	NWB with waffle boot	90
8	Deceased	n/a
9	Podus brace	73
10	Surgical shoe with peg	85
11	NWB/wheelchair bound	100
12	Surgical shoe with peg	100
13	Surgical offloading shoe	n/a
14	Surgical offloading shoe	n/a
15	Surgical shoe with peg	26
16	Orthotic shoe	92
17	Orthotic shoe	100
18	Surgical shoe with peg	100
19	Orthotic shoe with insert	100

ID—identification; Max PAR—maximum percentage area reduction; NWB—non-weightbearing; TCC—total contact casting

Fig 3. Case 1: Treatment visit (TV) 1: A 76-year-old male patient presented with a six-week-old diabetic foot ulcer (DFU) **(a)**. TV4: DFU closed after three treatments **(e)**. **Case 2**: TV1: Ulcer post-debridement in a 65-year-old male patient with a nine-week-old ulcer. Ray amputation of right foot, second metatarsal **(b)**. TV6: DFU closed after five treatments **(f)**. **Case 3**: TV1: A 53-year-old male patient with a three-month-old ulcer measuring 11.55cm^2 post-debridement **(c)**. TV12: percentage area reduction (PAR) of ulcer was 99% **(g)**. **Case 4**: TV1: A 65-year-old male patient with a 36-week-old ulcer measuring 25cm^2 **(d)**. PAR of 90% at TV12 **(h)**



Conclusion

In conclusion, results of this clinical trial are encouraging and indicate the potential for this multimodal wound matrix to serve as an important therapeutic modality for more expeditious management of DFUs, especially when considering the benefit to this patient population who is at significant risk for lower extremity amputation. Patients who have experienced the frustration of prolonged wound duration, including previously failed advanced therapies, may benefit extensively from this unique wound matrix. JWC

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Reflective questions

- What is a multimodal approach to wound healing?
- How does the wound matrix mentioned in the article differ from other cellular, acellular and matrix-like products or skin substitutes?
- How does the wound matrix cited in the article 'customise' wound healing?

The use of a multimodal wound matrix to treat a variety of hard-to-heal wounds: a case series surveillance

Objective: This case series examines the use of a multimodal wound matrix (MWM) trialled in a selection of clinical practice settings and on a variety of hard-to-heal wounds. The objective was to evaluate the effects of MWM and its performance in managing such wounds, regardless of clinical setting and ulcer type.
Method: Treatment of the MWM was conducted by independent wound care practitioners on wounds that were of >4 weeks duration. Treatment was once a week. Assessment was taken after four weeks and at week 12 of the study to assess percentage area reduction (PAR) compared to baseline measurements taken at the first treatment visit. Complete (100%) re-epithelialisation was also recorded.
Results: A total of 63 wounds were treated with MWM, and ulcer types were grouped as: diabetic foot ulcers (n=21); venous leg ulcers (n=18); pressure injuries (n=10); and others (n=14). Of the wounds, 78% had 100% re-epithelialisation, with an average PAR of 57% at four weeks and 86% at 12 weeks. The average time to resolution for those wounds that closed was 7.9 weeks.
Conclusion: Results from this series of independent case studies support the application of MWM to potentially benefit healing in hard-to-heal wounds of different aetiologies of any duration and in a variety of clinical settings.
Declaration of interest: The authors have no conflicts of interest to declare. The product used in this case series was provided by the study sponsor, Omeza LLC, US.

atypical wounds • diabetic foot ulcer • multimodal wound matrix • multiple aetiologies • pressure injury • venous leg ulcer • wound • wound care • wound dressing • wound healing

The management of hard-to-heal (chronic) wounds is often met with countless challenges across the continuum of care; yet prolonged healing times lead to higher rates of complication, such as infections and amputations, and are a significant healthcare burden.[1,2] When wounds or ulcers remain open, they negatively impact patients' quality of life and can result in significant pain and suffering.[3] Among the most common types of hard-to-heal wounds are diabetic foot ulcers (DFUs), pressure injuries (PIs) (also referred to as pressure ulcers), and venous leg ulcers (VLUs), which are often associated with peripheral neuropathy, immobility and venous hypertension, respectively.[4,5]

Atypical ulcers from inflammatory or haematologic causes, such as pyoderma gangrenosum, hereditary thrombosis syndromes, leg ulcers as a consequence of sickle cell disease, and soft tissue radionecrosis, can also cause significant morbidity.[5]

Individuals who develop hard-to-heal wounds usually have one or more underlying comorbid conditions, such as diabetes, vasculitis, immobility, malnutrition and/or immune suppression, that can negatively affect their innate healing process and increase the risk of their wounds becoming hard-to-heal.[6,7] In addition, ageing affects the innate healing process and, as a result, the prevalence of hard-to-heal wounds is increasing with an ageing population.[4,8]

The following is a collection of case studies examining the use of a multimodal wound matrix (MWM) tested in a selection of clinical practice settings and on a variety of hard-to-heal wounds. The objective was to evaluate the effects of MWM and its performance as well as its versatility in the management of such wounds, regardless of type of clinical setting.

Designed from a clinical perspective and taking a bioactive approach to tackle many of the common pathophysiologies observed in hard-to-heal wounds, the primary concept of the MWM is based on how to stimulate the body to transform the wound microenvironment from a non-healing to a healing state.[9] The concept of the formulation was based on several physiological principles of wound healing. Firstly, the human body is designed to repair and heal wounds, but if stalled in one of the healing phases or deprived of the necessary macronutrients and metabolites for the healing cascade to progress, the wound will not close. Additionally, all hard-to-heal wounds, regardless of their aetiology and associated comorbidities, exhibit common traits, such as

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oedema, biofilm colonisation and decreased local perfusion to the wound, further complicating the innate healing process.[10]

The MWM used in this case series was developed to provide hard-to-heal wounds with the components needed to support the healing cascade, as well as to inhibit bioburden, decrease inflammation and to support regeneration. The formulation is composed of both marine-sourced peptides and omega-3 fatty acids, medium chain triglycerides, plant-sourced polyunsaturated fatty acids, vitamins and minerals. The benefits of omega fatty acids have been well established and along with the cold-water fish peptides are the foundation on which MWM was developed.[9] The physical characteristics of MWM are attributed to other oils and waxes which make the product malleable, and able to intimately connect with the exposed tissue of the irregular wound bed, or fit into tunnelling wounds or where wound undermining occurs.[9]

Methods

The case studies described in this article were conducted by independent wound care certified podiatrists, surgeons, nurse practitioners and dermatologists from clinics geographically spread across the US, from July 2020 through December 2022. Sites of service included physicians' offices, wound care centres, outpatient clinics, and skilled nursing facilities. Sites were chosen to trial the MWM because of the clinician's established clinical wound care experience. The case studies were not part of a registered clinical study but collected as data by the clinicians treating refractory ulcers with MWM. Sites were not provided with a protocol. Clinicians were expected to follow the product's Instructions for Use (IFU) when treating identified patients. Standard of care was not evaluated nor stipulated at the treatment sites but left to the treating clinician's discretion.

Ethical approval and patient consent

The case series was performed in accordance with US and international standards of Good Clinical Practice and Health Insurance Portability and Accountability Act guidelines. Ethics Board approval was not required for these case studies. Prior to study enrolment, all patients provided written informed consent to publish the case details and associated deidentified image assessments.

Patients

The case series population included adult (≥18 years) patients who presented with hard-to-heal wounds of varying aetiologies and durations. Wound types included DFUs, VLUs, PIs, surgical ulcers, arterial ulcers, and atypical wound aetiologies. Patients were followed up to 12 weeks or until wounds had re-epithelialised (measured as 100 percent area reduction (PAR)), whichever came first. Of the patients, one was treated up to 16 weeks.

There were no inclusion or exclusion criteria applied to the patients evaluated in these case studies, except for the contraindications listed in the manufacturer's IFU which include a known sensitivity to cod liver oil and no use on third-degree burns. Case studies included patients who were smokers, opioid and recreational drug users, and those with a body mass index >40kg/m^2.

The target ulcers had been previously treated with standard therapies including offloading, compression therapy and/or moisture managing dressings. The type of offloading for DFUs was left to the clinicians' discretion, based upon the patient's needs. All patients had failed at least one advanced wound care treatment modality, including, but not limited to, hyperbaric oxygen therapy (HBOT), negative pressure wound therapy (NPWT), cellular, acellular, and matrix-like products, and other advanced dressings.

Intervention

Following the manufacturer's IFU, the MWM was generally applied to the ulcer once a week. The wound bed was cleansed and debrided as per clinician decision before the MWM was applied directly to each wound in strips. Approximately 3–5 minutes after application, the MWM was spread evenly throughout the wound. A nonadherent primary dressing was applied to the wound following the application of the MWM. Added absorptive secondary dressings and appropriate modalities, such as compression wraps and offloading devices, were used based on clinician preference.

Treatment was managed in a variety of clinical settings. Data capture of weekly wound measurements was obtained through various methods including automated digital devices, standard photography via handheld devices, and paper ruler measurements. Wounds were assessed weekly and managed with reapplication of the MWM treatment when deemed appropriate by the treating clinician.

Study endpoints and statistical analysis

Study endpoints included PAR of the wound after four weeks of MWM treatment and time to closure with full re-epithelialisation (PAR of 100%). Wounds were measured weekly using either an imaging modality or by a standard method of recording length (L) × width (W) and sometimes, but not consistently, depth (D), with L being the longest length, W the greatest width, and D the greatest depth. Final visit data from patients who had relocated or were non-adherent before the completion of treatment were included in the dataset and analysis.

Data presented in this case series was collected retrospectively. All analyses of data from this study were descriptive (without p-value generation) as the study was not powered for inferential analyses and no formal hypothesis testing was performed.

Results

A total of 63 patients were included in this retrospective case series review. Wound types included: DFUs (n=21); VLUs (n=18); PIs (n=10); and 'others' (n=14; comprising:

arterial (n=3); surgical (n=2); atypical (n=2); trauma (n=6) and one wound of unknown aetiology) (Table 1).

The age range of the patients in this dataset at the time of treatment with MWM was 31–97 years, with a mean of 72 and a median of 72 years (Table 1). The ratio of male to female patients was equivalent. Wound duration recorded before treatment with MWM ranged from 4–780 weeks (mean: 96.3; median: 30 weeks) (Table 2). Ulcer size ranged from 0.16–72.00cm^2 with a mean size of 9.23cm^2 (Table 2).

Outcomes by wound type

Diabetic foot ulcers

Median age of the patients with DFUs was 63 years (range: 52–84 years) (Table 1). Comorbidities included (but were not limited to): type 2 diabetes; hypertension; hypercholesterolaemia; obesity; congestive heart failure; ischaemic cardiomyopathy; osteomyelitis; renal insufficiency; and chronic kidney disease. DFU area ranged from 0.16–15.4cm^2, with a median size of 1.9cm^2 (Table 2). DFU duration prior to MWM treatment ranged from four weeks to 15 years (780 weeks). Average four-week PAR was 78% (final PAR 88%) (Table 3, Fig 1), with 18/21 patients having a 100% PAR, with a mean healing time of six weeks (range: 3–12 weeks) (Table 3). Of the patients with a DFU, three included in the analysis did not complete treatment: two patients required resolution of a wound infection, and one patient relocated and was lost to follow-up.

DFU case example

An example of a DFU case study treated with the MWM is that of a 65-year-old female patient who presented with a blister of three months' duration that progressed to a hard-to-heal DFU (≥4 weeks duration), exacerbated by a Charcot deformity (Fig 2a). Previous treatments had included collagen powder, gauze covering, absorbent dressing and pressure offloading. The patient's DFU was assessed, treated weekly with MWM (except for a single missed appointment leading to a two-week period between treatment). The DFU was re-epithelialised after eight applications of MWM (PAR of 100% at 10 weeks) (Fig 2b).

Table 1. Patient age distribution by wound type

Ulcer type	Patients, n	Age range, years	Age, median, years	Age, mean, years
DFU	21	52–84	63	64
VLU	18	31–97	67	66
PI	10	70–97	88	85
Other				
Arterial	3	56–87	86	80
Surgical	2			
Atypical	2			
Trauma	6			
Unknown	1			

DFU—diabetic foot ulcer; PI—pressure injury; VLU—venous leg ulcer

Venous leg ulcers

Among the 18 patients with VLUs, the median age was 67 years (range: 54–97 years) (Table 1). Patients presented with comorbidities, including (but not limited to): chronic venous disease; lymphoedema; obesity; venous insufficiency; sarcoidosis; and failed arterial bypass graft (patient required an arterial pump). Ulcer area size in this group ranged from 1.62–72.00cm^2, with a median size of 4.6cm^2, and a wound duration ranging from 12–208 weeks (Table 2). Patients with a VLU received an average of eight treatments with MWM. Average four-week PAR was 54%, and final PAR was 76% (Fig 1). Of the patients, 11 VLUs closed in 12 weeks and one patient's wound had 100% closure at 16 weeks (Table 3). The average time to wound resolution from first application of the wound matrix was 8.5 weeks (Table 3).

VLU case example

A 69-year-old female patient presented upon admission to a skilled nursing facility with a wound of >2 years duration to her right lower extremity. The patient's wound was exacerbated by venous insufficiency and prior trauma to the site. On initial evaluation, the

Table 2. Wound size and duration by wound type

	Ulcer size, cm^2			Ulcer duration, weeks		
	Range	Median	Mean	Range	Median	Mean
DFU	0.16–15.40	1.87	3.18	4–780	21	130.6
VLU	1.62–72.00	4.55	12.75	12–208	32	51.9
PI	0.05–5.81	0.54	1.77	5–208	45	24.0
Other	0.49–74.40	10.13	16.71	6–468	77	83.7
Total	0.16–74.40	3.00	9.23	4–780	30	96.4

DFU—diabetic foot ulcer; PI—pressure injury; VLU—venous leg ulcer

Table 3. Wound closure rates by wound type

Ulcer type	Total	Closed, n	Closed, %	12-week PAR	4-week PAR	Mean time to resolution, weeks
DFU	21	18	86	88.4	78.0	5.6
VLU	18	12	67	76.3	54.0	8.5
PI	10	7	70	87.7	59.0	7.1
Other	14	11	79	91.9	75.0	6.5
Total	63	49	77	91.9	75.0	6.5

DFU—diabetic foot ulcer; PAR—percentage area reduction; PI—pressure injury; VLU—venous leg ulcer

Fig 1. Multimodal wound matrix treatment results by percentage wound area reduction (PAR) at four weeks and at the end of treatment. DFU—diabetic foot ulcer; PI—pressure injury; VLU—venous leg ulcer

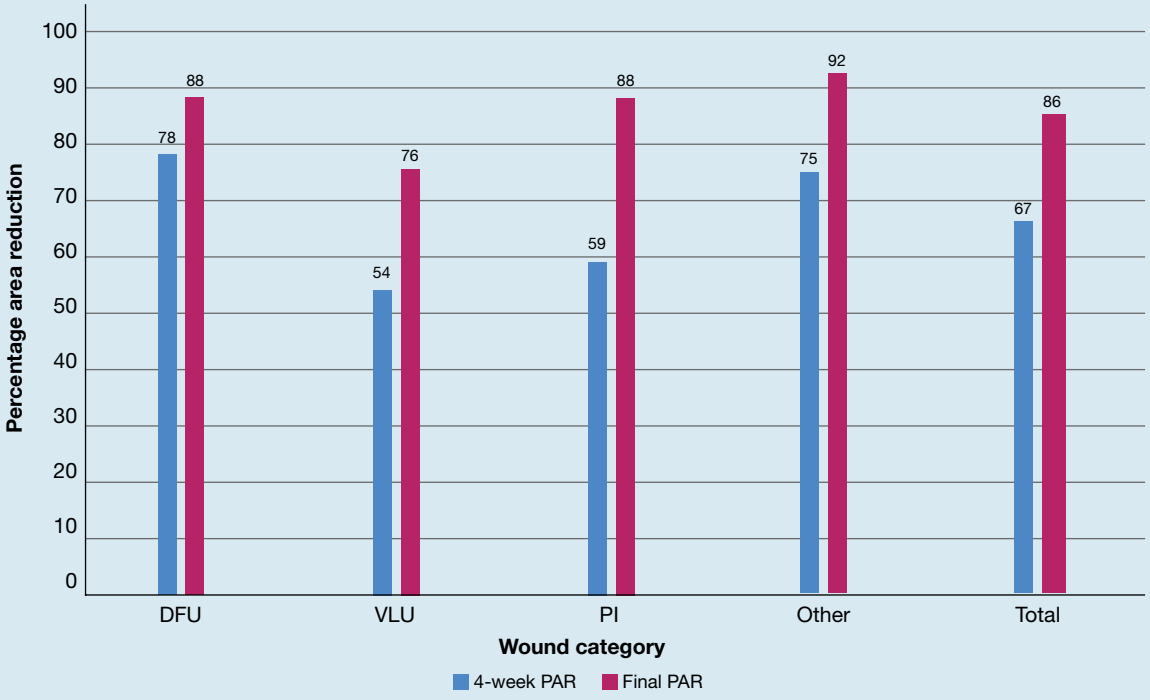
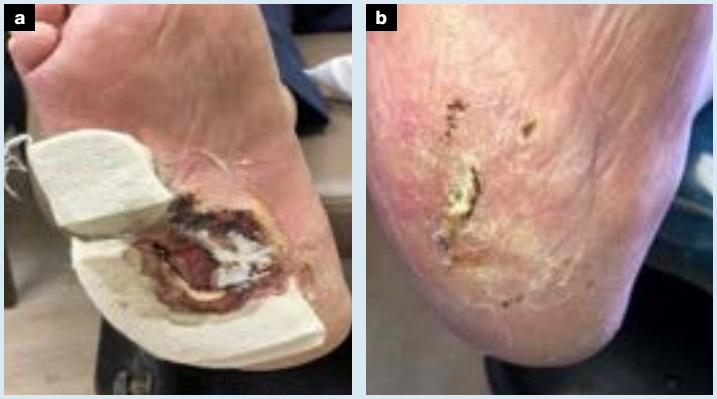


Fig 2. A 65-year-old female patient with Charcot deformity, of 16 weeks duration that was not responding to treatment **(a)**. The wound closed after eight treatments with multimodal wound matrix **(b)**

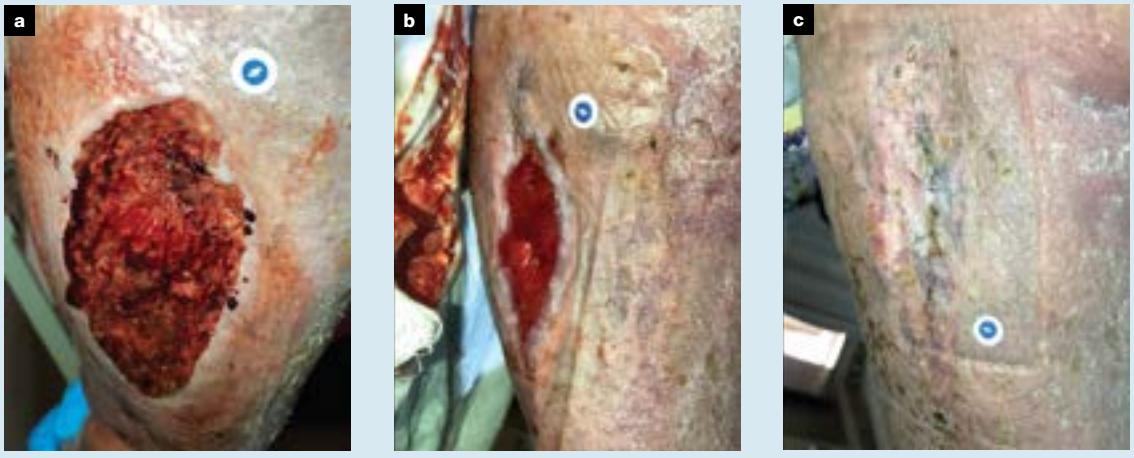


wound measured 7.08×4.48cm. (Fig 3a). Prior treatment to MWM application had included a polyurethane foam impregnated with a polyvinyl alcohol, an absorbent pad, and dressing changes every other day. After regular treatments with MWM, the PAR had improved by 85%, measuring 3.45cm^2 (Fig 3b). The wound resolved after 15 treatments (Fig 3c).

Pressure injuries

The median age of the 10 patients with PIs was 88 years (range: 70–97 years) (Table 1). Comorbidities included (but were not limited to): neuropathy; congestive heart failure with left ventricular ejection fraction; hypertension; atrial fibrillation; and stage 3 injury. Wound area ranged from 0.50–5.89cm^2, with a median size of 0.54cm^2 (Table 2), and wound duration prior to MWM treatment ranged from 5 weeks to 4 years (208 weeks) (Table 2). Patients previously received

Fig 3. A 69-year-old female patient with a venous leg ulcer on the lower extremity of two years' duration **(a)**. The ulcer after 10 treatments with the multimodal wound matrix (MWM) **(b)**. After 15 weekly treatments with MWM **(c)**



advanced wound dressings to manage their PIs. Average four-week PAR was 59%, and final PAR was 88% (Fig 1). The rate of total wound closure was 70% (7/10) and the average time to wound resolution from first application of the MWM was 7.1 weeks (Table 3). In one PI that was managed with MWM treatment for 12 weeks, a 99% closure was achieved.

PI case example

A 98-year-old female resident of a group home was treated for a left heel ulcer that had been present for >300 days. The patient had lived with dementia and was minimally verbal upon initial evaluation. She was immobile, primarily seen in a wheelchair or bed, and had evidence of peripheral arterial disease. There had been no prior debridement of the PI or consistent offloading to the site. The patient received an initial debridement of callus overlying the wound which measured 2.36×2.59cm (Fig 4a). Her clinician initiated a protocol that included application of MWM to the wound twice weekly and offloading with pillows behind the patient's leg to 'float' the heel. The four-week PAR was 76%, measuring 0.93×1.58cm. At week 12, a final PAR of 99.2% was achieved, with a total of 22 applications over 11 weeks of MWM treatment to the wound (Fig 4b).

Atypical and other aetiologies

Atypical ulcers present with unique characteristics and challenges, requiring accurate diagnosis and subsequent management. These wounds may not respond in the manner expected of more commonly diagnosed wounds, such as VLUs, DFUs or PIs. In this case series, 14 wounds of varying aetiologies were included. The group included patients with sickle cell disease, pyoderma gangrenosum, arterial disease, and hard-to-heal surgical and trauma ulcers. Of the patients, four presented with wounds located on their lower extremities. The age range of the patients was 56–87 years (Table 1). Wounds included in this group had a duration that ranged between 6–468 weeks, with a median of 77 weeks. The size of the ulcers ranged from 0.49–74.42cm^2, with a mean size of 16.71cm^2 and a median size of 10.13cm^2 (Table 2).

Among this mixed group of hard-to-heal wounds, the average four-week PAR was 75%, and the final PAR was 92% (Fig 1). Of the wounds, 11 (79%) healed in an

Fig 4. A 98-year-old female patient with a pressure ulcer of 42 weeks duration **(a)**. Ulcer at week 12 following 11 applications of multimodal wound matrix **(b)**

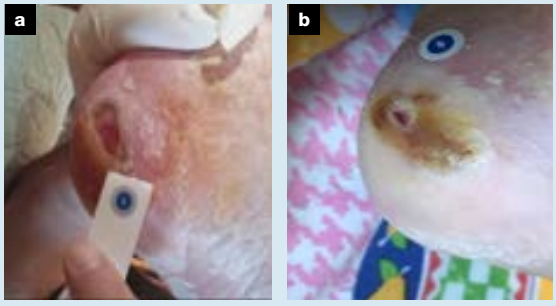
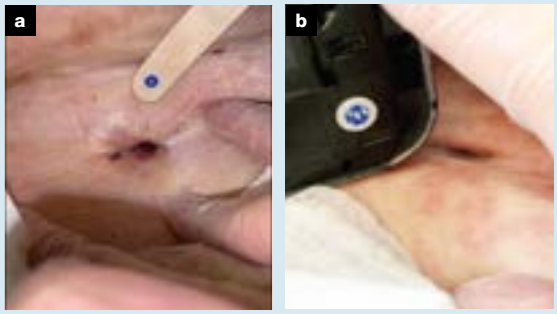


Fig 5. A 79-year-old female patient with a surgical wound of the abdomen that had not responded to >100 weeks of treatment **(a)**. The wound after nine treatments with the multimodal wound matrix **(b)**

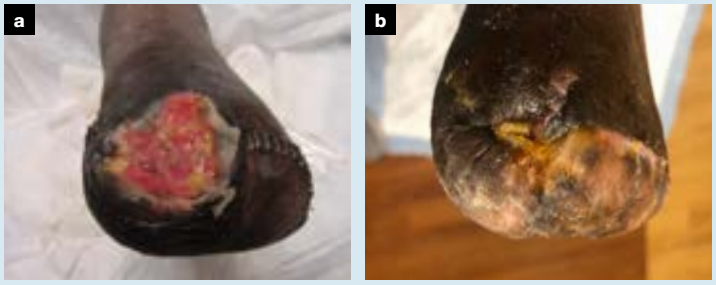


average of 6.5 weeks (Table 3) from start of the MWM weekly applications to resolution.

Atypical ulcer case study examples

Case 1: A 79-year-old female patient with a hard-to-heal, surgical wound of the abdomen with a persistent tunnel was evaluated after approximately two years of prior management. The wound was complicated by the presence of an underlying synthetic mesh that was implanted during the original surgical procedure. Upon initiation of MWM, the wound measured approximately 4.5×1.08×0.91cm (Fig 5a). After four weeks, the measurements had reduced to 0.61×0.41×0.3cm and the wound resolved after nine treatments (Fig 5b).

Fig 6. A 56-year-old male patient who smoked with trauma to a previous amputation of a gangrenous left hallux **(a)**. The wound closed after seven treatments with the multimodal wound matrix **(b)**



Case 2: A 56-year-old male patient, who was a smoker, with a history of diabetes, experienced a left leg trauma to a previous amputation of a gangrenous left hallux. The leg trauma had obliterated the patient's anterior tibial and peroneal arteries, leaving him with a single vessel runoff to the left foot via a posterior tibial artery. The patient had been hospitalised previously to receive intravenous antibiotics and a vascular assessment; a left superficial femoral artery occlusion was identified on an arteriogram and a stent was placed within the vessel. Following discharge, he presented to the wound care centre for follow-up care.

At presentation, the patient's left hallux amputation site was completely necrotic with eschar and he reported significant rest pain of the distal left foot (10/10). A referral was made by the attending physician for adjunctive HBOT and for smoking cessation assistance. The patient's pain was not alleviated by these interventions, and he was referred for additional vascular and podiatric surgery consultations. The patient was subsequently admitted to the hospital where he underwent a left transmetatarsal amputation. After discharge to home, he resumed outpatient HBOT for the original hard-to-heal left hallux amputation wound. A freshly stapled lesser digit amputation incision was present in addition to the periwound skin, which was darkly pigmented and dusky, and with continued hyperalgesia. These findings negated him as a candidate for subsequent NPWT. At this time, with the wound measuring 3.4×3.4cm, the MWM application was initiated and applied to the site every 5–7 days (Fig 6a). After three applications, there was significant improvement in the wound depth/tissue colour, with visible re-epithelialisation at the wound edges. Despite the patient's obvious ischaemia, a PAR of 65% was attained after four applications. The wound improved with each treatment and closed after the seventh application of MWM (Fig 6b). He was discharged from the wound specialist's service <2 months after the initial MWM application.

Discussion

While the underlying comorbid conditions contributing to the development of various acute and hard-to-heal wounds may be patient specific, the basic tissue reparative process is similar, regardless of the wound location on the body. The healing cascade contains a series of complex and coordinated events that include: phagocytosis; damaged extracellular matrix degradation; angiogenesis; neocollagenesis; and tissue remodelling.[4] Commonalities therefore exist in the care of these wounds, regardless of type or place of treatment. However, although there are guidelines for standard of care for hard-to-heal wounds, we nevertheless accept that the standard treatment at wound care sites can vary, primarily due to available resources at each site. For example, topical product availability to clinicians is often based on contracts within a healthcare system or determined by clinician preference. Parameters for consideration and guidelines are widely accepted and implemented, but not always carried through. For example, offloading for DFUs may limit a patient's ability to work or drive a car, and therefore the decision to implement may need to be adapted to meet the patient's basic needs.

The case studies described here include hard-to-heal ulcers that failed to progress into a healing trajectory, despite the use of previous advanced therapies and standard treatment. The phrase 'real-world evidence' to describe the data is not included here because these words hold a specific definition in clinical research, but these case studies are intended to reflect the everyday challenges faced in wound care settings. The ulcers in this series are unlikely to qualify for clinical trials because of the age of the wounds and the patients' extensive comorbidities, but they are representative of cases routinely seen in US wound clinics. The 63 complex wounds had a range of underlying conditions including ischaemia, diabetes, venous stasis disease, or constant pressure. Nevertheless, 77% of the ulcers closed in an average time of seven weeks, with a mean four-week PAR of 57% and a 86% PAR at 12 weeks (Table 3). The results described in this case series illustrate the ability of the MWM to support wound repair across the phases of wound healing in a wide variety of ulcers regardless of aetiology.

The current global wound care product market is an immense commercial enterprise, with revenues exceeding $22.25 billion USD.[11] Yet it is primarily

comprised of singularly focused advanced wound care treatments, such as products used mainly for infection control, tissue debridement or exudate management.[12–14] While these therapies have benefit, they may only have utility in certain patients or specific wound types. Additionally, autologous and allograft tissue therapies used in the treatment of complex wounds have limitations of cost, accessibility, procedural pain, contradictory evidence, and wound exclusion based on aetiology.[15] Leveraging multimodal products able to address a myriad of core local factors contributing to delays in wound healing are pivotal in supporting successful outcomes in this complex patient population. The development of the MWM was born out of this need.

Prior to these clinical studies, the safety of the MWM was assessed in clinical trials (registered clinical trials numbers: NCT4510376; NCT04510675; AND NCT04512274), where it showed no potential allergenicity or sensitisation, and no known product-related adverse events. The active ingredients of the product are sustainably sourced.[9] The formulation is composed of components that have supporting research, showing properties that have reported antimicrobial, anti-inflammatory and angiogenic factors. Omega fatty acids and other nutrients contained in the MWM have been studied extensively by researchers and their respective benefits have been validated well before the product was developed.[16] The composition and physical qualities of MWM, such as melting point and being anhydrous, are also design-specific features to deliver the active ingredients to the wound bed as a slow bolus and to promote the healing process.[9]

Modern wound care is delivered in a wide variety of settings by multidisciplinary providers, including a growing mobile wound care sector. Some advanced wound care therapies require special handling and storage (freezer/refrigerator), making them less than ideal for use in care settings not associated with hospital or clinic facilities. Use of the MWM in this limited series illustrated ease of product use, with an ability to convert previously recalcitrant wounds to a healing trajectory. Wider adoption of the MWM could provide substantial clinical improvement to the current standards of care and other advanced modalities. Versatility of the product was also observed as the types of wounds treated in this study reflected a range of aetiologies typically encountered in settings where wounds are commonly managed.

This case series provides background knowledge behind the development of the MWM and its potential for treatment in hard-to-heal wounds. Having treatments that are suitable for use across the continuum of care is of increasing importance. As our population ages, the number of patients with hard-to-heal wounds continues to rise. Shelf-stable, easy to use, patient-friendly products, such as the MWM, are a welcome addition to the wound care armamentarium.

Limitations

The major limitation of this data is that treatment was provided with no protocol, inclusion or exclusion criteria, nor standardised reporting. It is a collection of surveillance data from independent practitioners using a variety of methods to collect wound measurements and patient information in different care settings.

Another limitation is that there is variability in the aspects of care at the case studies' sites which affects evaluation of the treatment outcome of MWM. Future studies are needed to increase the patient population and results compared to structured clinical trials to reduce potential inherent bias.

Conclusion

The results of this case series support the potential use of this MWM for multiple wound types of any duration that are failing to respond to alternate treatments. To evaluate the effects of the matrix in a more controlled setting with standardised treatment, three clinical trials are being conducted for the treatment of DFUs, for refractory VLUs, and for wounds/ulcers of multiple aetiologies. Results and data from ongoing and future controlled clinical trials will provide data to further evaluate the efficacy and the limitations of the wound care matrix in the treatment of hard-to-heal wounds. JWC

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Reflective questions

- How effective can a multimodal approach be for wounds of multiple aetiologies?
- Which hard-to-heal wound aetiologies could benefit most from marine-sourced products?
- What are the common challenges to healing hard-to-heal wounds faced by health professionals, irrespective of care setting?