

Abstract

Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most commonly encountered bacteria in the burn unit and can be challenging to iradicate.¹ Copper has been previously shown to have antimicrobial activity against various pathogens.² There has also been reported wound healing effects with the use of copper.³ The present study was performed to investigate the antimicrobial and wound healing activity of two novel hydrogel formulations with copper and their ability to enhance the healing using a 3rd degree burn porcine model.⁴

Methods: Ninety-six (96) third degree burn wounds were created on pigs, within 20 minutes after wounding all wounds were infected with Methicillin Resistant *Staphylococcus aureus* (MRSA USA300) and treated with one of the following treatments: 1) Hydrogel Gauze with Copper H1-C (Copper H1-C)^, 2) Hydrogel Gauze with Copper H2-A (Copper H2-A)+, 3) Gelling Fiber Dressing with silver (GFD-Ag)*, or 4) Untreated Control●. All wounds were covered with a polyurethane film dressings and assessed on Days 3, 6, 14 and 21. Incisional and punch biopsies were obtained for histological and microbiology assessments, respectively.

Results: Copper H1-C and Copper H2-A showed a significant (p≤0.05) reduction in MRSA counts when compared to GFD-Ag and Untreated Control wounds at all assessment days. Both copper dressing showed more than 99.8% of reduction in all assessment days. On Days 14 and 21, wounds treated with Copper H1-C resulted in a MRSA reduction of 3.55±0.10 and 3.58±0.10 Log CFU/g bacterial, respectively. These values represent more 99.97% of reduction on both days. Wounds treated with Copper H1-C resulted in an increase in the epithelialization percentage on Days 14 and 21 (as compared to the other treatments groups. All treatment groups showed a significant (p≤0.05) increase in the epithelialization by Day 21.

Discussion: Overall, the hydrogel copper gauze treatments were effective in reducing MRSA infected wounds while enhancing the healing process. These findings suggest that this treatment may be an important armamentarium for wound care providers in combating wound infections and accelerating healing of wounds.

^, + Hydrogel Gauze with Copper prototype H1-C and H2-A (iFyber, LLC Ithaca, NY, USA), *KERRACEL™ Ag (3M, St. Paul, MN USA), ● Tegaderm™ (3M, St. Paul, MN USA)

Introduction

Third-degree burns are severe injuries that require immediate treatment to help prevent infection. ⁵ Copper has been shown to have antimicrobial properties is a good candidate for wound dressings.⁶ Studies of copper incorporated with hydrogels and dressings expressed encouraging results with wound healing and infections.^{7,8} A well-established porcine model was used to investigate the antibacterial and healing activity of two novel hydrogel cooper formulations.

References

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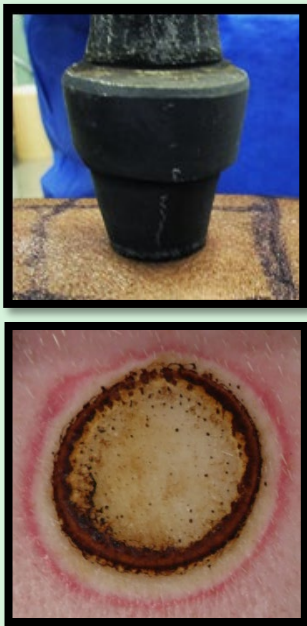
Materials and Methods

1. Experimental Animals:

Four (4) swine were used as our experimental animals due to the morphological, physiological, and biochemical similarities between porcine skin and human skin.⁹

2. Wounding Technique:

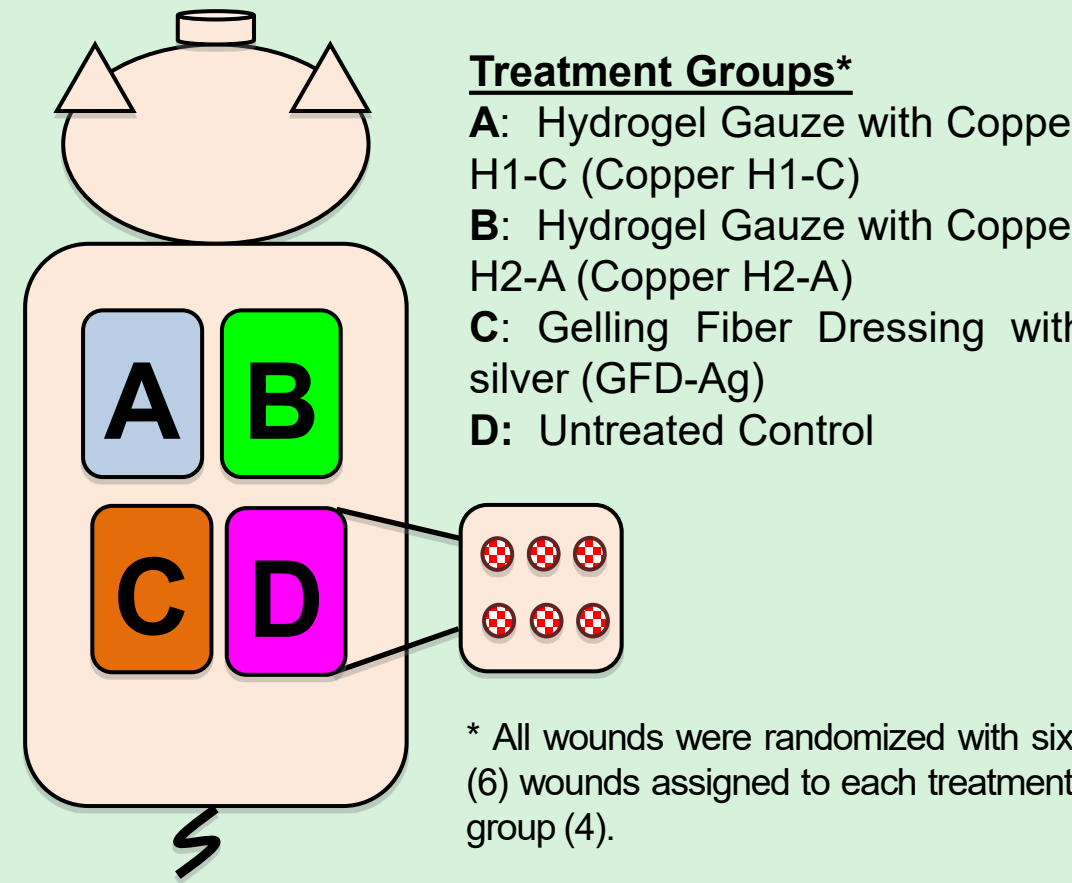
- Twenty-four (24) 3rd degree burn wounds were created using a branding iron (L & H Manufacturing Company Mandan, North Dakota 58554).
- Branding iron at 300°C was placed by 15 seconds to create wounds with 27mm diameter and a depth of approximately 3mm.



3. Inoculation:

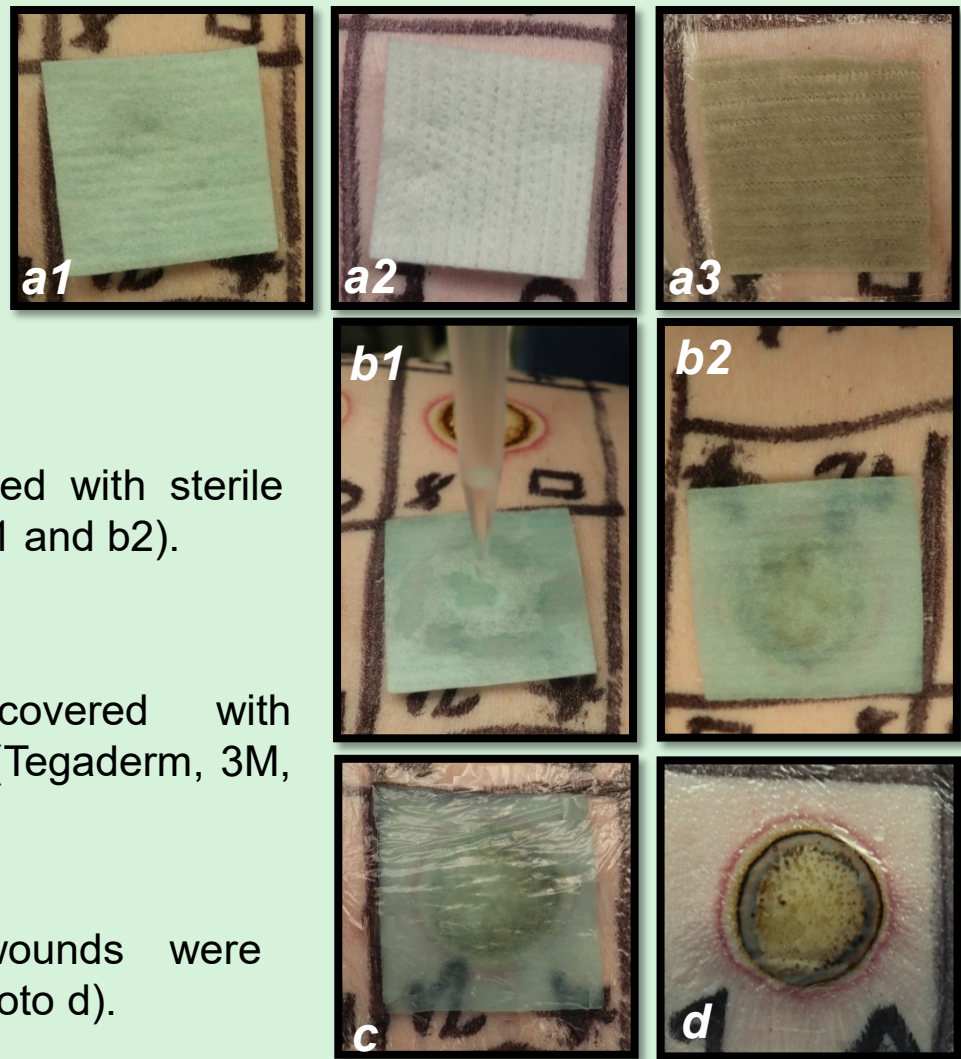
- After wounding, all wounds were inoculated with 25μL of Methicillin Resistant *Staphylococcus aureus* (MRSA USA300).

4. Experimental Design:



5. Treatment Regimen:

a. Wounds were treated after inoculation with either Copper H1-C (a1), Copper H2-A (a2) or GFD-Ag (a3).



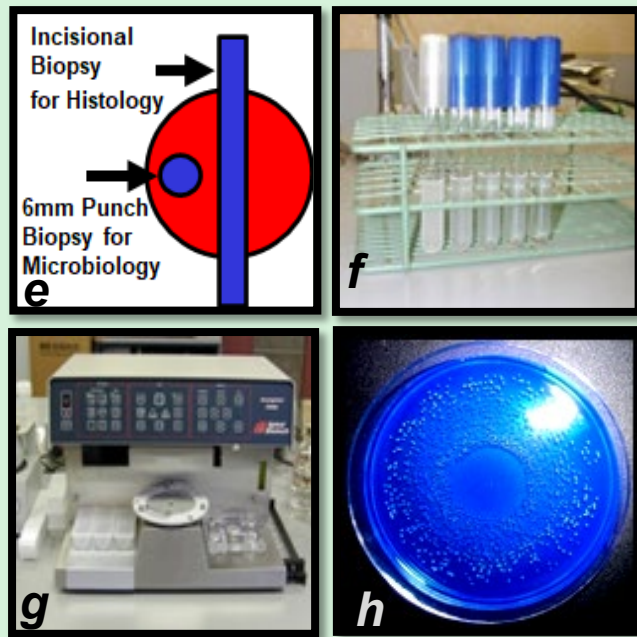
b. Dressings were saturated with sterile saline by using a pipette (b1 and b2).

c. Dressings were covered with polyurethane film dressing (Tegaderm, 3M, St. Paul, MNT)

d. Untreated Control wounds were covered with Tegaderm (photo d).

6. Microbiology Assessment:

- On Days 3, 6, 14 and 21 post treatment, wounds were recovered by using a 6mm punch biopsy (photo e).
- Biopsies were weighed, homogenized and combined with a scrub solution.
- Serial dilutions were made (photo f) and quantified using the Spiral Plater System (which deposits a defined amount (50μl) of suspension over the surface of a rotating agar plate: photo g)
- MRSA USA300 was isolated on ORSAB (Oxacillin Resistance Screening Agar Base) incubated at 37±2°C for 36-48 hours (photo h).The colony forming units per g (CFU/g) were calculated.



7. Histology Assessment:

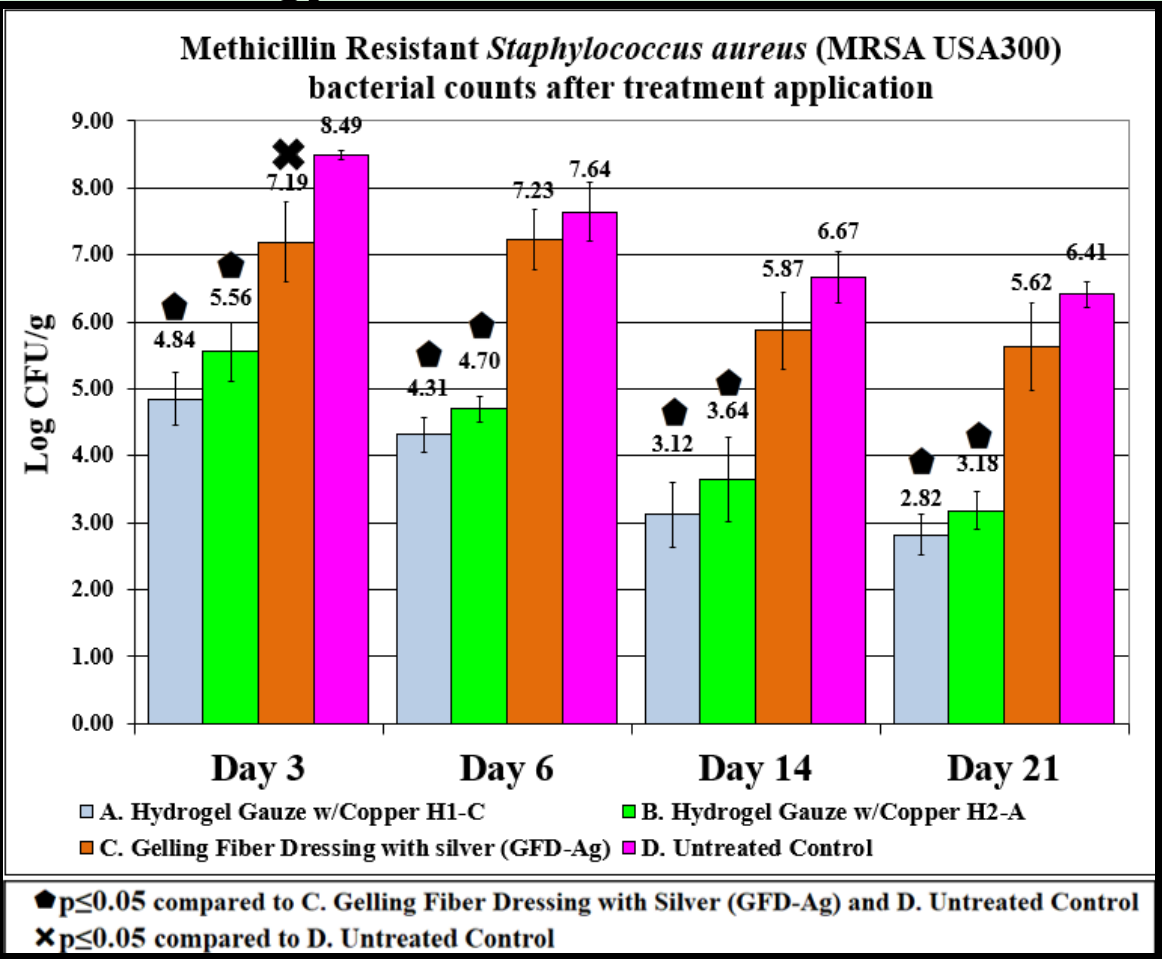
- On each assessment day, incisional biopsies were taken from the center of each wound (photo e). Each biopsy was placed in formalin and then stained with hematoxylin and eosin (H&E).
- The specimens were evaluated blinded via light microscopy and examined for the following elements: Percent of wound epithelialized (%), epithelial thickness (cell layers μm), white cell infiltrate and granulation tissue formation

8. Statistical Analysis:

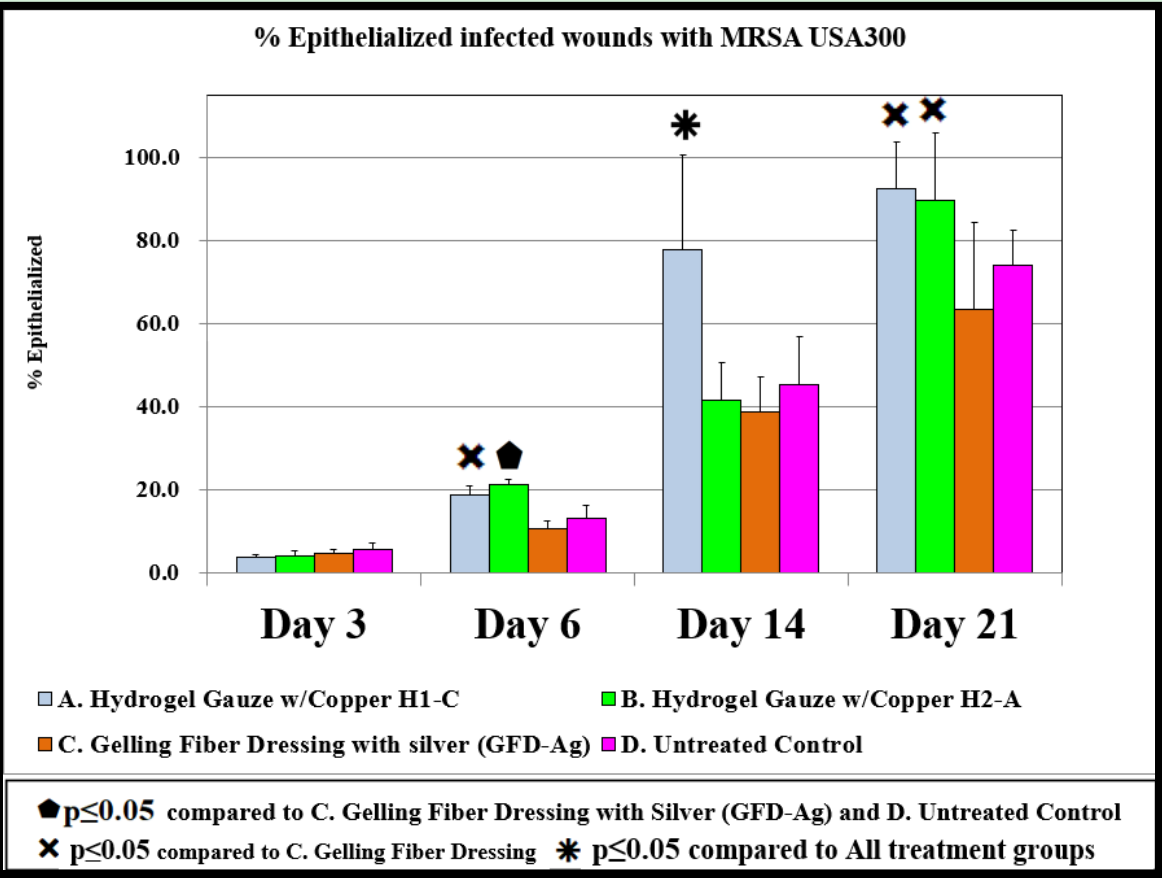
- A two-way analysis of variance (ANOVA) was used for statistical analysis for the microbiology and histology results. A p-value of less than 0.05 was considered significant.

Results

Microbiology Results:



Histology Results:



- Epithelial thickness (cell layers μm) and White cell infiltrate histological parameters expressed no significant differences among all treatment groups.

- Granulation Tissue Formation in all treatment groups have increased throughout each assessment time and Copper H2-A had significant difference (p≤0.05) when compared to Untreated Control wounds on Day 6.

Conclusions

- Both Copper H1-C and Copper H2-A treatments demonstrated the most beneficial effects on reducing MRSA USA300 bacterial counts and enhancement of wound healing.

- New therapies that can reduce bioburden while enhancing wound healing for both civilian and military wounds are needed, and the cooper dressings used in these studies show great promise.

Acknowledgements:

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- Copper H1-C treatment exhibited the lowest bacterial counts and significant (p≤0.05) reductions of 99% (over 2.34 Log CFU/g) when compared to GFD-Ag and Untreated Control during each assessment day.
- Highest reductions (p≤0.05 over 99%) for Copper H1-C wounds were on Day 3 (3.64±0.32 Log CFU/g) when compared to Untreated Control and on Day 6 (2.92±0.19 Log CFU/g) when compared to GFD-Ag.
- Wounds treated with Copper H2-A were significantly (p≤0.05) less in MRSA USA300 counts (over 97% of reduction) than GFD-Ag and Untreated Control wounds throughout all assessment days.
- GFD-Ag treated wounds had a significant difference (p≤0.05) of 1.30±0.53 Log CFU/g when compared to Untreated Control wounds on Day 3.

- By Day 6, Copper H1-C treated wounds had a significant difference (p≤0.05) of epithelialization when compared to GFD-Ag wounds.
- Wounds treated with Copper H2-A were significantly (p≤0.05) superior to GFD-Ag and Untreated Control wounds on Day 6.
- On Day 14, Copper H1-C treated wounds were significantly (p≤0.05) 35% greater than all other treatment groups.
- Both Copper H1-C and Copper H2-A on Day 21 had significant differences (p≤0.05) of 29% and 26.2% epithelialization when compared to the GFD-Ag treated wounds, respectively.
- All treatment groups significantly (p≤0.05) increased when comparing Day 21 to previous assessment days.

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