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# Anti-microbial Efficacy of Silicone-Based Nitric Oxide-Releasing Wound Dressings in a Dermal Porcine Wound Model


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## *Abstract:*

**Introduction:** Nitric oxide (NO) is a short-lived, diatomic, lipophilic gas with antimicrobial activity. Several in-vitro and in-vivo studies have demonstrated the efficacy of NO in microbial reduction. Recently, NO and its derivatives have been shown to exhibit broad-spectrum antimicrobial activity against bacteria, viruses, and parasites. The objective of this study was to assess the efficacy of various topical PDMS (silicone)-based nitric oxide formulations on microbial reduction and healing using a porcine wound model. A NO donor (SNAP; S-nitroso-N-acetylpenicillamine) was utilized to continually release NO gas from the PDMS film to the wound surface.

**Methods:** Twenty-seven (27) deep reticular dermal wounds (22mm x 22mm x 3mm deep) were made with a specialized electrokeratome in 4 animals. Methicillin Resistant Staphylococcus aureus USA300 (MRSA USA300) or Pseudomonas aeruginosa PA 090-010 (PA: military isolation) were inoculated in 2 animals per organism. After 24 hours, the dressings were removed, and three wounds were recovered for baseline enumeration. Wounds were randomly assigned to one of the following treatment groups: A- NO donor loaded at 10 wt%, B- PDMS Vehicle Control, C- Silver Sulfadiazine Positive Control and D- Untreated Control. Wounds were recovered on days 7 and 12 and assessed for bacterial burden and wound healing.

**Results:** Significant reductions ($p \leq 0.05$) were observed in wounds treated with NO donor 10 wt% infected either with MRSA USA300 or PA09-010, compared to baseline and untreated control. Wounds treated with the NO donor 10 wt% demonstrated a 99.8% reduction of MRSA compared with untreated wounds. In wounds infected with PA09-010 reductions exhibited values of 93.7% compared to untreated control. In wounds infected with MRSA USA300 and treated with NO donor 10 wt%, significant reductions ($p \leq 0.05$) were also observed against PDMS Vehicle Control and Silver Sulfadiazine. No detrimental effects on wound healing were observed by NO donor loaded PDMS films at 10 wt%.

**Discussion:** NO rapidly decreased wound bacterial count significantly, suggesting an important advance in the treatment of infected wounds. These studies may have important clinical implications in eradication of bacteria in open wounds with topically applied nitric oxide (NO)-releasing wound dressings.


## *Introduction:*

Common infections found in acute and chronic wounds are *Staphylococcus aureus* or *Pseudomonas aeruginosa*, which require prompt medical intervention due to their propensity to produce biofilms and resistance to antimicrobial therapy.[1] Nitric Oxide has tremendous potential as a novel antimicrobial therapeutic due to its broad-spectrum activity against multi-drug resistant bacteria, ability to diffuse rapidly through cell membranes, and the belief that NO uses a mechanism of action so basic to the biochemistry of bacteria that may be unlikely for NO-resistant strains to develop.[2] S-nitroso-N-acetylpenicillamine (SNAP) has excellent solubility in organic solvents and is widely explored to develop NO-releasing materials used as antimicrobial coatings and devices.[3] Antimicrobial efficacy of Nitric Oxide against common pathogens in wounds, inoculated with *S. aureus* and *P. aeruginosa* have been demonstrated from other researchers.[4,5] This study examined the in vivo efficacy of a silicone based NO formulation dressing on Methicillin Resistant *Staphylococcus aureus* USA300 (MRSA USA300) and *Pseudomonas aeruginosa* (PA 09-010) infected wounds, and healing capabilities on a porcine model.

## *References*


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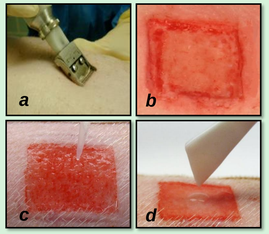
## Materials and Methods:

### *1. Experimental Animals:*

Four (4) swine were used as our experimental animal due to the morphological, physiological, and biochemical similarities between porcine skin and human skin.[6]

### *2. Wounding Technique:*

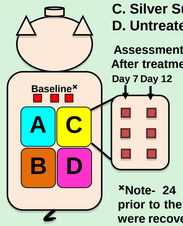
- An electrokeratome (photo a) was used to create 27 deep reticular dermal wounds measuring 12 mm x 12 mm x 3 mm deep (photo b) on each animal.




### *3. Inoculation:*

- A fresh culture of Methicillin Resistant *Staphylococcus aureus* USA300 (MRSA USA300) or *Pseudomonas aeruginosa* PA 090-010 (PA: military isolate previously obtained from Lee Cancio, MD, USAISR) were used for these studies.
- After wound creation, 25 µL of the inoculum suspension (photo c) was used to inoculate each wound by scrubbing the mixed inoculum by 30 seconds into each wound with a teflon spatula (photo d). Wounds were covered with a polyurethane film dressing to allow for biofilm formation for 24 hours.[7,8]

### *4. Experimental Design:*




**Treatments Groups**
**A. NO donor loaded at 10 wt%***
**B. PDMS Vehicle Control***
**C. Silver Sulfadiazine Positive Control***
**D. Untreated Control***

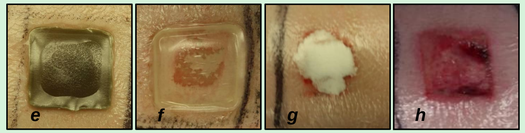
**Assessment Times (Days)**
**After treatment application**
**Day 7 Day 12**

**After treatment application all wounds were covered with polyurethane film dressings (Tegaderm, 3M, St. Paul MN)**

***Note- 24 hours biofilms was established prior to the first treatment. Baseline wounds were recovered after 24 hours.**

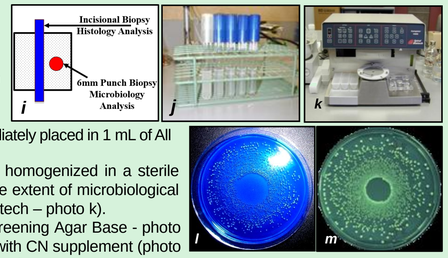
### *5. Treatment Application:*

- Test formulations were applied over the surface of the infected wounds after biofilm formation. (photo e - NO donor loaded at 10 wt%, photo f – PDMS Vehicle Control, photo g - Silver Sulfadiazine and photo h - Untreated Control)
- After treatment application treated wounds were covered with a polyurethane film dressing (Tegaderm, 3M).




### *6. Microbiology Recovery:*

- Baseline wounds were recovered 24 hours after inoculation prior to treatment (6mm punch biopsy – photo i).
- On day 7 and 12 after treatment application, three (3) wounds per treatment were recovered. Biopsies (6mm) were weighed and immediately placed in 1 mL of All Purpose Neutralizing Solution.
- Samples were combined with 4 mL of Neutralizing Solution and homogenized in a sterile homogenization tube. Serial dilutions (photo j) were made and the extent of microbiological contamination assessed using the Spiral Plater System (Spiral Biotech – photo k).
- MRSA USA300 was isolated on ORSAB (Oxacillin Resistance Screening Agar Base - photo l) and *P. aeruginosa* strains were isolated on Pseudomonas Agar with CN supplement (photo m). Plates were incubated at 37±2°C for 24-48 hours. The colony forming units per gram (CFU/g) were calculated and comparison of the means was analyzed.

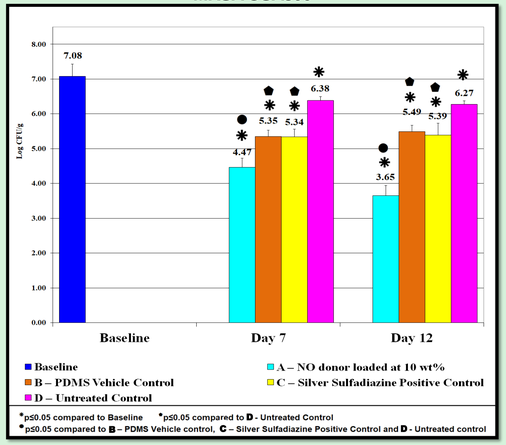



### *7. Histology Assessment:*

- On each assessment day, incisional biopsies were taken from the center of each wound (photo i). Each biopsy was placed in formalin and then stained with hematoxylin and eosin (H&E).
- The specimens were evaluated blinded and examined for the following elements:
  **Percent of wound epithelialized (%):** Measurement of the length of the wound surface that has been covered with epithelium.
  **Epithelial thickness (cell layers µm):** The thickness of the epithelium in µm will be measured on five equal distance points from each other in the biopsy and averaged.
  **White cell infiltrate (scored):** Measured by the presence and amount of subepithelial mixed leukocytic infiltrates. Mean Score: 1 = absent, 2 = mild, 3 = moderate, 4 = marked, 5 = exuberant.
  **Granulation tissue formation (approximate %):** The approximate amount of new granulation tissue formation (dermis) will be graded as follows: 0 = 0, 0.5 = 1-10%, 1 = 11-30%, 2 = 31-50%, 3 = 51-70%, 4 = 71-90%, 5= 91-100%

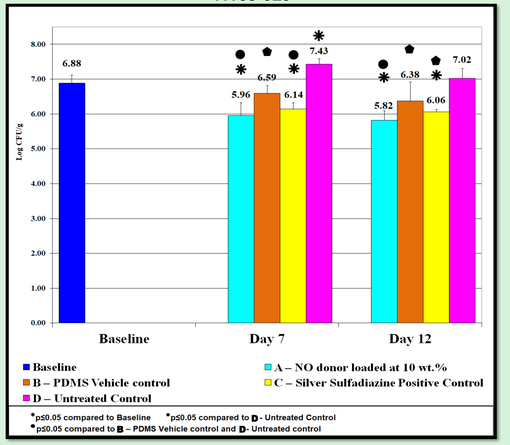
## Results:

**MRSA USA300**




- 24 hours after inoculation, the baseline for MRSA USA300 was 7.08±0.34 Log CFU/g.
- On Day 7, all treatment groups exhibited significant reduction when compared to Baseline, with highest 99.79% being from NO donor loaded at 10 wt%.
- PDMS Vehicle Control, Silver Sulfadiazine and Untreated Control showed a bacterial reductions of 98.15%, 98.21% and 80.02%, respectively compared to Baseline.
- Compared to Untreated Control, NO donor loaded at 10% wt showed a reduction of 98.78%, while PDMS Vehicle Control and Silver Sulfadiazine showed 90.76% and 91.03% reduction of MRSA levels, respectively.
- On Day 12, NO 10 wt% exhibited 99.96% reduction (3.43±0.05 Log CFU/g) when compared to Baseline and 99.76% reduction (2.62±0.19 Log CFU/g) when compared to Untreated Control.

**PA 09-010**




- 24 hours after inoculation, the baseline for PA 09-010 was 6.88±0.24 Log CFU/g.
- Both NO donor loaded at 10 wt% and Silver Sulfadiazine on Day 7 exhibited significant reduction of 88.10% and 81.81% when compared to Baseline wounds, respectively.
- Higher reduction was observed with NO donor loaded at 10 wt% and Silver Sulfadiazine were compared to Untreated Control (more than 94.89%), or 85.57% when compared to PDMS Vehicle Control.
- On Day 12, NO 10 wt% had a bacterial reduction of 1.07±0.04 and 1.20±0.00 Log CFU/g (91.42% and 93.74%) compared to Baseline and Untreated Control wounds, respectively.
- Silver Sulfadiazine had exceptional reductions of 84.99% and 89.05 when compared to Baseline and Untreated Control wounds, respectively.

### *Histology Results:*

- For white cell infiltration, NO donor loaded at 10 wt% was the best on Day 7 for both MRSA USA300 and PA 09-010 infected wounds.
- Overall, all treatment groups increased epithelization from Day 7 to Day 12 for MRSA USA300 and PA09-010 infected animals.

## *Conclusions*

- Overall, NO donor loaded at 10 wt% treated wounds perform better than all other treatment groups against both organisms.
- NO 10 wt% was more effective on eradicating MRSA USA300 than the *Pseudomonas aeruginosa* PA 09-010 infected wounds.
- Better efficacy in bacterial reduction was observed from NO donor loaded at 10 wt% when compared to positive controls in wounds infected with MRSA USA300 and PA 09-010.
- These studies may have important clinical implications in rapid eradication of bacteria residing in open wounds with topically applied, anti-microbial, nitric oxide-releasing wound dressings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