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Case Report

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A Three-Step Multimodal Biofilm-Focused Protocol in Complex Acute and Chronic Wounds: Clinical Outcomes from ICU Real-World Patients

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ABSTRACT

Introduction: Prevention and management of biofilm in acute and chronic wounds in ICU settings is critical, as biofilm significantly impedes wound healing. Approximately 90% of all wounds are affected by biofilm. It is not feasible to eliminate biofilm using a single wound-cleaning technology. Early intervention and the combined use of multiple technologies for biofilm removal are essential to promote wound healing.

Rapid and effective treatment is vital to prevent severe complications such as infections or amputations in intensive care units. Drawing on extensive knowledge of available technologies and their mechanisms of action, we adopted a multimodal approach to treat wounds with biofilm. The primary objective was to prevent and manage biofilm in both acute and chronic wounds.

Methods: Five patients with infected acute and traumatic wounds—including chronic osteomyelitis tunnel wound, gunshot injuries, shrapnel wounds, surgical incisions, and fasciotomies—underwent Three-Step Multimodal Biofilm-Focused Protocol consisting of three sequential steps:
Step 1: Cleansing and moistening wounds with a solution containing active pure HOCl (pH 2.5) for at least 30 minutes.
Step 2: Cleansing and moistening wounds with a polyhexamethylene biguanide (PHMB) and betaine solution, left in place for 30 minutes, followed by cleaning with a debridement pad.
Step 3: Application of a synergistic ionic silver (0.01%)–menthol liquid dressing with unique properties (cleansing, disinfecting, removing waste and slough, destroying biofilm). This dressing offers broad-spectrum antibacterial activity and accelerates healing. Wet pads were left in the wounds for the remainder of the day.

Results / Discussion: All wounds demonstrated improvement, with the formation of new granulation tissue. Subsequent treatments included negative pressure therapy, secondary surgical closure, or skin grafting. Gunshot and shrapnel wounds healed successfully, limb amputation was avoided, and the chronic osteomyelitis tunnel wound contracted.

This Three-Step Multimodal Biofilm-Focused Protocol enhanced wound healing, prevented amputations, and accelerated recovery. The method was later applied to other complex wounds in ICU patients.

Keywords: Complex Wounds, Wound-Bed Preparation, Biofilm Disruption, Hypochlorous Acid Irrigation, Polyhexamethylene Biguanide (PHMB), Betain, Synergistic Ionic Silver Dressing, Menthol-Based Liquid Dressing, Moisture Balance, Chronic Wound Management, Traumatic Injuries, Blast and Shrapnel Wounds, Osteomyelitis-Associated Wounds, Tunnel Wounds / Sinus Tract Wounds, Fungal Maceration, Negative Pressure Wound Therapy Compatibility, Multimodal Wound Care, Wound Healing Acceleration, Infection Control, Granulation Tissue Formation, Real-World Clinical Cases

Introduction

A wound represents a disruption in tissue integrity caused by trauma, surgery, infection, or underlying disease. When inadequately managed, wounds may deteriorate, deepen, or progress into a chronic non-healing state, resulting in prolonged morbidity, increased healthcare costs, and a higher risk of limb loss. Effective wound care requires early removal of necrotic tissue and contaminants, control of microbial burden, and correction of physiologic parameters essential for healing. Chronic wounds typically exhibit an alkaline environment

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(pH 7.1–8.9), a biochemical state that impairs oxygen release, angiogenesis, and cellular repair. Without timely intervention, even acute wounds may rapidly transition toward chronicity [1].

The shift from acute to chronic occurs particularly quickly in contaminated or complex wounds, including diabetic foot ulcers, burns, surgical-site complications, and fasciotomies. Among the most challenging are combat-related and high-energy traumatic injuries, where delayed evacuation, ischemia, devitalized tissue, and heavy environmental contamination markedly increase the risk of deep infection and sepsis, making limb salvage significantly more difficult. Battlefield studies identify delayed debridement, inadequate cleansing, and ischemia as leading predictors of amputation and infection-related failure [2]. These findings underscore the need for rapid and effective cleansing strategies to stabilize the wound environment and prevent progression toward surgical amputation whenever possible.

A key contributor to wound deterioration is biofilm—a structured microbial community that confers high antimicrobial tolerance, drives persistent inflammation, and delays granulation. Expert consensus emphasizes that biofilm-associated wounds require multimodal management, including mechanical disruption and chemical antimicrobial action using well-tolerated technologies such as polyhexanide, hypochlorous solutions, and ionic silver [3,4]. In high-risk settings such as burn ICUs, HOCl-based decontamination protocols have significantly reduced resistant organisms and cross-contamination, demonstrating strong bioburden control that may lower downstream complications and limb-threatening infections [5].

Several antimicrobial cleansing technologies have demonstrated efficacy in reducing bacterial burden, promoting granulation, and accelerating wound closure. Polyhexanide-based solutions support treatment of critically colonized wounds [3]. Hypochlorous solutions provide broad-spectrum activity without cytotoxicity, making them suitable for fragile tissue states [5]. Ionic silver exhibits potent bactericidal effects, including membrane lysis and cytoplasmic leakage [6]. Clinically, synergistic ionic silver (0.01%)–menthol liquid dressings have shown faster granulation, higher culture-negativity rates, and reduced hospitalization in diabetic foot ulcers—factors strongly associated with decreased amputation risk [7,8]. Controlled-release ionic silver dressings have also demonstrated faster wound-area reduction compared to alternatives, supporting their role in managing complex chronic wounds [9].

In severe trauma or implant-associated infections, combined approaches such as continuous antimicrobial instillation with negative-pressure therapy have achieved rapid resolution of multidrug-resistant infections without hardware removal, illustrating the value of multimodal strategies in limb preservation [10].

Given the complex progression of acute contaminated wounds toward chronicity—and the practical challenges encountered in real-world ICU, trauma, and limb-salvage settings—there is a clear clinical need for a simple, reproducible, biofilm-focused wound-cleansing protocol that supports infection control, optimizes wound-bed preparation, promotes granulation, and reduces amputation risk. Building on published evidence across diverse technologies and clinical contexts [1–18], we developed a Three-Step Multimodal Biofilm-Focused Protocol designed to meet these needs in both acute and chronic complex wounds.

Materials and Methods

In this report, we present the real-world treatment experience of five patients with highly complex wounds considered difficult to manage in both civilian and combat medicine. The series includes:

- One deep tunnel wound associated with chronic osteomyelitis in a quadriplegic patient
- Three severe traumatic blast- and shrapnel-related wounds sustained during military operations
- One case of painful bilateral fungal–maceration wounds caused by prolonged moisture inside military boots

Despite differing etiologies, all wounds shared several critical characteristics. Approximately 90% exhibited visible biofilm or heavy bacterial burden, contributing to delayed healing, rapid bacterial spread, and, in several cases, reduced responsiveness to systemic antibiotics. Prior to initiating the Three-Step Multimodal Protocol, several patients received systemic antibiotics, yet many demonstrated limited response or microbial resistance—particularly in wounds with heavy biofilm. Some patients also underwent surgical debridement; however, fibrin, slough, or necrotic tissue reappeared within 48 hours, or further debridement was not feasible.

These challenges rendered traditional wound-care approaches insufficient and highlighted the clinical need for a strategy capable of addressing contamination, biofilm, moisture imbalance, and pain—while preserving tissue viability and preparing the wound bed for closure. In response, clinicians implemented the solution-based Three-Step Multimodal Biofilm-Focused Protocol described below.

Three-Step Multimodal Biofilm-Focused Protocol:

Step 1 – HOCl-Based Initial Cleansing

Wounds were irrigated and moistened with a low-pH pure hypochlorous acid (HOCl) solution (pH ≈ 2.5) and kept in contact for at least 30 minutes. This step reduced surface bacteria, loosened slough, and hydrated necrotic debris.

Hypochlorous acid (HOCl) is a critical component of the innate immune system, playing an essential role in pathogen defense. It is highly effective against a broad spectrum of microorganisms—including bacteria, viruses, fungi, and protozoa—by inducing oxidative damage to microbial cell walls, membranes, proteins, and nucleic acids, ultimately causing cell lysis and death [11]. Importantly, HOCl does not harm periwound skin or granulation tissue.

Step 2 – PHMB Solution Cleansing and Moisturizing

A polyhexamethylene biguanide (PHMB) plus betaine solution was applied and allowed to remain for 30 minutes, providing additional antimicrobial action. This was followed by mechanical cleaning with a debriding pad to remove loosened slough and biofilm.

PHMB is a cationic polymer with surfactant properties that exerts its bactericidal effect by penetrating microbial membranes and condensing bacterial DNA [12]. The PHMB–betaine solution combines two active components: Polyhexanide (PHMB) 0.1% – a selective, broad-spectrum antimicrobial agent and Betaine 0.1% – a unique surfactant that breaks bonds and dissolves biofilm layers.

PHMB demonstrates broad-spectrum activity against bacteria, fungi, parasites, and certain viruses, with a high therapeutic index. It has been widely used in clinical, home, and industrial settings for many years without evidence of resistance development. PHMB is safe, non-cytotoxic, and does not damage newly forming wound tissue [12,13].

Step 3 – Synergistic Ionic Silver (0.01%)–Menthol Liquid Dressing

Finally, wounds were treated with pads soaked in a multi-component ionic silver–based liquid dressing, providing: moisture balance, biofilm disruption through surfactant-enhanced penetration, removal of residual debris and slough, reduction of odor and pain, continuous antimicrobial activity, a stable environment conducive to granulation. Wet pads were left in place for 24 hours, ensuring prolonged contact and consistent cleansing.

In daily clinical practice, we applied the three-step treatment protocol to more than 10 patients with complicated wounds of diverse origins, including traumatic injuries, chronic wounds, deep tissue infections, atypical wounds, and fungal infections. In this article, we present five representative cases illustrating wounds of different etiologies.

Results

All five wounds in this series progressed successfully toward healing following the multimodal wound-bed treatment protocol. Although each case presented unique challenges—such as chronic infection, deep tissue destruction, heavy contamination, early biofilm formation, or moisture-associated fungal overgrowth—the clinical trajectories followed a similar pattern: stabilization, purification of the wound bed, development of healthy granulation tissue, and eventual closure or full epithelialization.

The following narratives detail the clinical course of each patient.

Case 1 – Chronic Osteomyelitis Tunnel Wound

The first patient presented with a 16 cm-long deep sinus (tunnel wound) with a 3 cm entrance, associated with chronic osteomyelitis of the lower limb. The wound exhibited heavy purulent discharge, persistent slough, and a strong odor. Previous interventions—including systemic antibiotics, repeated debridements, and surgical procedures—produced only temporary improvement; slough reappeared within 48 hours, and the sinus remained unchanged at 16 cm, indicating entrenched infection and active biofilm.

Following implementation of the multimodal protocol—twice-daily cleansing with hypochlorous acid solution, followed by polyhexamethylene biguanide (PHMB) plus betaine, and then wicks soaked in synergistic ionic silver (0.01%)–menthol liquid dressing —the wound began to stabilize. Discharge decreased noticeably within the first few days, odor resolved, and healthy granulation tissue started forming along the tunnel walls.

After 25 months of consistent treatment, the transformation was substantial.

The original 16 cm tunnel contracted to a maximum depth of 0.5 cm, and the 3 cm entrance narrowed to 0.7 cm (Figure 1). The tunnel cavity, once filled with purulence, was instead occupied by firm, healthy granulation tissue, and the wound was nearly fully resolved.

Figure 1: Chronic Osteomyelitis Wound After 25 Months of Consistent Treatment

Case 2 – Multiple Severe Combat Wounds

The patient sustained three severe combat-related wounds of the lower limb, all characterized by deep tissue loss, heavy contamination, and early biofilm formation. Two wounds involved extensive soft-tissue destruction of the leg, while the third affected the below-knee amputation (BKA) stump. Due to severe infection in the stump and two additional wounds on the hip, the patient was considered a candidate for high amputation (above-knee). This risk further emphasized the urgency of implementing an aggressive, biofilm-focused wound management strategy to preserve limb viability.

At presentation on 03 Nov 2023, all wounds contained fibrin, necrotic tissue, and exudate, and previous cleaning attempts had not prevented slough from rapidly reappearing. (Figure 2)

Figure 2: Case 2 Initial Stage

To stabilize these wounds, the full Three-Step Multimodal Biofilm-Focused Protocol was initiated.

Once treatment began, the wounds improved rapidly. Within days, contaminated surfaces became cleaner and more stable,

odor resolved, and healthy granulation tissue began spreading across the wound beds. By the second week, all wounds demonstrated uniform granulation and adequate perfusion, with no recurrence of slough or necrosis.

At the end of seven weeks, all three wounds had completely closed—one achieved primary closure, another was successfully grafted with full integration, and the amputation stump reached complete epithelialization. Healing remained stable with no signs of infection. The treatment prevented above-knee amputation and promoted faster rehabilitation (Figure 3).

Figure 3: Case 2 All Stages

Case 3 – Traumatic Hand and Forearm Wounds
This patient presented with two severe traumatic wounds involving the dorsal hand and proximal forearm, both caused by high-energy injury. The wounds exhibited deep soft-tissue loss, exposed structures, heavy contamination, and early biofilm formation.

Prior surgical debridements produced only short-term improvement; within 48 hours, fibrin and necrotic tissue consistently reappeared, indicating persistent biofilm activity and poor wound stability.

To halt this deterioration, the full Three-Step Multimodal Biofilm-Focused Protocol was initiated.

Once treatment began, tissue quality improved rapidly. Within days, the wound surfaces became cleaner and more stable, odor resolved completely, and granulation spread evenly across the wound beds. By the second week, exposed structures were covered by healthy granulation tissue, and wound edges appeared thickened, vascular, and suitable for reconstruction.

By 14 November 2023, both wounds demonstrated successful early reconstruction, with grafted and granulating areas showing strong integration, uniform color, and no recurrence of necrotic tissue. The overall appearance reflected a rapid transition from contaminated traumatic defects to stable, well-healed wounds. Healing remained steady, with no signs of infection or wound breakdown (Figure 4).

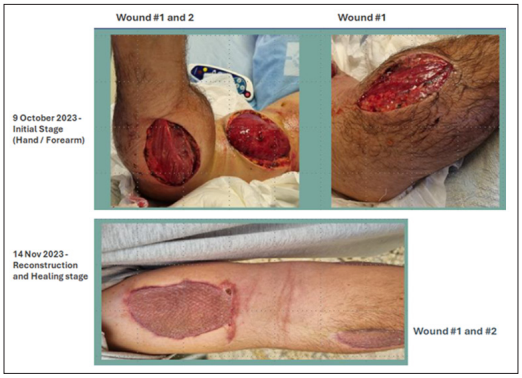

Figure 4: Case 3 All stages

Case 4 – Multiple High-Energy Traumatic Wounds: This patient presented with several traumatic open wounds across the lower limb following a high-energy blast injury. The wounds varied in depth and configuration, with irregular edges, exposed soft tissue, heavy contamination, and clear signs of early biofilm formation. Some defects extended deeply enough to expose disrupted fascia, while others demonstrated active bleeding and unstable wound beds. Despite initial surgical debridement, necrotic tissue and fibrin rapidly reappeared within 48 hours, indicating persistent microbial activity and the need for an intensified wound-bed preparation strategy (Figure 5).

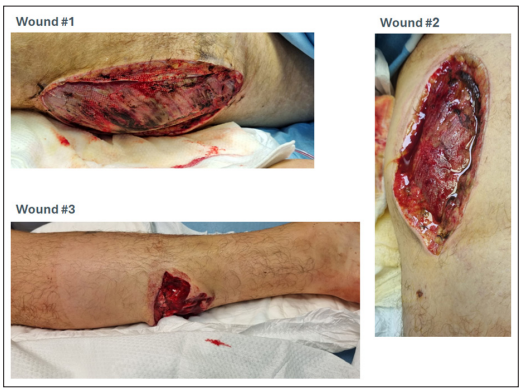

Figure 5: Case 4: Initial stage

The full Three-Step Multimodal Biofilm-Focused Protocol was initiated immediately.
Within a few days, the wounds improved remarkably: contaminated surfaces became visibly cleaner, irregular wound beds stabilized with a uniform layer of healthy granulation, odor disappeared completely, and exudate levels dropped. The rapid reformation of fibrin—which had been a major barrier to progress—was no longer observed. By the seventh day, both major wounds had transformed from unstable traumatic defects into well-perfused, granulating surfaces suitable for reconstruction.

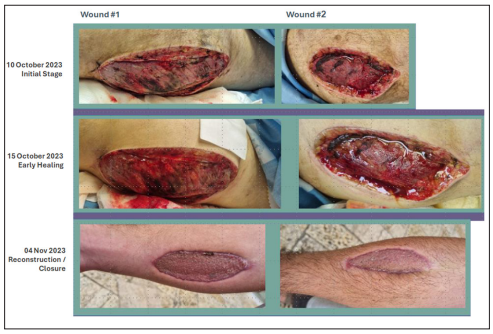

Figure 6: Case 4 All stages

Over the following weeks, granulation continued to mature, and by 04 November 2023, less than one month from the initial injury, all wounds had progressed to full closure. Reconstructed and grafted sites demonstrated excellent integration, smooth epithelialization, and no signs of infection or tissue breakdown. The scars appeared stable and well-vascularized, indicating durable healing (Figure 6).

At follow-up, the patient showed complete restoration of the wound sites with preserved limb integrity and functional mobility. No infection recurred, and despite the severity of the initial trauma, amputation was avoided. The overall course demonstrated rapid stabilization, successful reconstruction, and full healing within a remarkably short period (Figure 7).

Figure 7: Case 6 Follow-up

Case 5 — Severe Bilateral Heel Maceration with Fungal Overgrowth

The patient presented with a highly complicated bilateral heel condition characterized by extensive maceration, fungal overgrowth, and epidermal breakdown resulting from prolonged use of tight, non-ventilated military boots. The skin of both heels was markedly softened, overhydrated, and unstable, with widespread peeling, flaking, erythema, and superficial erosions. Moisture accumulation and occlusion created ideal conditions for fungal proliferation, leading to pain, irritation, and a severely impaired skin barrier. The presentation was symmetrical on both heels and consistent with advanced boot-related fungal infection and maceration.

Treatment was initiated using the full Three-Step Multimodal Biofilm-Focused Protocol. A noticeable clinical response was evident by the fourth day of treatment: maceration diminished visibly, wet and unstable surfaces became drier and more cohesive, inflammation subsided, and the patient reported reduced pain and burning. By the second week, both heels demonstrated uniform improvement, with re-establishment of the epidermal barrier and progressive reduction of fungal scaling.

After seventeen days of treatment, the skin of both heels had effectively normalized, with resolution of fungal overgrowth, restoration of barrier function, and complete stabilization without secondary infection or further deterioration. The patient regained full comfort during ambulation, and the heels appeared dry, intact, and fully recovered (Figure 8).

Figure 8: Case 6 – All stages

Discussion

The cases presented in this series demonstrate a consistent pattern: hypochlorous acid (HOCl) irrigation, PHMB combined with betaine, followed by a 24-hour application of synergistic ionic silver (0.01%)–menthol liquid dressing significantly changed the trajectory of healing. Each of the five cases—ranging from blast trauma and osteomyelitis-related sinus tracts to fungal overgrowth and maceration—showed rapid stabilization, accelerated granulation, and reliable wound closure when all three steps were combined. Each technology contributes a unique mechanism to the synergistic effect of the entire method.

HOCl provides a strong bactericidal effect through oxidation of bacterial cellular components and plays an important role in regulating wound pH. Chronic wounds are predominantly alkaline, which correlates with delayed healing. The optimal growth pH for most microorganisms isolated from wounds ranges between 5.5 and 9.0, for example Staphylococcus aureus thrives at pH 7.0–7.5, Candida at 7.0–8.0, and Klebsiella at 5.5–7.0. Pure HOCl exhibits a high oxidation potential (1150–1170 mV), requiring a low pH of

approximately 2.5, which is beneficial for wound healing because low pH inhibits pathogen growth.

HOCl solution is produced by electrolysis of pure water and salt without stabilizers or preservatives. It demonstrates proven efficacy and high therapeutic indices against Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli, in addition to a broad spectrum of other bacteria and fungi [4]. HOCL is non-toxic to granulation tissue and human cells. It can be safely used in diverse wound types, including surgical sites, peritoneal lavage, CNS exposure, and fistula drainage [14,15].

Polyhexamethylene biguanide (PHMB) combined with betaine provides dual-action benefits. PHMB is a broad-spectrum antimicrobial agent that penetrates bacterial membranes and condenses DNA, while betaine acts as a surfactant that breaks bonds and dissolves biofilm layers [3]. This solution significantly reduces bacterial load and biofilm and is indicated for critically colonized or infected wounds, burns, acute and chronic wound decontamination, surgical site infection prevention, and fungal infections [4,16]. It is effective against pathogens such as Candida, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, MRSA, VRE, and various molds. PHMB plus betaine has no reported resistance, does not suppress granulation or epithelialization, and is well tolerated with low allergenic potential. Dressing changes are painless and compatible with standard wound dressings.

The third component of the protocol, synergistic ionic silver (0.01%)–menthol liquid dressing, adds its own benefits. This multi-component, pH-neutral solution combines ionic silver, menthol, glycerol, a surfactant, and a buffered hypertonic environment. It supports moisture balance, disrupts biofilm through enhanced penetration, reduces odor and pain, and maintains antimicrobial activity while minimizing cytotoxicity to healthy tissue. The solution is suitable for wounds of various etiologies and stages [7,8,17]. Our experience also includes successful prevention of ulcer development in severe bullous erysipelas using this solution [18].

The Three-Step Multimodal Biofilm-Focused Protocol is highly versatile and can be integrated with other advanced wound technologies, including negative-pressure wound therapy, low-pressure irrigation systems, ultrasonic debridement, pulse lavage, and surgical techniques. Across all cases—acute, chronic, traumatic, fungal, and osteomyelitic—the same treatment logic produced the same outcome: rapid stabilization, predictable granulation, and fast closure. Deep traumatic wounds healed within seven weeks, osteomyelitic tunnels contracted dramatically within 25 months, and fungal maceration normalized in less than seventy-two hours. No wound showed deterioration, recurrence, or treatment resistance during therapy. Even in severe blast injuries, amputation was avoided.

These findings support the conclusion that the combined use of HOCl, PHMB plus betaine, and synergistic ionic silver (0.01%)–menthol liquid dressing represents a highly effective and biologically rational strategy for managing complex wounds. Its safety, comfort, and consistency across diverse wound types reinforce its role as an advanced multimodal approach in contemporary wound care.

Conclusion

This case series demonstrates that combining HOCL irrigation, PHMB + betain cleansing, and a synergistic ionic silver (0.01%)–menthol liquid dressing provides a highly effective approach for managing complex wounds. Each component of the Three-Step Multimodal Biofilm-Focused Protocol contributes to achieving infection control and promoting healing. Different ingredients act on a different spectrum of pathogens and together achieve synergistic effectiveness.

Across all cases - acute traumatic wounds, chronic osteomyelitic tunnel, deep soft-tissue injuries, and fungal maceration—the same protocol produced the same pattern of response: rapid cleaning, fast granulation, elimination of infection, and reliable closure. Severe blast wounds healed in about seven weeks, osteomyelitic tunnel contracted dramatically within 25 months, and severe bilateral fungal heel maceration improved markedly within the first 48–72 hours and progressed to full restoration of the skin barrier within 17 days. No deteriorations, complications, or amputations occurred.

These outcomes highlight that Three-Step Multimodal Biofilm-Focused Protocol offers a robust, safe, and reproducible solution for both acute and chronic complex wounds. Its consistent performance across diverse wound types supports its role as an advanced multimodal strategy in modern wound management.

In daily clinical practice we applied Three-Step Multimodal Biofilm-Focused Protocol on more than 10 patients with complicated wounds of different origin. We continue to investigate the effect of the Three-Step Multimodal Biofilm-Focused Protocol on various wounds of different etiologies.

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