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Effects of a Novel Non-Biologic Desiccant to Remove Bacteria Using a Deep Dermal Wound Porcine Model

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Abstract:

Debridement plays a critical role in wound bed preparation and management. In addition to removing necrotic tissue, debridement can eliminate bacteria that are frequently harbored within the tissue.[1] Infected wounds particularly with drug-resistant bacteria such as *Staphylococcus aureus* have a high-risk of impending the healing process. The purpose of this pilot study was to a examine the ability of a novel debridement method which uses a novel molecular cleaning technology, to remove both necrotic tissue and bacteria from infected wounds using a porcine wound model.[2,3] Thirty deep dermal wounds (22mmx22mmx3mm) were created and inoculated with Methicillin Resistant *Staphylococcus aureus* (MRSA USA300). Wounds were covered for 72 hours to allow biofilm formation. Baseline wounds (3) were assessed prior treatment application and remaining wounds were assigned to one of three treatment groups: 1) Novel Debridement Formulation [NDF*], 2)Gauze with sterile saline, or 3) Untreated control. All wounds were treated for 30 seconds and then rinsed with 10ml of sterile saline. After treatment application a sterile gauze was used to remove the slough and wounds were covered with a polyurethane film. Amount of slough was assessed using digital planimetry. Biopsies were taken on days 4,8 and 11 post-treatment for microbiology and histological assessment. After initial treatment, over 80% more slough was removed with NDF as compared to controls. NDF also achieved bacterial reductions of more than 99.77% and 99.86 when compared to baseline bacterial counts and untreated group in all assessment days, respectively. NDF treated wounds resulted in reductions of 89.40%, 97.52% and 98.97% when compared to Gauze with sterile saline group in assessment Days 4, 8 and 11, respectively. NDF treated wounds showed a more than 1 Log CFU/g bacterial reduction compared day 11 to day 4. An initial increase in epithelialization was noted with NDF on day 4 compared to other treatment groups. Overall, the NDF appeared to be the most effective treatment group to reduce MRSA counts. Our results suggest that this novel treatment may have added clinical beneficial effects in wound bed preparation. Additional animals are needed to substantiate these findings.

*Revity – Epien Medical, St. Paul, MN

Introduction:

The presence of biofilms in wounds can be an important barrier to effective treatments.[4,5] Many patients in hospitals acquire nosocomial infections that become a challenge to prevent and treat[6]. Such infections are often caused by antibiotic-resistant organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA). An additional challenge when attempting to halt bioburden proliferation is the microorganism's ability to colonize a surface by forming a protective biofilm matrix.[7] MRSA forming extracellular polymeric substance (EPS) makes treatment more difficult to manage. Debridement techniques have shown limited ability to mechanically remove bacteria from a wound bed.[1] NDF* is a topical formulation that can be used by healthcare practitioners for wound cleansing. The purpose of this study was to evaluate the ability of NDF* to remove non-viable tissue in wound debridement and also examine its ability to reduce the bacterial load in wounds inoculated with methicillin-resistant *Staphylococcus aureus* (MRSA).

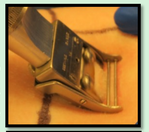
Materials and Methods:

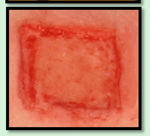
1. Experimental Animals:

Swine were used as our experimental animal due to the morphological, physiological, and biochemical similarities between porcine skin and human skin.[8]

2. Wounding Technique:

A specialized electrokeratome was used to create thirty (30) deep reticular dermal wounds measured (22mm x 22mm x 3mm deep) on the paravertebral and thoracic area.

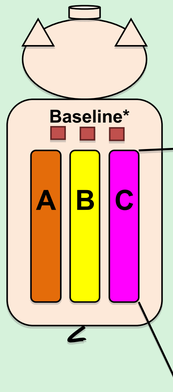




3. Inoculation:

- After creation of wounds, 25µl of Methicillin Resistant *Staphylococcus aureus* (MRSA USA300) was used to inoculate each wound by scrubbing 10^6 CFU/ml) inoculums into each wound with a teflon spatula (30 seconds).
- Nine (9) wounds were assigned to each treatment group (3 groups total) and 3 wounds were used as a baseline
- All wounds were then covered with a polyurethane film for 72 hours (to allow biofilm formation).

4. Experimental Design:



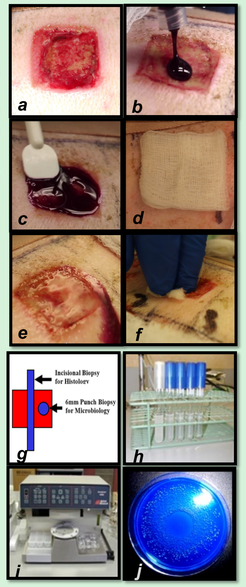
Treatment Groups
A: Novel Debridement Formulation [NDF* Revity®]
B: Saline Irrigation
C: Untreated Control

Assessment Times (Days After Treatment Application)

*Baseline wounds were recovered at 72 hours after inoculation and prior to treatment.

5. Treatment Regimen:

a. After 72 hours, all wounds were debrided.
b. Wounds treated with NDF received 500ul.
c. NDF treatment was spread with spatula and allowed to stay in place for 30 seconds
d. Saline Irrigation wounds each had a premoisten gauze (500 µL of sterile saline) placed over the wound which was allowed to stay in place for 30 seconds.
e. After 30 seconds, all wounds were rinsed with a 10mL syringe of sterile saline (image showed rinsing after NDF application).
f. After rinse wounds were gently wipe with moistened sterile PBS gauze and then covered with Tegaderm.



6. Wound Recovery:

Microbiology Analysis:

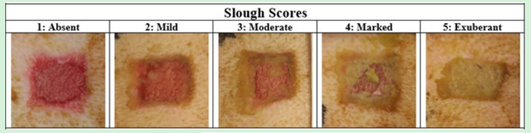
- Baseline wounds were recovered before treatment application. On days 4, 8 and 11 post treatment, three wounds per group were recovered by using a 6mm punch biopsy (photo g).
- Biopsies were homogenized and combined with a scrub solution.
- Serial dilutions were made (photo h) and quantified using the Spiral Plater System (which deposits a defined amount (50µl) of suspension over the surface of a rotating agar plate: photo i) MRSA USA300 was isolated on ORSAB (Oxacillin Resistance Screening Agar Base) incubated at 37±2°C for 36-48 hours (photo j).The colony forming units per g (CFU/g) were calculated.

Histology Analysis:

- From the same wound incisional biopsies were also taken (photo g).
- Incisional biopsy was obtained through the center of the wounds including normal adjacent skin on both sides.
- 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. Mean Score: 1 = absent, 2 = mild, 3 = moderate, 4 = marked, 5 = exuberant, Granulation Tissue Formation. 0 = 0, 0.5 = 1-10%, 1 = 11-30%, 2 = 31-50%, 3 = 51-70%, 4 = 71-90%, 5= 91-100% and New Blood Vessel Formation: Presence of new blood vessels (non-quantitative). Mean Score: 1 = absent, 2 = mild, 3 = moderate, 4 = marked, 5 = exuberant.

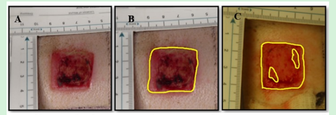
Clinical Observations:

- The amount of slough and coagulum was score using the scales below.



Digital Photography & Measurement of the Slough:

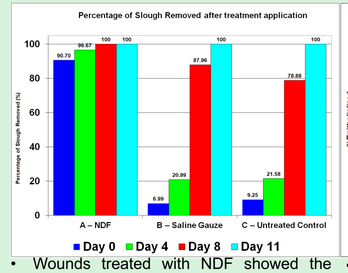
- Photographs was taken before and after treatment by using two rulers that was placed tangential. The wound area that includes slough was traced by digital imaging with ImageJ.



Scaling of Photograph (**A**) and measurement of slough removal [before (**B**) and after (**C**)]

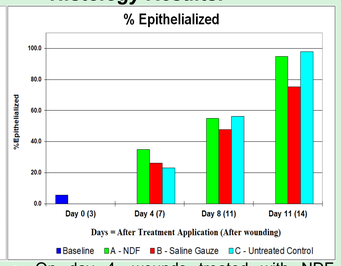
Results:

Slough Removal Results:



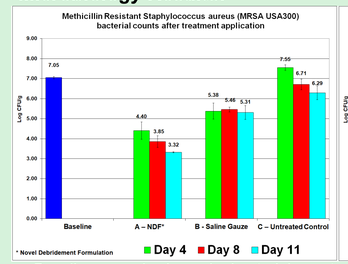
- Wounds treated with NDF showed the highest percentage of slough removal on every assessment day.
- These wounds exhibited 90.70% of slough removed as early as day 0.
- All wounds reached 100% at day 11.

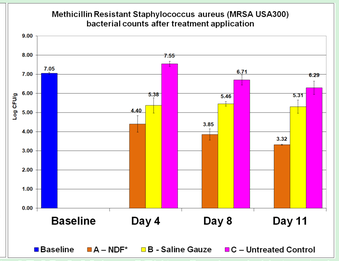
Histology Results:



- On day 4, wounds treated with NDF exhibited the highest amount of re-epithelialization (34.8%) when compared against the other treatment groups.
- On days 8 and 11, wounds treated with NDF results in 54.8 and 94.8% of re-epithelialization.
- All other parameters had similar results.

Microbiology Results:





- Baseline wounds showed a bacterial count of 7.05±0.06 Log CFU/g. On day 4, Untreated Control wounds showed the highest MRSA counts at 7.55±0.14 Log CFU/g.
- NDF also achieved bacterial reductions of more than 99.77% and 99.86 when compared to baseline bacterial counts and untreated group in all assessment days, respectively.
- NDF treated wounds resulted in reductions of 89.40%, 97.52% and 98.97% when compared to Gauze with sterile saline group in assessment Days 4, 8 and 11, respectively. NDF treated wounds showed a more than 1 Log CFU/g bacterial reduction compared day 11 to day 4.

Conclusions

- Wounds treated with NDF had a higher percentage of slough removal and MRSA reduction. Revity treated wounds had a desirable effect on slough removal the day of treatment (day 0) and 4 days after this single application the count reached more than 99 % of bacterial reduction compared with the baseline and untreated wounds.
- The effects were noticeable when compared against the other groups. Ultimately, NDF was able to reduce the MRSA microbial counts by half compared with Untreated Control on every assessment day. Additional studies with more animals would be needed to substantiate these claims and acquire statistical data.

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