UNIVERSITY OF MIAMI
MILLER SCHOOL
of MEDICINE

Efficacy of Nitric Oxide-Releasing Dressings to Reduce Bioburden and Enhance Healing in a Second Degree Porcine Wound Model

Stephen C. Davis[1], Ivan Jozic[1], Joel Gil[1], Michael Solis[1], Ryan Strong[1], Roger Cassagnol[1], Beatriz Abdo Abujamra[1], Pravalika Irukulla[2], Elizabeth Brisbois[2,3], Hitesh Handa2[,3] and Ronald J. Shebuski[2]

[1]Dr. Phillip Frost Department of Dermatology and Cutaneous Surgery, Miller School of Medicine, University of Miami, Miami, Florida, [2]Nytricx, Inc. Athens, GA, [3]College of Engineering, University of Georgia, Athens, GA

UNIVERSITY OF MIAMI
MILLER SCHOOL
of MEDICINE

Abstract

Background:
Burn wound healing is slower when compared to other mechanisms of injury and made challenging by frequent complications like infections which further delay healing.[1-3] Nitric Oxide (NO) has been demonstrated to have antimicrobial efficacy and to accelerate the wound healing process through multiple mechanisms.[4] The objective of this study was to evaluate NO-releasing dressings against wound infections by common pathogens, using a porcine model due to morphological and immunological similarities to human skin.[5,6]

Methods:
Thirty second degree burn wounds measuring 27 mm in diameter were created on each of six animals. The wounds were immediately inoculated with either methicillin-resistant Staphylococcus aureus (MRSA USA300) or Pseudomonas aeruginosa (PA09-010)[7] and then treated with either (A) 10 wt.% SNAP, (B) TPS10, (C) PDMS Vehicle Control, (D) Silver Sulfadiazine Positive Control (SSD), or (E) Untreated Control and then covered with polyurethane film dressing. Treatments were replaced every three days and wounds were recovered for microbiological and histology analysis on days 6, 9, or 21.

Results:
The NO-releasing dressings 10 wt.% SNAP and TPS10 produced significant reductions ($p \leq 0.05$) in MRSA bioburden at all assessment times compared to the PDMS Vehicle Control, SSD, and Untreated Control groups. Against PA09-010, the TPS10 and SSD groups had PA09-010 counts significantly lower ($p \leq 0.05$) than PDMS Vehicle Control and Untreated Control at all assessment times. Against both pathogens granulation tissue formation and epithelial thickness were significantly ($p \leq 0.05$) greater in TPS10 treated wounds on day 21 compared to SSD and Untreated Control, respectively.

Conclusion:
The NO-releasing dressings were effective in decreasing the bioburden of both MRSA and P. aeruginosa in burn wound infections. The broad-spectrum antimicrobial efficacy, in addition to enhanced wound healing, observed in this study makes NO therapies a promising therapeutic for the management of burn wounds. Further investigation should be conducted against additional wound pathogens and evaluate effects on healing in non-infected wounds.

Introduction

Thermal injuries have a high incidence of complications including infection or kidney injury.[1] These infections, often due to increased burn-related susceptibility, further impede wound healing that is already reduced compared to other mechanisms of injury.[2,8] Recent works have demonstrated that Nitric Oxide (NO), an endogenous gasotransmitter, has broad spectrum antimicrobial efficacy and enhances wound healing. Emerging delivery modalities, such as SNAP, have enabled NO application to wounds for these therapeutic effects.[9] This study investigated the efficacy of SNAP dressings against MRSA and *P. Aeruginosa* burn wound infections.

References

1. Foppiani JA, Weidman A, Hernandez Alvarez A, et al. A meta-analysis of the mortality and the prevalence of burn complications in Western populations. J Burn Care Res. 2024;45(4):932-944. doi:10.1093/jbcr/irae064
2. Jabeen S, Clough ECS, Thomlinson AM, Chadwick SL, Ferguson MWJ, Shah M. Partial thickness wound: Does mechanism of injury influence healing? Burns. 2019;45(3):531-542. doi:10.1016/j.burns.2018.08.010
3. Uberoi A, McCready-Vangi A, Grice EA. The wound microbiota: microbial mechanisms of impaired wound healing and infection. Nat Rev Microbiol. 2024;22(8):507-521. doi:10.1038/s41579-024-01035-z
4. Bahadoran Z, Mirmiran P, Hosseinpanah F, Kashfi K, Ghasemi A. Nitric oxide-based treatments improve wound healing associated with diabetes mellitus. Med Gas Res. 2025;15(1):23-35. doi:10.4103/mgr.MEDGASRES-D-24-00020
5. Sullivan TP, Eaglstein WH, Davis SC, Mertz P. The pig as a model for human wound healing. Wound Repair Regen. 2001;9(2):66-76. doi:10.1046/j.1524-475x.2001.00066.x
6. Dawson HD, Loveland JE, Pascal G, et al. Structural and functional annotation of the porcine immunome. BMC Genomics. 2013;14:332. doi:10.1186/1471-2164-14-332
7. Pastar I, Nusbaum AG, Gil J, et al. Interactions of methicillin resistant Staphylococcus aureus USA300 and Pseudomonas aeruginosa in polymicrobial wound infection. PLoS One. 2013;8(2):e56846. doi:10.1371/journal.pone.0056846
8. Benitez JP, Zuluaga M, Trochez JP, et al. Infecções em feridas em queimados: Revisão de 35 anos de avanços, desafios diagnósticos e estratégias baseadas em evidências. Rev Bras Cir Plást. 2025;40(CP). doi:10.1055/s-0045-1809394
9. Hopkins SP, Pant J, Goudie MJ, Schmiedt C, Handa H. Achieving long-term biocompatible silicone via covalently immobilized S-nitroso- N-acetylpenicillamine (SNAP) that exhibits 4 months of sustained nitric oxide release. ACS Appl Mater Interfaces. 2018;10(32):27316-27325. doi:10.1021/acsami.8b08647

Materials and Methods

1. Experimental Animals:

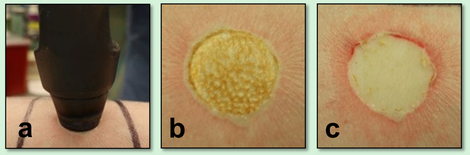
- Six specific-pathogen-free (SPF) female swine were used in this study due to the anatomical similarities between human and porcine skin.[5]

2. Wounding Technique:

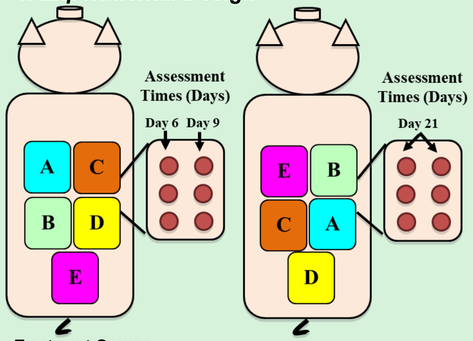
- A branding iron set to 200° C was held for 6 seconds vertically over the skin with pressure from gravity alone (a) to create thirty (30) second degree burn wounds per animal measuring 27 mm in diameter (b). Blisters were removed with a sterile Teflon spatula before inoculation (c).

2. Bacterial Inoculation:

- Inoculum suspensions containing 10^6 CFU/mL of either MRSA USA 300 or PA09-010 were used .
- 25 µL of the inoculum suspension was deposited individually into the center of the wounds via pipette and spread with a sterile Teflon spatula.



4. Experimental Design

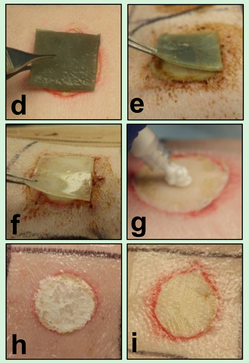


Treatment Groups

A. 10 wt. % SNAP
B. TPS10
C. PDMS Vehicle Control
D. Silver Sulfadiazine Positive Control
E. Untreated Control

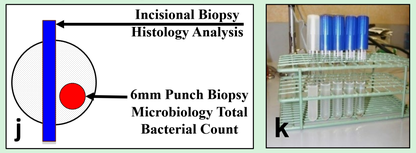
5. Treatment Regimen

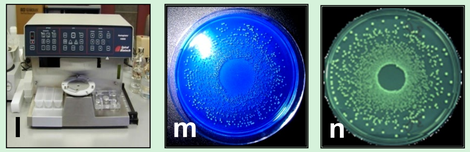
- All treatments were applied immediately following wound inoculation.
- 10 wt. % SNAP (d), TPS10 (e), and PDMS Vehicle Control (f) were applied and covered with polyurethane film dressings .
- 200 mg of Silver Sulfadiazine was applied (g) and spread with a sterile spatula (h).
- All wounds were covered individually with polyurethane film dressing (i).
- Every 3 days treatment was reapplied.



6. Microbiology Assessment

- Six wounds were assessed per treatment per assessment time for each organism. 6 mm punch biopsies (j) were collected and homogenized in Tenbroeck homogenization tubes.
- Serial dilutions (k) were plated using a Spiral Plater System (l) onto selective media: Oxacillin Resistant Screening Agar Base (ORSAB) plates (m) for MRSA USA300, PA Agar (n) plates for PA09-010.
- After 24 hours growth, colonies were counted and the quantity of colony forming units per g (CFU/g), were calculated.





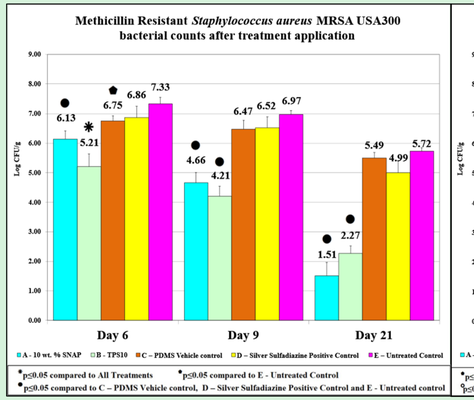
7. Histology Assessment

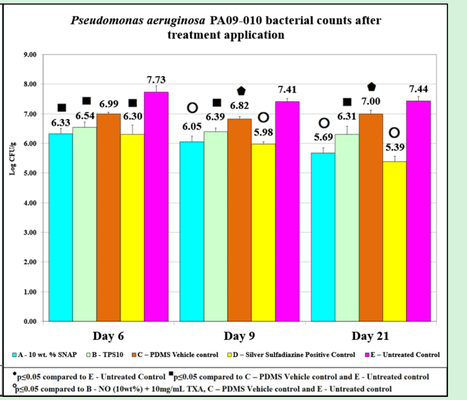
- Incisional biopsies (j) were collected from the center of the wound and stained with Hematoxylin and Eosin (H&E).
- The following parameters were assessed for all histological samples:
- Percentage of re-epithelization (percent of wounded area covered by newly formed epidermis).
- Epithelial thickness (averaged thickness of newly formed epithelium).
- White cell infiltration (leukocytic infiltrates to assess inflammation).
- Granulation tissue (percent of wound bed with granulation tissue).

Acknowledgements
This study was supported by Nytricx Inc,

Contact Information
Stephen C. Davis. Research Professor
University of Miami, Miller School of Medicine Dr. Phillip Frost Dept. of Dermatology & Cutaneous Surgery
sdavis@med.miami.edu Ph: 305.243.4897

Results





MRSA USA300 Results

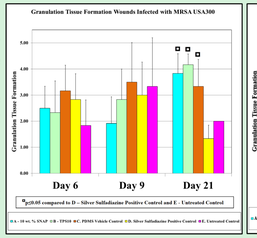
- Compared to Untreated Control, the 10 wt.% SNAP, TPS10 and PDMS Vehicle Control groups had significantly lower ($p \leq 0.05$) MRSA counts on day 6.
- The 10 wt.% SNAP and TPS10 treatment groups had MRSA bioburden reductions of over 99.5% on day 6 when compared to Untreated Control, PDMS Vehicle Control and Silver Sulfadiazine Positive Control.
- Silver Sulfadiazine, the Positive Control, did not significantly reduce MRSA burden compared to Untreated or Vehicle Control groups at all assessment times.
- 10 wt.% SNAP and TPS10 treated wounds had greater than 99.9% reductions in MRSA counts compared to Untreated Control wounds on day 21.

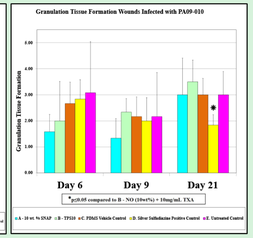
PA09-010 Results

- On day 6, 10 wt.% SNAP, TPS10, and Silver Sulfadiazine Positive Control significantly ($p \leq 0.05$) reduced PA09-010 compared to both PDMS Vehicle Control and Untreated Control.
- The 10 wt.% SNAP and Silver Sulfadiazine Positive Control treatments produced further significant reductions on days 9 and 21 compared to TPS10.
- On day 21, Silver Sulfadiazine Positive Control achieved a greater than 98.2% reduction in PA09-010 compared to Untreated Control.
- 10 wt.% SNAP and Silver Sulfadiazine Positive Control treatment groups had PA09-010 counts on day 21 significantly lower than those observed on day 6 and 9.

Histology Results

- Granulation tissue for TPS10 was significantly increased on day 21 compared to Silver Sulfadiazine Positive Control
- On day 21, Silver Sulfadiazine treated wounds had the highest re-epithelization against both organisms but was not significant compared to 10 wt.% SNAP.
- Epithelial Thickness, Granulation Tissue Formation, and White Cell Infiltration were not significantly different on days 6 and 9 between all treatment groups against both organisms.





Conclusions

- This study demonstrates that NO-releasing SNAP dressings have considerable therapeutic potential in the management of burn wound infections due to the observed antimicrobial efficacy against MRSA and *P. aeruginosa*.
- Wound healing following SNAP application was comparable to or superior to Silver Sulfadiazine treated wounds and SNAP did not appear to impede wound healing.
- Future work involving additional animals and assessment times should be conducted to substantiate these findings.