



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



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Abstract:

Introduction:

Nosocomial infections are common in many healthcare provider and debridement plays a critical role in wound bed management. Debridement can eliminate necrotic tissue and bacteria that are harbored within the tissue.¹ Infected wounds with drug-resistant bacteria (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) have a high-risk of impending the healing process. Novel debridement method (novel molecular technology) was used in this study to remove necrotic tissue and bacteria from infected wounds using a porcine model.^{2,3}

Methods:

Deep dermal wounds (120 wounds) measuring (22mmx22mmx3mm) were created and inoculated with Methicillin Resistant *Staphylococcus aureus* (MRSA USA300) or *Pseudomonas aeruginosa* PA09-010(military isolation). Wounds were covered for 72hours to allow biofilm formation and baseline wounds (3) were assessed prior treatment application, then remaining wounds were treated for 30seconds with: 1) Regenerative Debridement Technology [RDT*], 2) Saline Gauze, or 3) Untreated control. Wounds were rinsed with 10ml of sterile saline then 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:

Immediately after treatment over 85% more slough was removed with RDT* with a significant reduction ($p \leq 0.05$) on days 4 and 8 compared to controls. MRSA USA300 and PA09-010 was significant ($p \leq 0.05$) reduced with RDT* (99.70% and 81.14%) compared to baseline and untreated in all assessment days, respectively. More than 1 LogCFU/g bacterial reduction compared day 11 to day4 was observed in wounds treated RDT* in both microorganisms. An initial increase in epithelialization was noted with RDT on day4 compared to other treatment groups. IL-1 α expression was reduced (62%) in wounds treated with RDT* compared with Gauze with sterile saline. TNF α were increased on day4 with RDT* and reduced on Days 8 and 11, as compared with baseline wounds. MMP-9 was reduced on day 4 compared to control wounds.

Discussion:

Overall, RDT* was most effective reducing MRSA than PA09-010 in wounds. No detrimental results were observed in wounds treated with RDT* in epithelialization comparing with both controls. These results may have significant clinical implications when treating patients with acute and/or chronic wounds.

*REVITY® – Epien Medical, Inc. Minneapolis, 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 treat⁶. Such infections are often caused by antibiotic-resistant organisms such as Methicillin Resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*. 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) and *Pseudomonas aeruginosa*.

References

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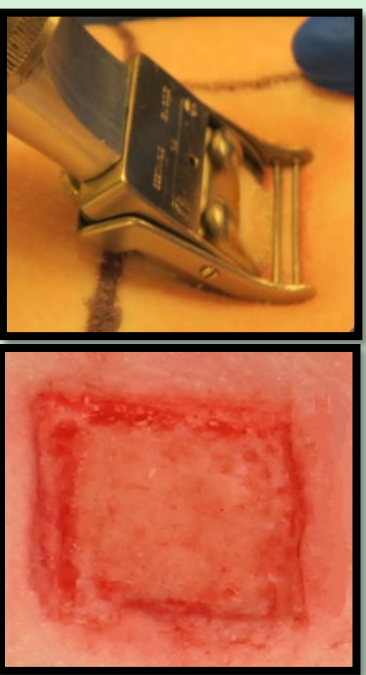
Materials and Methods:

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:

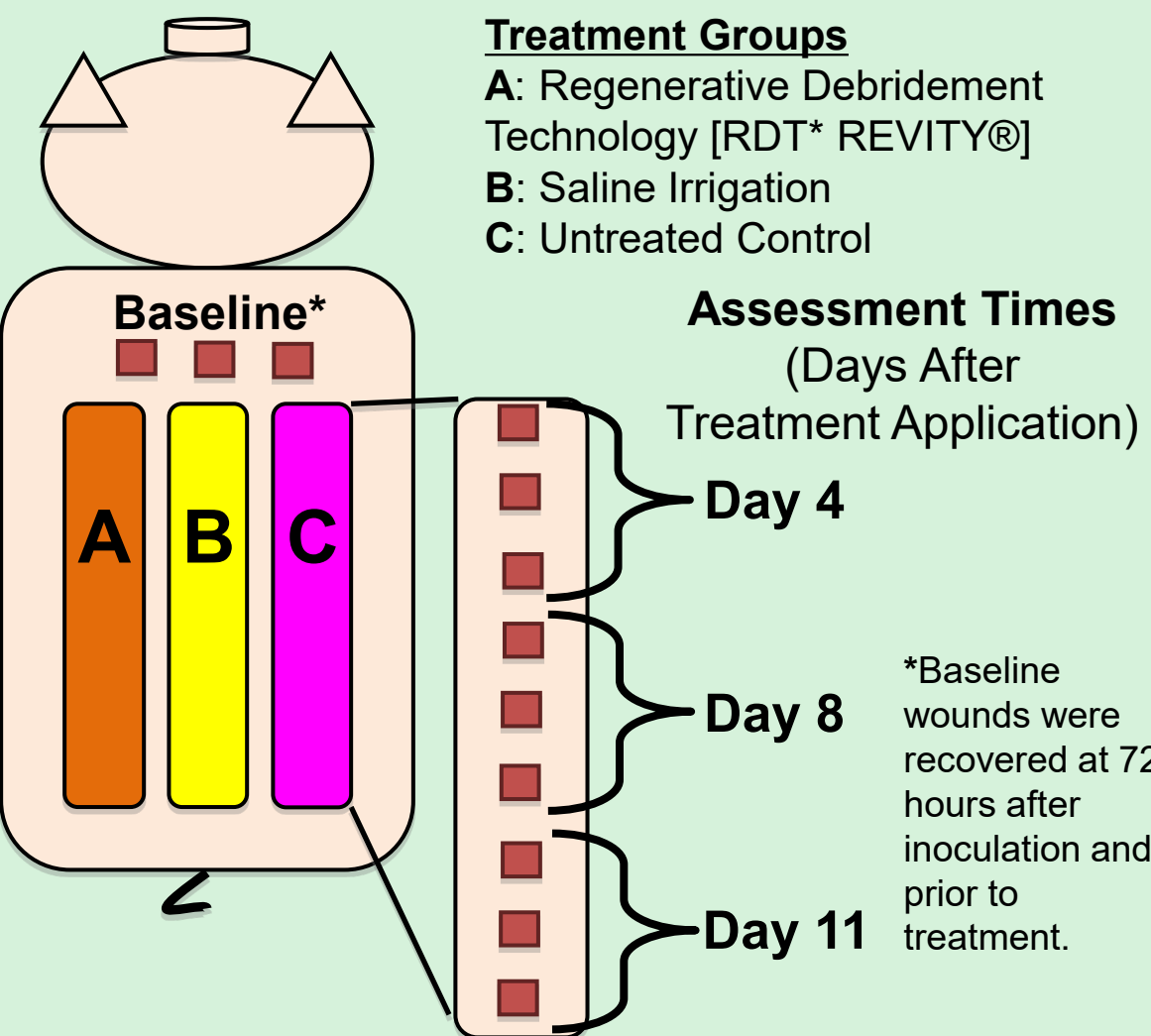
A specialized electrokeratome was used to create thirty (30) deep reticular dermal wounds measured (22mm x 22mm x 3mm deep) on the paravertebral and thoracic area.



3. Inoculation:

- After creation of wounds, 25 μ l of Methicillin Resistant *Staphylococcus aureus* (MRSA USA300) or *Pseudomonas aeruginosa* was used to inoculate each wound by scrubbing (10⁶ 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)

Day 4

Day 8

Day 11

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

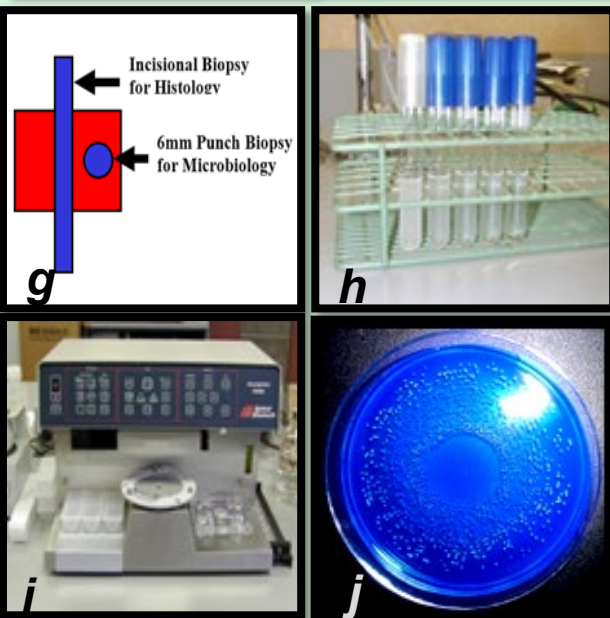
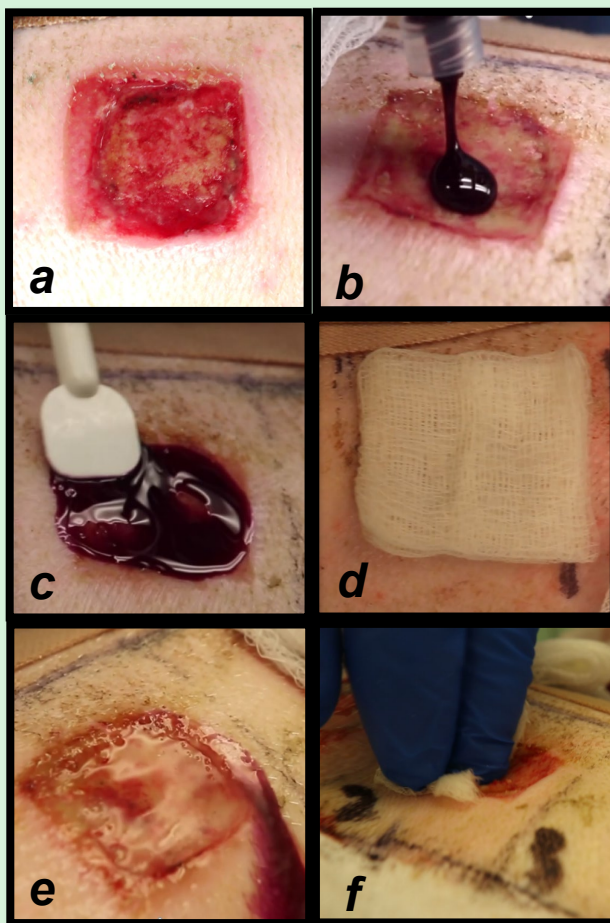
5. Treatment Regimen:

- After 72 hours, all wounds were debrided.
- Wounds treated with RDT received 500ul.
- RDT treatment was spread with spatula and allowed to stay in place for 30 seconds
- Saline Irrigation wounds each had a premoisten gauze (500 μ L of sterile saline) placed over the wound which was allowed to stay in place for 30 seconds.
- After 30 seconds, all wounds were rinsed with a 10mL syringe of sterile saline (image showed rinsing after RDT application).
- 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 (50 μ l) 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 \pm 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. Incisional biopsy was obtained through the center of the wounds including normal adjacent skin on both sides (photo g).
- The specimens were evaluated blinded via light microscopy and examined for the following elements: Percent of wound epithelialized (%), Epithelial thickness (cell layers μ m), 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.

Molecular Analysis:

- From the same wound only for the animal infected with MRSA a 4mm pouch biopsy was taken to analysis iL-1 α , TNF α and MMP-9

Clinical

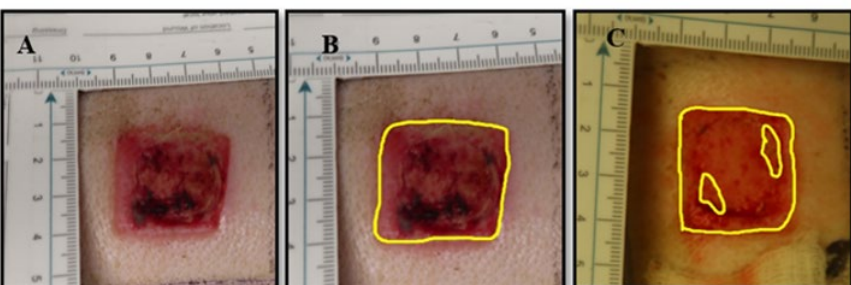
Observations:

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



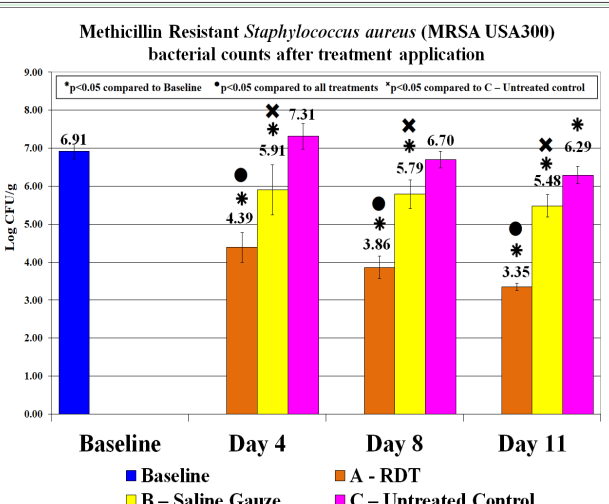
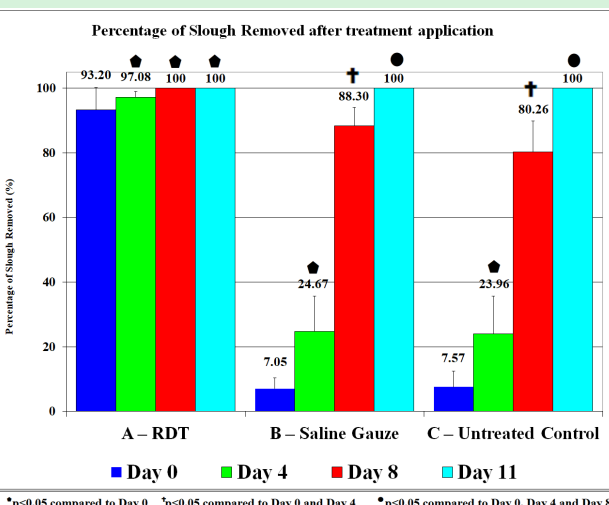
Digital Photography & Measurement of the Slough:

- 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.

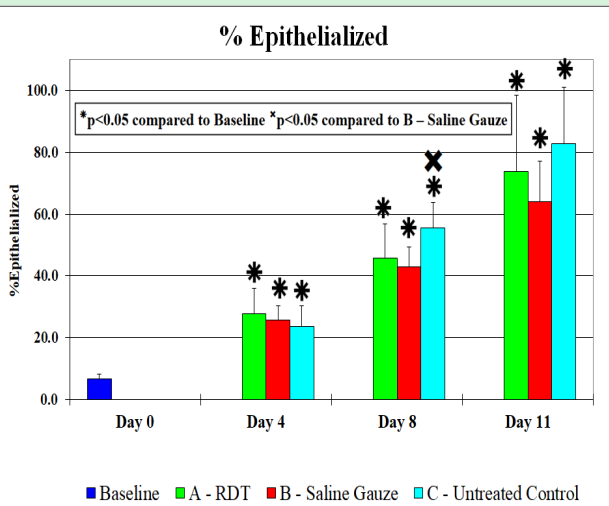


Scaling of Photograph (A) and measurement of slough removal [before (B) and after (C)]

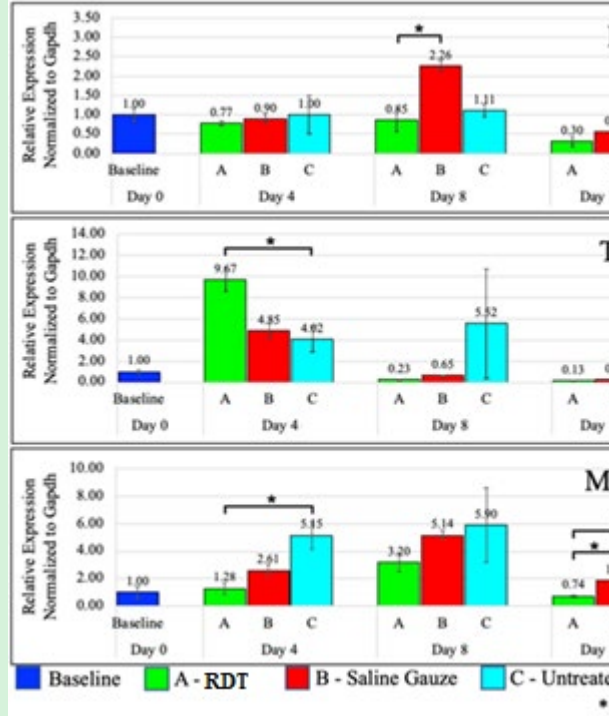
Methicillin Resistant *Staphylococcus aureus* (MRSA USA300)



- RDT treated wounds resulted in reductions of 96.97%, 98.81% and 99.25% when compared to Gauze with sterile saline group in assessment Days 4, 8 and 11, respectively ($p < 0.05$). RDT treated wounds showed a more than 1 Log CFU/g bacterial reduction compared day 11 to day 4.



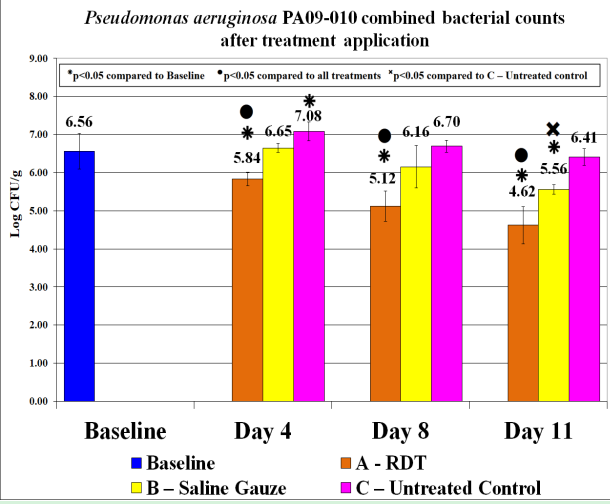
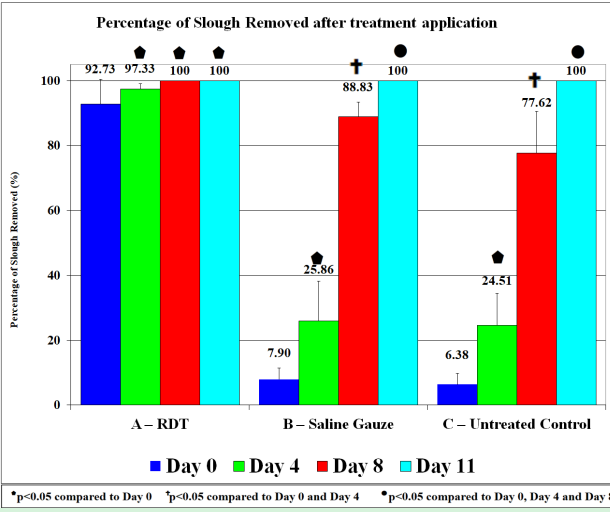
- On days 8 and 11, wounds treated with RDT results in 45.8 and 73.7% of re-epithelialization. RDT showed $p \leq 0.05$ compared to baseline. All other parameters had similar results.



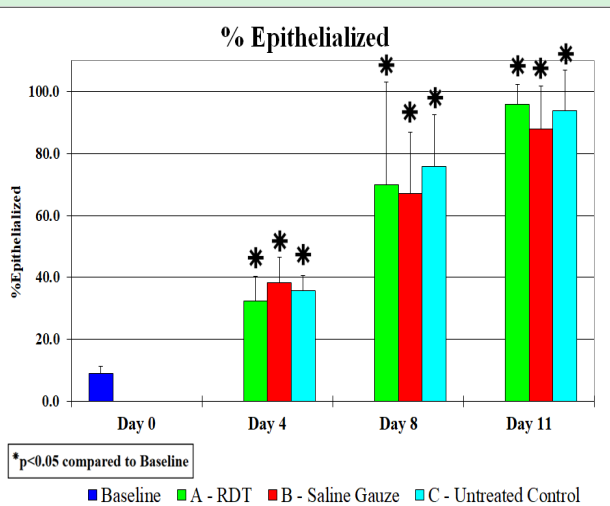
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Results:

Pseudomonas aeruginosa PA09-010



- RDT had a bacterial reductions of more than 99.70% and 99.86 compared to baseline bacterial counts and untreated group in all assessment days, respectively. ($p \leq 0.05$).



- RDT treated wounds resulted in reductions of 96.97%, 98.81% and 99.25% when compared to Gauze with sterile saline group in assessment Days 4, 8 and 11, respectively ($p \leq 0.05$). RDT treated wounds showed a more than 1 Log CFU/g bacterial reduction compared day 11 to day 4.

- On day 4, wounds treated with RDT exhibited the highest amount of re-epithelialization (27.9%) when compared against the other treatment groups.
- On days 4 and 8, wounds treated with RDT results in 32.2 and 69.9% of re-epithelialization. RDT showed $p \leq 0.05$ compared to baseline. All other parameters had similar results.

Conclusions

- Wounds treated with RDT had a higher percentage of slough removal and MRSA or PA 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 a higher bacterial reduction compared with the baseline and untreated wounds. Further analysis against a Positive Control group, such as Mupirocin, SSD and/or other conventional antimicrobial/healing therapies, would provide more insight on Revity's effectiveness to compete against what is currently utilized in wound care settings.