Comparative Evaluation of Commonly Used Antimicrobial Wound Dressings and Nitric Oxide Treatment of Infected and Non-Infected Full-Thickness Wounds on Swine (*Sus scrofa*)

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OBJECTIVE

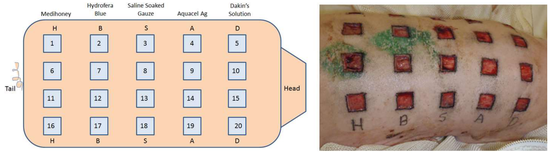
Develop an evidenced-based uninfected and CA-MRSA exposed full-thickness wound model on swine (*Sus scrofa*). Inform the medical community of best practices for wound healing, comparing the non-antibiotic antimicrobial dressings Aquacel Ag, Hydrofera Blue, Medihoney Calcium Alginate, wet-to-dry Dakin's hypochlorite solution-soaked gauze, and saline-soaked gauze (control). With best practices identified in the established models, evaluate topical nitric-oxide delivering advanced development candidate technologies.

ABSTRACT

Skin and soft tissue infections (SSTI) represent nearly $25 billion in U.S. annual medical costs. Drug-resistant bacteria pose a dire threat to public health, and the alarming dearth of new antibiotics compels the characterization of novel, pro-healing, broad-spectrum antimicrobials. To better inform wound care providers, we developed evidenced-based uninfected and commonly acquired MRSA-exposed full-thickness wound models on swine to test wound healing and post-exposure prophylactic antimicrobial efficacy of Commercial-off-the-Shelf (COTS) antimicrobial dressings. With best practices identified, we comparatively challenged the performance of topical PhoGel48 containing Nitricil™: a nitric-oxide (NO) delivering advanced development technology developed under US Army SBIR contract W81XWH-11-C-0029. We found that the NO-delivering gel formulations provided improved wound healing and antimicrobial activity versus the FDA approved treatments tested, and potentially meet a critical need for improved SSTI therapies. Of all test groups, only the NO-releasing drug groups were capable of providing improved antimicrobial activity versus wet-to-dry gauze controls, and were not contraindicated for wound healing. The linear wound healing measurements of PhoGel48 (0.3% NO) was statistically superior to saline control ($p < 0.05$).

METHODS

Post-Exposure Prophylaxis Full-Thickness Wound Healing Model



Evidenced-Based Wound Healing Evaluation of Antimicrobial Treatments

COTS Comparison
1. Aquacel Ag (1.2% Ionic Silver)
2. Hydrofera Blue (Foam with Anti-Infective Dyes)
3. Medihoney Calcium Alginate (Manuka Honey)
4. Dakin's Solution (0.125% Sodium Hypochlorite)
5. Saline-Soaked Gauze (Control)

Uninfected Full-Thickness Wounds | **MRSA-Exposed Full-Thickness Wounds**

Identify Best Healing Antimicrobial Treatment (Best Practices)

Evaluate PhoGel48 Containing Nitric Oxide (NO) Releasing Nitricil™
1. PhoGel48 (0.3% NO)
2. PhoGel48 (1.8% NO)
3. Best Practice 1
4. Best Practice 2
5. Saline-Soaked Gauze (Control)

Uninfected Full-Thickness Wounds | **MRSA-Exposed Full-Thickness Wounds**

Day Zero: Wound and Exposure
1. Twenty 2x2cm Full thickness wounds excised from the back of swine *(Sus scrofa)*
2. Wounds inoculated with 10^8 CFU in 250 µL, or Sterile Saline
3. Tegaderm-Covered for 24hrs

Dressing Changes
1. COTS Phase: *Aquacel Ag, Hydrofera Blue and Medihoney changed every three days. Dakin's and Saline wet-to-dry gauze changed twice daily.*
2. *PhoGel48 Phase: Medihoney changed every three days, all others changed once daily.*

Evaluation Metrics
1. Linear Wound Healing Measurements (Day 10)
2. % Re-epithelialization (Histology) (Day 10)
3. Semi-Quantitative Microbiology (Days 1,4,7,10)
4. Bacterial Identity Verified at Random Using Vitek® 2 Bacterial Identification

Study Strength
COTS Comparison Uninfected n = 4
COTS Comparison MRSA Exposed n = 4
PhoGel48 Evaluation Uninfected n = 6
PhoGel48 Evaluation MRSA Exposed n = 4

Figure 1. Wound Model and Treatment Evaluation Methods

RESULTS

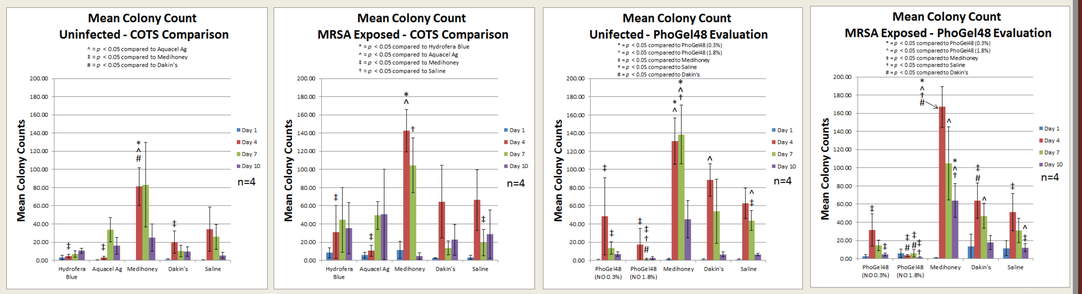
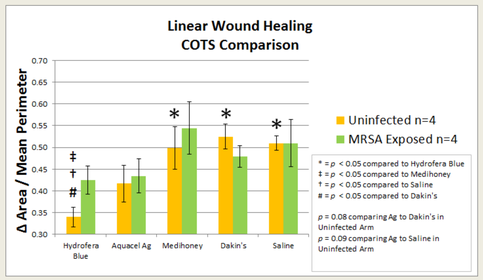


Figure 2. Semi-quantitative microbiology for uninfected and MRSA exposed COTS comparisons, and uninfected and MRSA exposed PhoGel48 evaluations (left to right), swabbed on days 1, 4, 7 and 10 . Wound fluid swabs were placed in 10mL PBS. One microliter of vortexed solution was plated directed onto sheep-blood agar and incubated at 37°C for 24 hours. Colonies were characterized by phenotype and counted. Plate containing >200 colonies were labeled too-many-to-count (TMTC) and assigned a maximum value of 200.



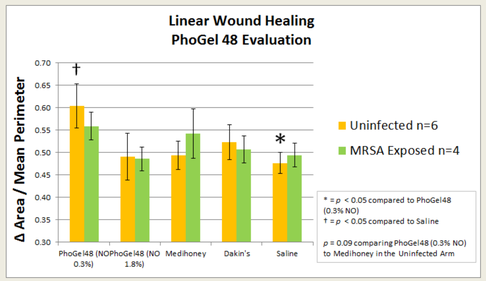


Figure 3. Linear Wound Healing calculated using the Gilman's Equation for COTS Comparison (left) and PhoGel48 Evaluation (right).

Figure 4. Representative wounds on day 10 (photograph, left). Uninfected PhoGel48 animal displayed. Tattooed areas are the original wound area (minus any contraction). Red areas considered final wound areas. Treatment groups: H = Medihoney, S = Saline, D = Dakin's, N_b = 1.8% PhoGel48, N_a = 0.3% PhoGel48.

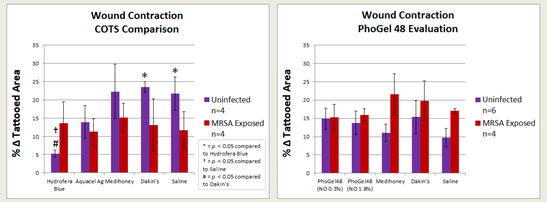


Figure 5. Wound contraction calculated by the change in tattooed wound area for COTS Comparison (left) and PhoGel48 Evaluation (right).

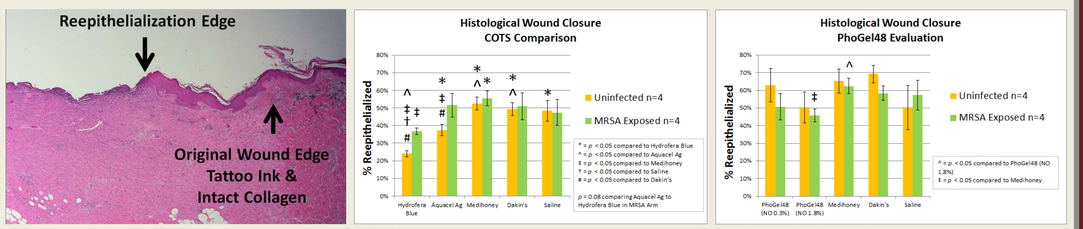


Figure 6. Percent re-epithelialization measurement (left), by treatment in COTS comparison (center), and PhoGel48 evaluation (right).

POST-HOC ANALYSES

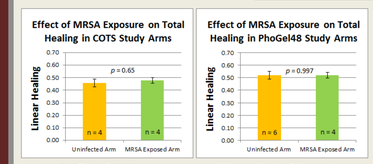


Figure 7. Effect of MRSA exposure on overall linear healing (all wound measurements averaged across all animals in the arm). Left: COTS comparison phase. Right: PhoGel48 evaluation phase. MRSA exposure did not significantly alter healing rates the either study.

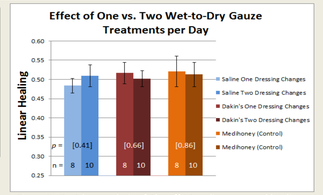
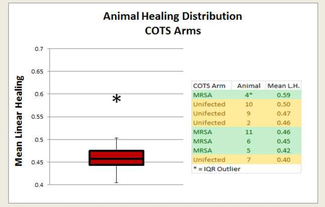


Figure 8. Post-hoc analysis of the effect on linear wound healing of one versus two wet-to-dry gauze changes per day. Sample groups are not statistically distinguishable.



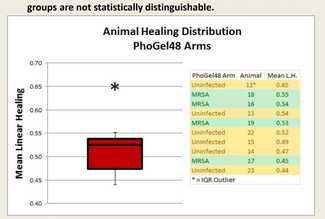


Figure 9. Uninfected and MRSA infected overall animal healing scores pooled. Left: COTS comparison phase. Right: PhoGel48 evaluation phase. In both experiments, an outlier corresponded to an animal that bled excessively during surgery and the first days of dressing changes.

DISCUSSION

The rise of hospital-acquired SSTIs involving Multi-Drug Resistant Organisms (MDRO) and the alarming shortage of new antimicrobial treatments compel the identification of best wound care practices, and the discovery of novel non-antibiotic therapeutic approaches. Our data suggest that COTS product choice should include consideration for infection risk mitigation, and the efficiency of wound healing. The COTS tested here versus wet-to-dry Saline- or Dakin's-soaked gauze are either contraindicated for wound healing, or are not effective microbial proliferation suppression agents. Our post-hoc data analysis suggests that while painful, multiple wet-to-dry dressing changes per day may aid in removal of surface microbes; the practice does not accelerate wound healing. Interestingly, we observed that the small population of heavily bleeding, highly vascular animals were statistically far superior wound healers versus the remaining subject population.

We find significant promise in the exploration of broad-spectrum anti-microbial treatments based on topical nitric oxide, which also provide accelerated pro-healing benefits. The PhoGel48 products developed under Army SBIR and tested in this work should be challenged with an expanded panel of single- and poly-MDRO exposures for *in-vivo* antimicrobial efficacy and wound healing performance.

CONCLUSIONS

- **Aquacel Ag and Hydrofera Blue antimicrobial dressings are contraindicated for wound healing versus control.**
- **Of COTS compared to Saline, only Dakin's solution-soaked wet-to-dry gauze provided both comparable levels of wound healing and suppression of microbial proliferation.**
- **There is no wound-healing benefit to multiple wet-to-dry dressing changes in a single day.**
- **Both PhoGel48 0.3% and 1.8% nitric-oxide delivering formulations appear to have enhanced post-MRSA exposure prophylaxis properties, as well as demonstrating microbial proliferation suppression in the uninfected model.**
- **The PhoGel48 0.3% nitric-oxide delivering compound was the only treatment to demonstrate improved linear wound healing versus Saline-soaked wet-to-dry gauze.**
- **Highly vascular subjects demonstrated significantly accelerated wound healing in these studies.**

We would like to thank the Tripler Army Medical wound-care nursing staff, the Department of Surgery, and the DCI Animal Research Section for their significant contributions to the success of this research effort. This work was supported by the TATRC - AMMED Advance Medical Technology Initiative (AAMTI) and by U.S. Army Medical Command core funding provided to the TAMC Department of Clinical Investigation. The NO delivering products tested in this project were developed by Novan Inc. with the support of an Army 10.3 SBIR Phase II award (W81XWH-11-C-0029, COR MAJ Robert Brodnick). The study protocol was approved by the Institutional Animal Care and Use Committee at Tripler Army Medical Center. Investigators complied with the policies as prescribed in the USDA Animal Welfare Act and the National Research Council's 'Guide for the Care and Use of Laboratory Animals. Facilities are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). The views expressed in this publication/presentation are those of the author(s) and do not reflect the official policy or position of the Department of the Army, Department of Defense, or the US Government.