

The Antimicrobial Effects of a Standard-Issue Copper-Based Antimicrobial Military Wound Dressing To Reduce Bioburden in a 2nd Degree Burn Porcine Wound Model

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Abstract

Background: The most common burns clinically are second-degree burns, that need to be managed to prevent infection and detrimental in healing.¹ Copper has been used for centuries with its antimicrobial activity reputation against various pathogens by penetrating cells and promotes wound healing.^{2,3} Studies has shown the efficacy of copper wound healing and reducing bioburden on second-degree burn wounds.⁴ The present study was performed to investigate the antimicrobial activity of two novel copper dressings to reduce Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Acinetobacter baumannii* bioburden using an infected second degree burn wound porcine model.^{5,6}

Methods: Forty-eight (48) 2nd degree burn wounds (8.5 mm diameter) were created on each of four animals and immediately inoculated with either methicillin-resistant *Staphylococcus aureus* (MRSA USA300) or *Acinetobacter baumannii* ATCC19606 (AB19606). Six wounds on each animal were treated with either (A) Copper-based wound dressings [CWD]*, (B) Cu-Dressing Formulation 1, (C) Cu-Dressing Formulation 2, (D) Vehicle Control 1, (E) Vehicle Control 2, or (F) Untreated Control, then covered with polyurethane film. All dressings were applied on day 0 and day 3. On days 3 and 6 after treatment application wounds were recovered for bioburden counts.

Results: Both Cu-Dressing Formulations significantly ($p \leq 0.05$) reduced over 95% of MRSA USA300 and AB19606 at all timepoints when compared to Untreated Control. Compared to all treatment groups, Cu-Dressing Formulation 1 exhibited the lowest ($p \leq 0.05$) bacterial counts on Day 6 against both pathogens. Throughout the study both Cu-Dressings significantly reduced ($p \leq 0.05$) MRSA USA300 and AB19606 comparative to CWD. Cu-Dressing Formulation 1 exhibited significant differences ($p \leq 0.05$) of 2.25 ± 0.44 Log CFU/g (99.44% of reduction) for MRSA USA300 and 1.71 ± 0.07 Log CFU/g (98.06% reduction) for AB19606 on day 6 compared to Untreated Control wounds. All treatments against both microorganisms established significant microbial reductions ($p \leq 0.05$) between both assessment days with the most acknowledged from the Cu-Dressings.

Conclusion: Cu-Dressing Formulations demonstrated superior efficacy on both tested pathogens, exhibiting significant antimicrobial activity within infected 2nd degree burn wounds. These findings encourage further development and investigation of copper therapies as a promising, novel treatment approach for burn wound infections in patients.

* CWD – MedCu (MedCu Technologies, Herzliya, Israel)

Introduction

Second-degree burns are globally a health challenge as they penetrate the dermal layer, and immediate treatment is necessary for healing and preventing infection.⁷ Copper exerts toxicity to microorganisms through various mechanisms, with copper oxide particles incorporated into textile fibers for fabric production.⁸ Copper dressings have shown to have antimicrobial properties against different microbes and promote wound healing.⁹ A well-established porcine model was used to investigate the antibacterial effects of two novel copper dressing formulations.

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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.⁶

2. Wounding Technique:

- Forty-eight second-degree burns were created on each animal with cylindrical brass rods heated at 100°C.
- Held vertically for 6 seconds on the skin, creating an 8.5 mm diameter x 0.8 mm deep burn wound.
- After creating burns, the blister was removed with a sterile spatula.¹⁰

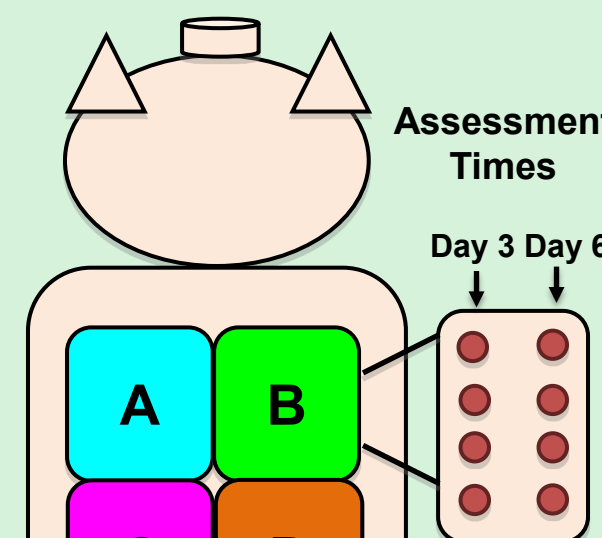


3. Inoculation:

- After wounding, all wounds were inoculated (10^6 CFU/mL) with 25µL of either Methicillin Resistant *Staphylococcus aureus* (MRSA USA300) or *Acinetobacter baumannii* ATCC19606 (AB19606).



4. Experimental Design:



* Treatment application was within 20-30 minutes after inoculation for Biofilm prevention. After treatment application, all wounds were covered with polyurethane film dressings (Tegaderm, 3M, St. Paul MN)

Treatment Groups

- A: Copper-based wound dressings [CWD]*
- B: Cu-Dressing Formulation 1*
- C: Cu-Dressing Formulation 2*
- D: Vehicle Control 1*
- E: Vehicle Control 2*
- F: Untreated Control*

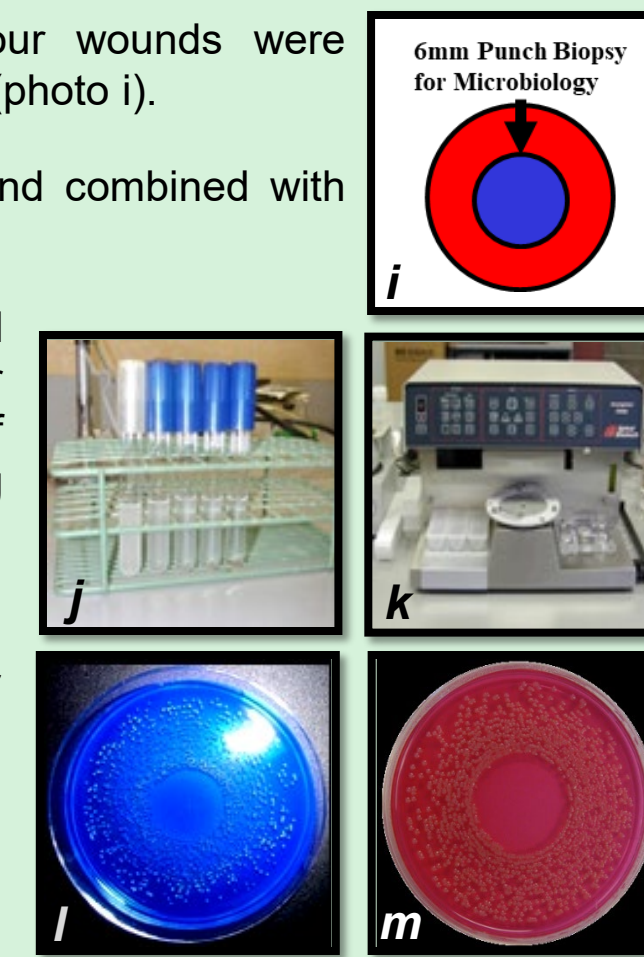
5. Treatment Regimen:

- All wounds except for Untreated Control were treated after inoculation (MRSA USA300 or AB19606) with 5x5 cm dressing of either:
 - CWD
 - Cu-Dressing Formulation 1
 - Cu-Dressing Formulation 2
 - Vehicle Control 1
 - Vehicle Control 2
- All the dressings were saturated with sterile saline by using a pipette (see photo f, as example).
- Afterwards, all dressings were covered with polyurethane film dressings [Tegaderm, 3M, St. Paul MN] (photo g).
- Untreated Control wounds were covered with only Tegaderm dressings (photo h).



6. Microbiology Assessment:

- On Days 3 and 6 post treatment, four wounds were recovered by using a 6mm punch biopsy (photo i).
- Biopsies were weighed, homogenized and combined with an All-Purpose Neutralizer Solution.
- Serial dilutions were made (photo j) and quantified using the Spiral Plater System which deposits a 50µL aliquot of suspension over the surface of a rotating agar plate: photo k).
- MRSA USA300 was isolated on ORSAB (Oxacillin Resistance Screening Agar Base: photo l) and Leed's Medium (photo m) was used to isolate AB19606. All plates were incubated at $37 \pm 2^\circ\text{C}$ for 24-48 hours. The colony forming units per g (CFU/g) were calculated.



7. 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.

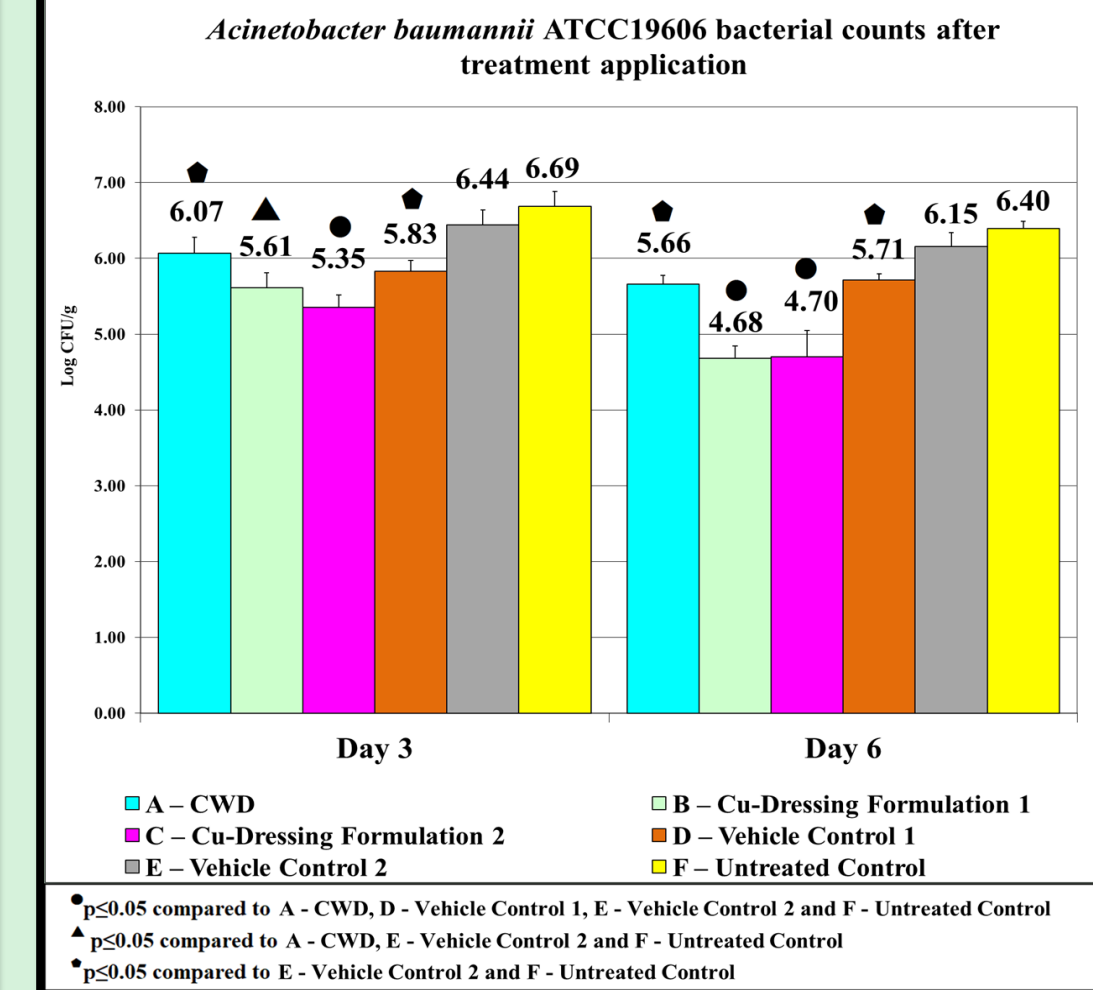
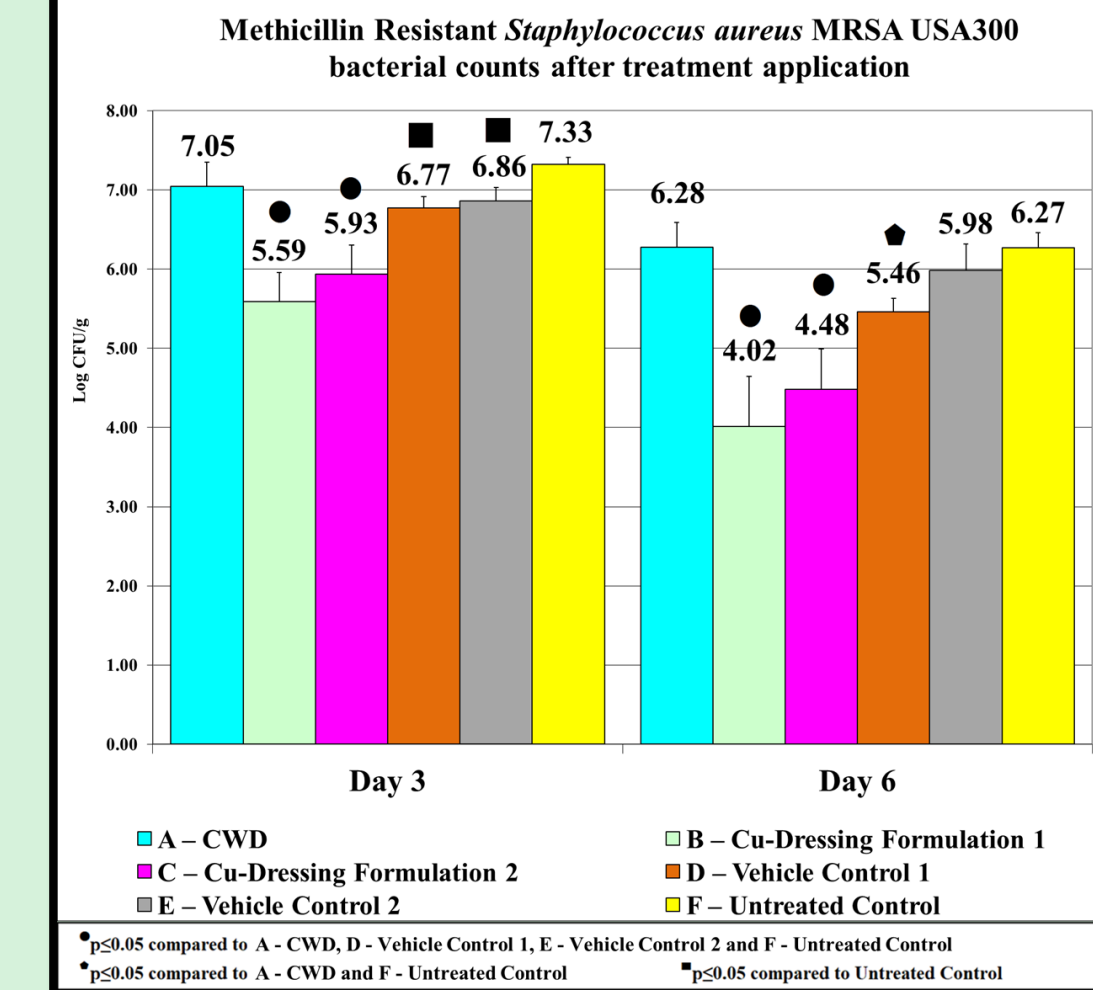
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Results



- Significant reductions ($p \leq 0.05$) for Cu-Dressing Formulation 1 and 2 on Day 3 were 1.74 ± 0.82 Log CFU/g (98.16%) and 1.39 ± 0.29 Log CFU/g (95.95%) when compared to Untreated Control, respectively.
- Both Cu-Dressing Formulation 1 and 2 exhibited significant ($p \leq 0.05$) MRSA reductions of 96.49% and 92.41% (1.46 ± 0.06 and 1.12 ± 0.06 Log CFU/g), respectively when compared to CWD treated wounds.
- On Day 3, Cu-Dressing Formulation 1 had significant ($p \leq 0.05$) reductions of 93.44% and 94.66% when compared to Vehicle Control 1 and 2, respectively.
- By Day 6, Cu-Dressing Formulation 1 and 2 had significant differences ($p \leq 0.05$) of 2.25 ± 0.44 and 1.79 ± 0.31 Log CFU/g (99.44% and 98.37%), respectively when compared to Untreated Control.
- When compared to CWD treated wounds, Cu-Dressing Formulation 1 wounds had a significant ($p \leq 0.05$) reduction of 99.45% (2.26 ± 0.32 Log CFU/g).
- Cu-Dressing Formulation 1 had significant reductions ($p \leq 0.05$) of 96.40% and 98.92% when compared to both Vehicle Control 1 and 2, respectively.
- On Day 6, a significant ($p \leq 0.05$) difference of 1.80 ± 0.20 Log CFU/g (98.41%) was observed when comparing Cu-Dressing Formulation 2 to CWD.
- When comparing Cu-Dressing Formulation 2 to Vehicle Control 2, a significant reduction ($p \leq 0.05$) of 1.50 ± 0.18 Log CFU/g (96.87%) was distinguished.

- On Day 3, both Cu-Dressing Formulations exhibited the lowest AB19606 counts compared to all treatment groups.
- Cu-Dressing Formulation 1 and 2 treated wounds had significant differences ($p \leq 0.05$) of 1.07 ± 0.01 Log CFU/g (91.53%) and 1.33 ± 0.03 Log CFU/g (95.36%), respectively when compared to Untreated Control.
- Wounds treated with Cu-Dressing Formulation 2 had a significant ($p \leq 0.05$) reduction of 91.85% when compared to Vehicle Control 2 wounds on Day 3.
- By Day 6, both Cu-Dressing Formulation 1 and 2 treated wounds were significantly ($p \leq 0.05$) lower in AB19606 counts than all other treatment groups.
- Compared to Untreated Control wounds, both Cu-Dressing Formulation 1 and 2 had significant reductions ($p \leq 0.05$) of 98.06% (1.71 ± 0.07 Log CFU/g) and 97.98% (1.69 ± 0.26 Log CFU/g), respectively.
- On Day 6, 90.65% and 90.26% of AB19606 reductions were observed when comparing wounds treated with Cu-Dressing Formulation 1 and 2 to Vehicle Control 1 treated wounds, respectively.
- Cu-Dressing Formulation 1 significantly ($p \leq 0.05$) exhibited a difference of 1.47 ± 0.16 Log CFU/g (96.61%) when compared to Vehicle Control 2.
- Cu-Dressing Formulation 2 have a significant ($p \leq 0.05$) reduction of 96.47% (1.45 ± 0.16 Log CFU/g) when compared to Vehicle Control 2 on Day 6.

Conclusions

- Both Cu-Dressing Formulations were the most effective against both pathogens throughout the duration of the study and significant ($p \leq 0.05$) to all treatment groups on Day 6.
- These findings highlight the need for further investigation into the efficacy of these copper dressings on burned wounds and evaluate their potential wound healing capabilities.