

Joel Gil<sup>1</sup>, Michael Solis<sup>1</sup>, Ryan Strong<sup>1</sup>, Roger Cassagnol<sup>1</sup>, Jie Li<sup>1</sup>, Aaron Strickland<sup>2</sup> and Stephen C. Davis<sup>1</sup>

<sup>1</sup>Dr. Phillip Frost Department of Dermatology and Cutaneous Surgery, Miller School of Medicine, University of Miami, Miami, Florida, <sup>2</sup>iFyber, LLC, Ithaca, NY, United States.



## Abstract

**Background:** Methicillin-resistant *Staphylococcus aureus* (MRSA), *Acinetobacter baumannii* and *Candida albicans* have been shown presence in wound infection.<sup>1-3</sup> Copper has been previously shown to have antimicrobial activity against various pathogens.<sup>4</sup> There have also been reported wound healing effects with the use of copper.<sup>5</sup> The present study was performed to investigate the antimicrobial activity of two novel copper dressings to reduce the bioburden using a full thickness in a porcine model.<sup>6</sup>

**Methods:** Full thickness wounds (punch biopsy 10 mm, n=48 per animal) were created on six animals and immediately inoculated with either methicillin-resistant *Staphylococcus aureus* (MRSA USA300) *Acinetobacter baumannii* ATCC19606 (AB19606) or *Candida albicans* ATCC64550 (CA64550). Wounds were treated (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. Wounds were recovered for microbiological counts on days 3 and 6, after treatment application.

**Results:** Cu-Dressing Formulation 2 significantly reduced MRSA USA300, AB19606 and CA64550 burden ( $p \leq 0.05$ ) at all timepoints compared to all treatment's groups. Cu-Dressing Formulation 1 significantly reduced MRSA USA300 and AB19606 relative to CWD ( $p \leq 0.05$ ). Both copper formulations showed more than 99% reduction six days after initial treatment compared to those wounds left untreated against all microorganisms tested ( $p \leq 0.05$ ). Wounds infected CA64550 and treated with Cu-Dressing Formulation 2 exhibited  $3.24 \pm 0.32$  Log CFU/g (99.95% of reduction) on day 6 compared to untreated control wounds ( $p \leq 0.05$ ). CWD and both Cu formulations showed significant microbial reduction ( $p \leq 0.05$ ) between day 3 and day 6.

**Conclusion:** Cu-Dressing Formulations demonstrated broad-spectrum antimicrobial activity in infected full thickness wound model. These findings support the use of copper therapies as promising new treatments for wound infection. Clinical trials examine the use of Cu-dressings are warranted.

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

## Introduction

Wounds infections are a health and economical challenge as they need immediate action to manage inflammation and prevent the infestation of microorganisms.<sup>7</sup> As an essential mineral, copper has various mechanisms that are effective by controlling the distributions of ions.<sup>8</sup> The impact of copper dressings have shown to have antimicrobial properties against different microbes and promote wound healing.<sup>9</sup> A well-established porcine model was used to investigate the antibacterial effects of two novel copper dressing formulations.

## References

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## Materials and Methods

### 1. Experimental Animals:

Six (6) swine were used as our experimental animals due to the morphological, physiological, and biochemical similarities between porcine skin and human skin.<sup>6</sup>

### 2. Wounding Technique:

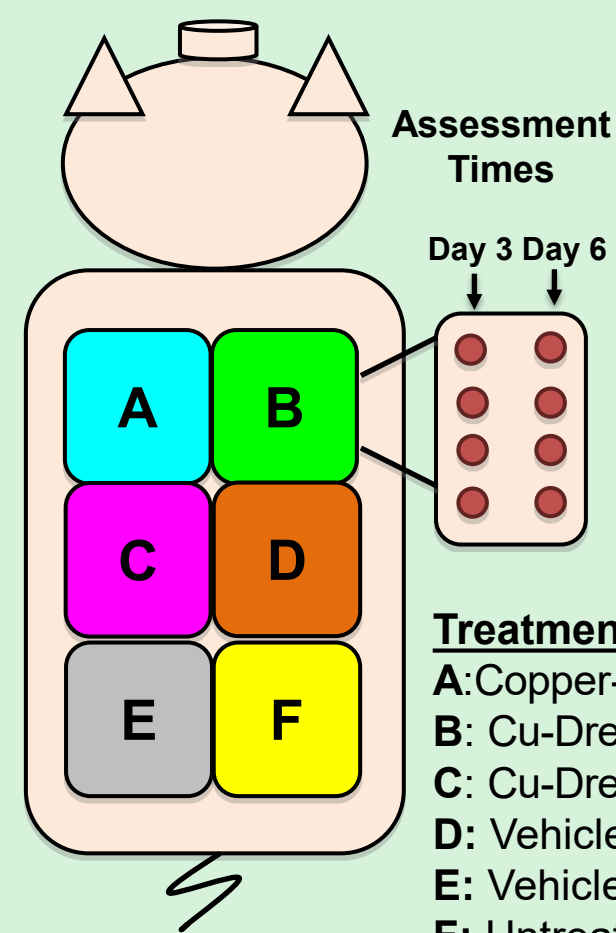
Forty-eight full thickness wounds were created on each animal with a 10mm punch biopsy.<sup>10</sup>

Each two animals were inoculated with one of the pathogens mentioned below.

### 3. Inoculation:

After wounding, all wounds were inoculated ( $10^6$  CFU/mL) with 25 $\mu$ L of either Methicillin Resistant *Staphylococcus aureus* (MRSA USA300), *Acinetobacter baumannii* ATCC19606 (AB19606) or *Candida albicans* ATCC64550 (CA64550).

### 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, AB19606 or CA64550) with 3x3 cm dressing of either:

- a) CWD
- b) Cu-Dressing Formulation 1
- c) Cu-Dressing Formulation 2
- d) Vehicle Control 1
- e) 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:

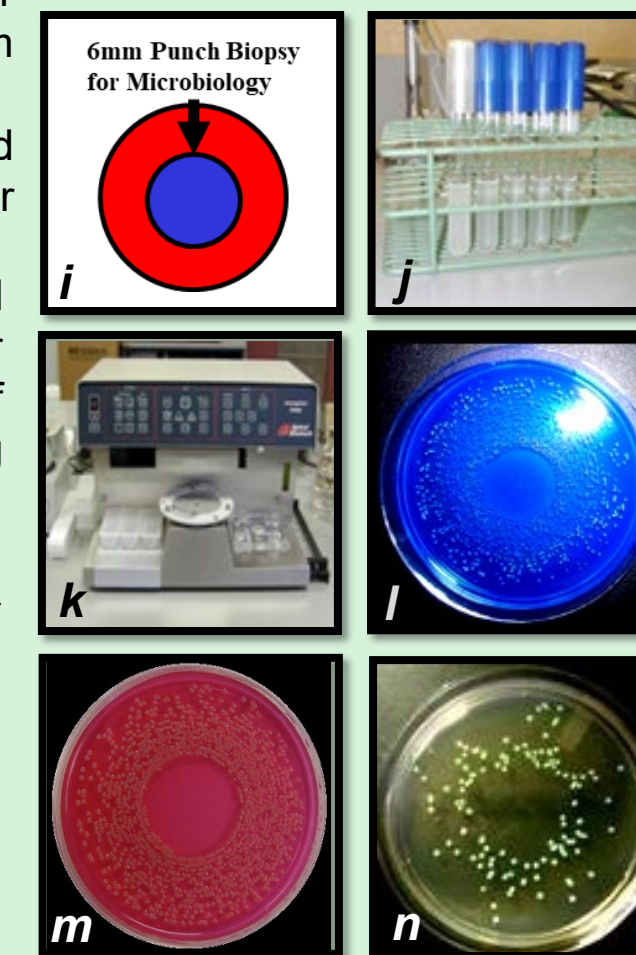
- On Days 3 and 6 post treatment, four wounds were recovered by using a 6mm punch biopsy (photo i).
- Biopsies were weighed, homogenized and combined with an All-Purpose Neutralizer Solution.
- Serial dilutions were made (photo j) and quantified using the Spiral Plater System which deposits a 50 $\mu$ L 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), Leed's Medium (photo m) was used to isolate AB19606 and CHROMagar™ *Candida* was used to isolate CA60550 (photo n). All plates were incubated at  $37 \pm 2^\circ\text{C}$  for 24-48 hours and up to 5 days at  $30^\circ\text{C}$  for CA. 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.

### Acknowledgements:

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## Results

### MRSA USA300 Results:

- Wounds treated with Cu-Dressing Formulation 1 had a significant reduction ( $p \leq 0.05$ ) of 94.84% ( $1.74 \pm 0.82$  Log CFU/g) when compared to Untreated Control wounds.
- On Day 3, Cu-Dressing Formulation 2 exhibited a significant ( $p \leq 0.05$ ) difference of  $1.98 \pm 0.13$  Log CFU/g (98.95%) when compared to Untreated Control wounds.
- On Day 6, Cu-Dressing Formulation 1 and 2 had significant differences of  $1.70 \pm 0.13$  and  $2.14 \pm 0.15$  Log CFU/g (98.00% and 99.27%), respectively compared to Untreated Control.
- When comparing CWD to Untreated Control, a significant reduction ( $p \leq 0.05$ ) of 95.15% ( $1.31 \pm 0.30$  Log CFU/g) was observed.

### AB19606 Results:

- 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  $1.43 \pm 0.05$  Log CFU/g (96.26%) and  $1.74 \pm 0.02$  Log CFU/g (98.16%), respectively when compared to Untreated Control.
- By Day 6, both Cu-Dressing Formulation 1 and 2 treated wounds had significant ( $p \leq 0.05$ ) AB19606 reductions of 98.98% and 99.51% ( $1.99 \pm 0.01$  and  $2.31 \pm 0.28$  Log CFU/g), respectively when compared to Untreated Control wounds.
- CWD had a significant difference ( $p \leq 0.05$ ) of  $1.12 \pm 0.03$  Log CFU/g (92.48%) on Day 6. Compared to Untreated Control,

### CA64550 Results:

- On Day 3, 94.07% and 94.52% of CA64550 reductions were observed when comparing wounds treated with CWD and Cu-Dressing Formulation 1 to Untreated Control, respectively.
- Wounds treated with Cu-Dressing Formulation 2 significantly ( $p \leq 0.05$ ) exhibited a difference of  $2.02 \pm 0.09$  Log CFU/g (99.04%) when compared to Untreated Control.
- By Day 6, Cu-Dressing Formulation 2 had the most CA64550 ( $p \leq 0.05$ ) reduction of 99.95% ( $3.34 \pm 0.32$  Log CFU/g) when compared to Untreated Control.
- CWD and Cu-Dressing Formulation 1 had significant ( $p \leq 0.05$ ) reductions of  $1.76 \pm 0.10$  and  $1.93 \pm 0.04$  Log CFU/g (98.24% and 98.82%) when compared to Untreated Control on Day 6.

## Conclusions

- The Cu-Dressing Formulations were effective against all three pathogens throughout the duration of the study, with Cu-Dressing Formulation 2 being the most significant ( $p \leq 0.05$ ) to all treatment groups.
- Further studies are warranted to explore the effectiveness of these copper dressings on full thickness wounds and to ascertain their potential wound healing capabilities.

