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Effects of a Regenerative Debridement Technology to Remove Bacteria Using a Deep Dermal Wound Porcine Model

Joel Gil[1], Jie Li[1], Tongyu Cao Wikramanayake[1], Alexander Higa[1], Michael Solis[1], Steven Kavros[2] and Stephen C. Davis[1]

[1]Dr. Phillip Frost Department of Dermatology and Cutaneous Surgery, Miller School of Medicine, University of Miami, Florida, [2] EPIEN Medical, Inc., St. Paul, MN, Vascular Surgery Associates, Blaine, MN.

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Abstract:

Background:

Nosocomial infections are common in many healthcare provider settings, including military treatment facilities. 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 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]

Methods:

Three pigs were used for this study. On each animal 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) Regenerative Debridement Technology [RDT*], 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, histological and molecular (rt-PCR) assessments.

Results:

After initial treatment, over 80% more slough was removed with RDT as compared to controls.. RDT 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. RDT 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. RDT 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 RDT on day 4 compared to other treatment groups. Molecular results showed on Day 8 a 62% of reduction in IL-1α expression in wounds treated with RDT compared with Gauze with sterile saline. Levels of TNFα were significanly increased on day 4 with RDT treatment in then became substantially reduced on Days 8 and 11, as compared with baseline wounds. MMP-9 was also found to significantly reduced on day 4 as compared to control wounds.

Conclusion:

Overall, the RDT appeared to be the most effective treatment group to reduce MRSA counts and modulate healing. These results may have significant clinical implications when treating injured military personnel with acute or chronic wounds

*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] RDT* 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 RDT* 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).

Contact Information: Stephen C. Davis / Research Professor
Dr. Phillip Frost Department of Dermatology and Cutaneous Surgery.
Miller School of Medicine, University of Miami
sdavis@med.miami.edu. Ph: 305.243.4897

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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]

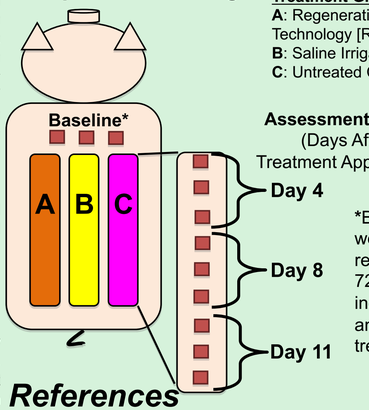
2. Wounding Technique:

An 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:



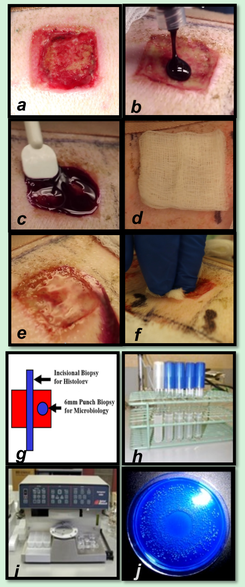
Treatment Groups
A: Regenerative Debridement Technology [RDT* REVITY®]
B: Saline Irrigation
C: Untreated Control

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 RDT received 500ul.
c. RDT 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 RDT application).
f. After rinse wounds were gently wipe with moistened sterile PBS gauze and then covered with Tegaderm.



6. Wound Recovery:

Microbiology Analysis:

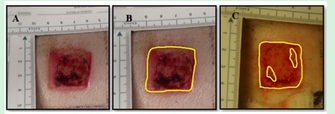
- Baseline wounds were recovered before treatment application. On days 4, 8 and 11 post treatment, three wounds per group were recovered by using a 6mm punch biopsy (photo g).
- Biopsies were homogenized and combined with a scrub solution.
- Serial dilutions were made (photo h) and quantified using the 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.

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.



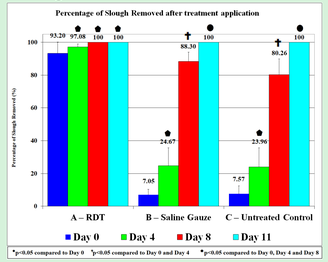
Scaling of Photograph (**A**) and measurement of slough removal [before (**B**) and after (**C**)]

Molecular Analysis:

- RNA was extracted and purified from collected porcine skin wound biopsies stored in RNAlater using Qiazol and RNeasy Mini Kit (Qiagen) following manufacturer's instructions. Real-time qPCR will be carried out using One-Step RT-PCR Kit (Quanta Biosciences Inc.) to assess gene expression of TNFα, IL-1 and MMP-9.

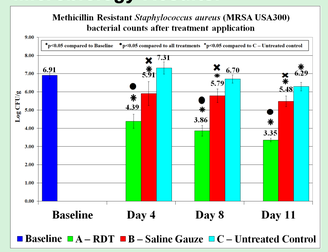
Results:

Slough Removal Results:

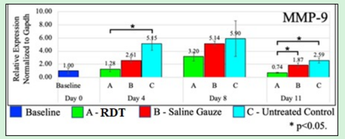


- RDT treated wounds showed the highest percentage of slough removal on every assessment day (p<0.05).

Microbiology Results:

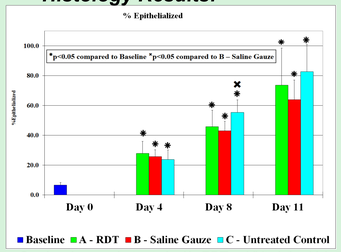


- RDT treated wounds resulted in reductions of 96.97%, 98.81% and 99.25% when compared to Gauze with saline on assessment Days 4, 8 and 11, respectively (p<0.05).



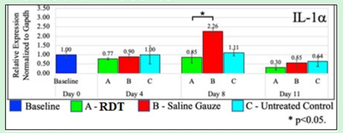
- Expression of MMP-1 and MMP-9 was increased in all the samples with or without RDT treatment, with untreated samples showing the most robust increase. MMP-9 expression levels in the RDT-treated samples were closest to baseline and were significantly lower than Saline Gauze treated or untreated wounds.

Histology Results:

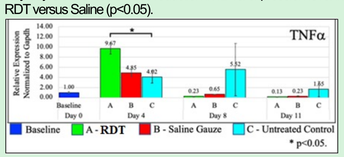


- On day 4, wounds treated with RDT exhibited the highest amount of re-epithelialization when compared against the other treatment groups. On days 8 and 11, wounds treated with RDT results in 45.8 and 73.7% of re-epithelialization. Untreated wounds more epithelialized than saline gauze on day 8 (p<0.05).

Molecular Results:



- By day 8 there was a 62% reduction in IL-1α expression level in RDT versus Saline (p<0.05).



- On day 4 TNFα levels were higher in RDT treated versus untreated samples (p<0.05) .

Conclusions

- Wounds treated with RDT had a higher percentage of slough removal on days 0, 4 and 8 after a single application.
- RDT was able to reduce the MRSA microbial counts by half compared with control treatments.
- RDT treated wounds showed reduced expression of IL-1 and MMP-9 levels throughout the study compared to controls. An initial robust increase in TNFα expression levels was seen with RDT treated wounds which rapidly reduced during the wound healing progress.
- RDT may have important clinical application for removing slough, reducing bacterial counts and mediating the wound healing process.

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