# The Antimicrobial and Wound Healing Effects of an Occlusive Cold Plasma Foil Using a Deep Dermal Wound Porcine Model


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## Abstract


**Introduction:**

Methicillin resistant Staphylococcus aureus (MRSA) is associated with chronic wounds and remains a prevalent pathogen with emerging resistances.[1] Cold atmospheric plasma therapy has been demonstrated to improve wound healing in early clinical evaluation and other works have reported antimicrobial activity.[2,3] The efficacy of cold plasma therapy was investigated in this study against MRSA wound infections. A porcine model was used because of the similarities to human skin.[4]

**Methods:**

Deep dermal wounds (22mm x 22mm x 3mm) were created on the paravertebral and thoracic areas of two animals. Wounds were inoculated with $10^6$ CFU/mL of MRSA USA300 and covered to allow for biofilm formation before treatment application. In the first animal, cold plasma therapy was compared to sham dressings and in the second animal, treatment regimen duration and frequency were evaluated. Microbiological baseline was recovered from designated wounds prior to treatment application. Remaining wounds were recovered once for histology and microbiology analysis at assessment times: Days 4, 8 and 11 or days 4 and 11 for the first and second animals, respectively.

**Results:**

The bioburden of MRSA USA300 in wounds was reduced significantly ($p \leq 0.05$) by cold plasma therapy compared to sham dressings at all assessment times. The reductions ranged from 97.8% to 99.1% on day 4 to day 11. The results of the treatment regimens for the second animal demonstrate that cold plasma therapy reduces MRSA in a dose-dependent manner with greater reductions observed for longer treatment durations and more frequent treatment applications. Treatment application for 4 minutes, 5x/week reduced MRSA counts by 99.87% compared to untreated wounds. No detrimental effects on healing were observed in the histological analysis of cold plasma treated wounds.

**Discussion:**

The dose-dependent manner of microbial reduction without impeding healing makes this a promising approach to the management of MRSA infections. Further investigation should be conducted against additional pathogens and full thickness or burn injuries to assess clinical potential and validate this study's findings.


## Introduction

Wound infections delay healing and biofilms make management of these infections challenging by increasing antibiotic resistance.[5] Cold or nonthermal plasma involves ionization of atmospheric air to generate plasma and has emerged as a novel therapy with promising clinical improvements in chronic wound healing.[6] This study evaluated the antimicrobial and wound healing potential of cold plasma therapy dressings using an established porcine infection model.

## References


1. Almuhayawi MS, Alruhaili MH, Gattan HS, et al. Staphylococcus aureus induced wound infections which antimicrobial resistance, methicillin- and vancomycin-resistant: Assessment of emergence and cross sectional study. Infect Drug Resist. 2023;16:5335-5346. doi:10.2147/IDR.S418681
2. Abu Rached N, Kley S, Storck M, Meyer T, Stücker M. Cold plasma therapy in chronic wounds-A multicenter, randomized controlled clinical trial (plasma on chronic wounds for epidermal regeneration study): Preliminary results. J Clin Med. 2023;12(15):5121. doi:10.3390/jcm12155121
3. Niedźwiedź I, Waśko A, Pawlat J, Polak-Berecka M. The state of research on antimicrobial activity of cold plasma. Pol J Microbiol. 2019;68(2):153-164. doi:10.33073/pjm-2019-028
4. 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
5. Metcalf DG, Bowler PG. Biofilm delays wound healing: A review of the evidence. Burns Trauma. 2013;1(1):5-12. doi:10.4103/2321-3868.113329
6. Abu Rached N, Kley S, Storck M, Meyer T, Stücker M. Cold plasma therapy in chronic wounds-A multicenter, randomized controlled clinical trial (plasma on chronic wounds for epidermal regeneration study): Preliminary results. J Clin Med. 2023;12(15):5121. doi:10.3390/jcm12155121
7. Davis SC, Ricotti C, Cazzaniga AL, Welch E, and Mertz PM. Microscopic and Physiological Evidence for Biofilm-Associated Wound Colonization In-vivo Wound Repair Regen 2008, 16(1):23-9.


## Materials and Methods

### 1. Experimental Animals:

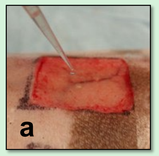
- Two female specific-pathogen-free swine.

### 2. Wounding Technique:

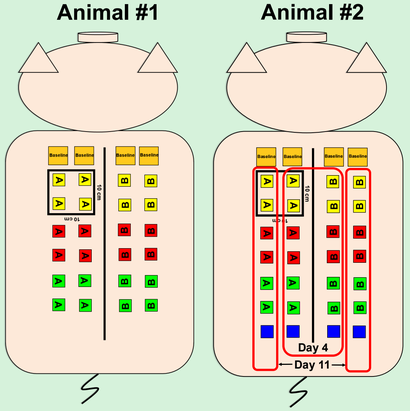
- Deep dermal wounds (22mm x 22mm x 3mm) (a) were created with an electrokeratome. 28 and 32 wounds were created for animals #1 and 2, respectively.

### 3. Inoculation:

- Wounds were each inoculated with 25 µL of a $10^6$ CFU/mL suspension of MRSA USA 300. (a) Wounds were covered with polyurethane film dressing for 24 (animal #1) or 72 hours (animal #2) to allow for biofilm formation before treatment application.[7]




### 4. Experimental Design




**Treatment Groups**
**A: Plasma Dressing**
**B: Sham Dressing**

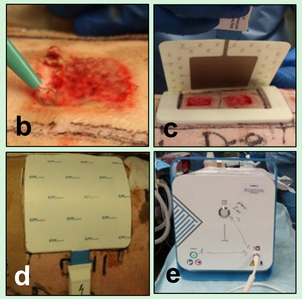
**Assessment Days**
- Day 4
- Day 8
- Day 11

**Treatment Groups**
**A: 2 minutes plasma application**
**B: 4 minutes plasma application**

**Treatment Regimens**
- Treatment 2x per week
- Treatment 3x per week
- Treatment 5x per week
- Untreated Control

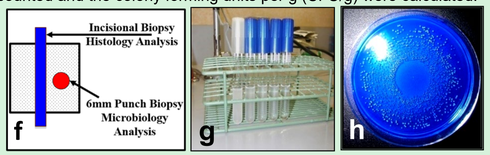
### 4. Treatment Regimen

- After biofilm formation, all wounds were debrided with a 4 mm curette. (b)
- For animal #1, twelve wounds each were treated with either plasma or sham dressing on days 0, 4, and 8 for 2 minutes. For animal #2, wounds were treated with plasma dressing either 2, 3 or 5 times a week and for either 2 or 4 minutes.
- Plasma dressing application involved adhesive foam around wounds (c), attaching the plasma dressing to form a seal (d) then the connected CPT device (e) was run for desired time. Dressings were removed and wounds were then covered with non-adherent gauze.




### 5. Microbiology Assessment

- Wounds were recovered for microbiology and histology on days 4,8 and 11 (animal #1) after treatment initialization or on days 4 and 11 (animal #2).
- 6 mm punch biopsies (f) of wounds were collected and homogenized with neutralizing solution. Serial dilutions (g) of these samples were plated using a Spiral Plater System onto Oxacillin Resistance Screening Agar Base plates (h). Colonies from overnight growth were counted and the colony forming units per g (CFU/g) were calculated.




### 6. Histology Assessment

- Incisional biopsies (g) were recovered from the center of the wound and stained with Hematoxylin and Eosin and Masson's Trichrome. Samples were assessed for re-epithelization (%), measurement of the length of the wound surface that has been covered with epithelium., White cell infiltrate. 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. The approximate amount of new granulation tissue formation: 0 = 0, 0.5 = 1-10%, 1 = 11-30%, 2 = 31-50%, 3 = 51-70%, 4 = 71-90%, 5= 91-100%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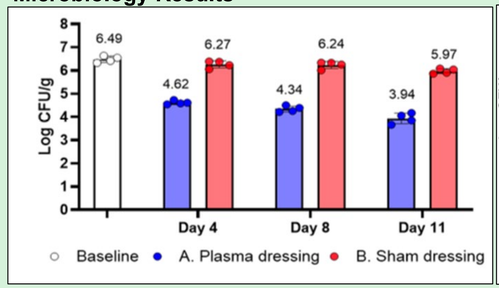
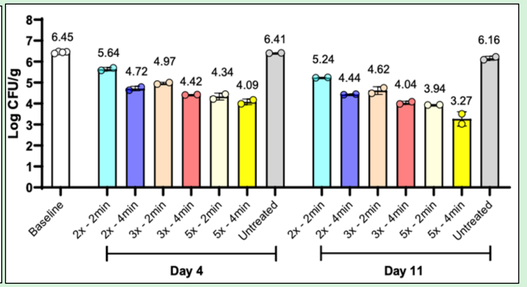
## Results

### Microbiology Results



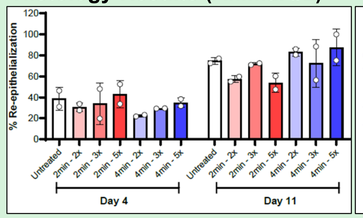

#### Animal #1

- Compared to the sham dressing, the plasma dressing reduced microbial burden at all assessment times. By day 11, the plasma dressing treated wounds had bacterial counts that were >2 Log CFU/g lower or 99% reduced compared with sham dressing wounds.
- When compared to baseline counts, the plasma dressing treated wounds had MRSA USA 300 counts that were 2.55 log CFU/g lower or 99.7% reduced.

#### Animal #2

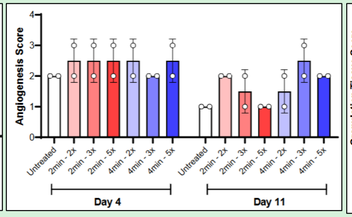
- All MRSA counts were reduced from baseline recovery to day 4 and then day 11. The greatest reduction was for 5x/week plasma dressing treatment at 4 minutes each, with a reduction of >3 log CFU/g or a greater than 99.9% reduction.
- Wounds treated with plasma dressing for 4 minutes had lower MRSA counts than those of 2-minute treatments for all treatment frequencies and at both assessment times.
- Untreated control wounds had sustained high MRSA bioburden and at day 11 was reduced only 0.29 log CFU/g from baseline.

### Histology Results (Animal #2)



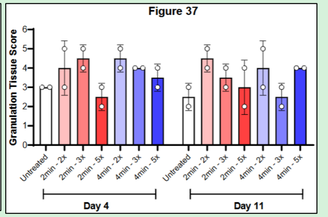

#### Re-epithelialization

- Re-epithelization was moderate for all treatment groups on day 4. without a discernable difference between plasma treated and control wounds.
- Increased plasma treatment frequency and duration generally increased epithelization compared to untreated control.




#### Angiogenesis

- Angiogenesis scores were consistently greater for plasma treated wounds compare to untreated control.
- The improvement in angiogenesis after plasma treatment was more notable at the early timepoint but persisted to day 11. Untreated wound angiogenesis score were also lower by day 11.




#### Granulation Tissue

- Plasma dressing treated wounds displayed higher or comparable granulation tissue scores on day 11 compared to the untreated control.
- Granulation generally increased with plasma treatment from day 4 to 11, while untreated was reduced.

## Conclusions

- The cold plasma dressing therapy evaluated in this study demonstrated potent antimicrobial efficacy against MRSA infected wounds. The dose-dependent nature observed for these findings supports the therapeutic potential of cold plasma. The dual action of antimicrobial and wound healing activity could improve the management of chronic wounds or drug-resistant infections.
- This preliminary study shows promising results and additional pre-clinical and clinical studies are warranted.