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Effects of a Novel Non-Biologic Desiccant to Remove Candida and Dermatophytes Using a Deep Partial Thickness Wound Porcine Model

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Abstract:

Introduction:

The prevalence of *Candida* and dermatophyte infections is increasing.[1,2] Trichophyton rubrum is one of the most prominent human pathogenic dermatophytes with almost 70% of chronic dermatophytoses.[2] *Candida albicans* is the most common species and can produce major complications in wounds such as burns.[3] Effects of a novel debridement method which use a novel molecular cleaning technology, to remove both necrotic tissue and dermatophytes from infected wounds using a porcine wound model was studied.[4,5]

Methods:

Deep partial thickness wounds (58wounds) measuring (10mm x 7mmx 0.5mm) were created and inoculated with either *Trichophyton rubrum* ATCC28188 (TR) or *Candida albicans* ATCC64550 (CA). Wounds were covered for 72hours to allow properly colonization. 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) Clotrimazole1% Positive Control+, or 3) Gauze with sterile saline. Wounds were treated for 30 or 60seconds and then rinsed with 5ml of sterile saline, then a sterile gauze was used to remove the slough and covered with a polyurethane film. For microbiology assessment, wounds were cultured using a 6mm punch biopsy. Wounds treated for 30seconds were assessed 20minutes and after 24hours wounds treated for 30 or 60seconds.

Results:

RDT* treated wounds showed a significant ($p \leq 0.05$) lowest CA and TR counts at 20minutes and 24hours (treated 30 or 60seconds) as compared to positive and untreated controls. At 24hours (treated 30 or 60 seconds) when comparing RDT* to untreated control showed 2.68±0.07 and 3.44±0.20 Log CFU/g a significant reduction ($p \leq 0.05$) in wounds infected with CA. Wounds infected with TR and treated (30 or 60seconds recovered at 24hours) with RDT* resulted in a significant reduction ($p \leq 0.05$) of 3.55±0.27 and 3.98±0.07 Log CFU/g respectively. Results showed a reduction ($p \leq 0.05$) after 60seconds treatment with RDT* compared with 30 seconds.

Discussion:

Overall, wounds treated with RDT* showed lower fungal counts at 20 minutes and 24 hours. Positive control had a good reduction compared to untreated in wounds infected with CA, however it did not appear as effective against TR. These results showed the important benefits of Revity in removing fungal infections in wounds.

*REVITY® – Epien Medical, Inc. Minneapolis, MN

+LOTRIMIN® AF - Bayer Healthcare LLC, Whippany, NJ, 07981

Introduction:

Infections with *C. albicans* and dermatophytes have been increased every years.[2] The presence of those organism in wounded area had implications for patients.[3] 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 fungal infections in wounds inoculated with *Trichophyton rubrum* ATCC28188(TR) or *Candida albicans* ATCC64550(CA).

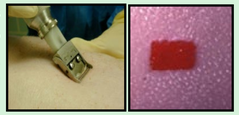
Materials and Methods:

1. Experimental Animals:

Swine were used as our experimental animal due to the morphological, physiological, and biochemical similarities between porcine skin and human skin.[6]

2. Wounding Technique:

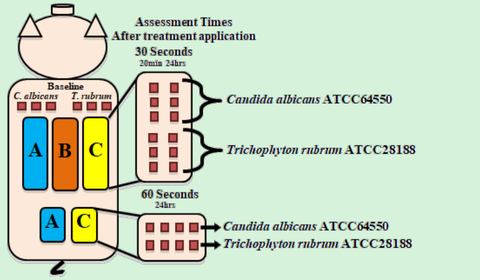
A specialized electrokeratome was used to create fifty-eight (58) deep partial thickness wounds measuring (10 mm x 7 mm x 0.5 mm deep) on the paravertebral and thoracic area.



3. Inoculation:

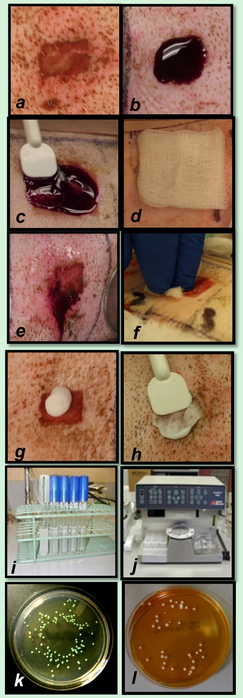
- After creation of wounds, 100µl of *Trichophyton rubrum* ATCC28188 (TR28188) and *Candida albicans* ATCC64550 (CA64550) was used to inoculate each wound by scrubbing 10^6 CFU/ml) inoculums into each wound with a teflon spatula (30 seconds).
- Sixteen (16) wounds were assigned to each treatment group (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 colonization).

4. Experimental Design:



5. Treatment Regimen:

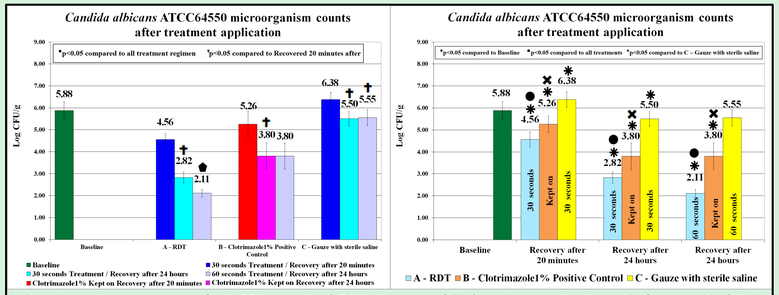
a. After 72 hours, all wounds were treated.
b. Wounds treated with RDT received 200ul.
c. RDT treatment was spread with spatula and allowed to stay in place for 30 or 60 seconds
d. Saline Irrigation wounds each had a premoisten gauze (200 µL of sterile saline) placed over the wound which was allowed to stay in place for 30 or 60 seconds.
e. After 30 or 60 seconds, all wounds were rinsed with a 5mL 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.
g. Clotrimazole 1% Positive Control wounds received 200mg of treated.
h. Positive control was spread with sterile spatula.



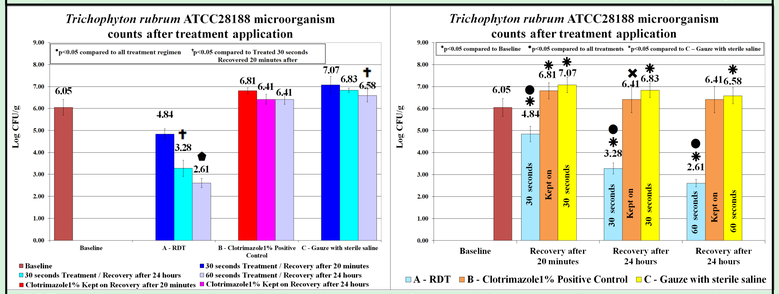
6. Wound Recovery:

- On Day 0 (72 hours after inoculation), three wounds from each organism were biopsied (6mm punch) as a baseline. Then three treated wounds were biopsied (6mm punch biopsy) 20 minutes after treatment application for each treatment group. The remaining wounds were cultured at 24 hours after treatment application.
- The biopsies (6mm) were weighed and immediately placed in 1 mL of All Purpose Neutralizing Solution.
- The sample was combined with an additional 4 mL of Neutralizing Solution and homogenized in a sterile homogenization tube.
- Serial dilutions (photo i) were made from all culture samples and the extent of microbiological contamination assessed using the Spiral Plater System (Spiral Biotech, Norwood, MA – photo j). This system deposits a 50µL aliquot of the scrub bacterial suspension over the surface of a rotating agar plate. BBL™ CHROMagar™ Candida was used to isolate CA64550 (photo k) and Dermatophyte Test Medium (photo l) was used to isolate (TR28188). All plates were incubated aerobically (24 hours – 5 days) at 30oC, after which the number of viable colonies were counted.

Results:



- Baseline wounds showed CA64550 counts of 5.88±0.41 Log CFU/g. Wounds treated with Gauze with Sterile Saline by 30 seconds exhibited the highest fungal counts among the entire study. RDT treated wounds resulted with the lowest fungal ($p \leq 0.05$) recovery either treated by 30 or 60 seconds.
- Wounds treated with RDT by 60seconds and recovered 24 hours after treatment application showed a significant reduction ($p \leq 0.05$) compared to all treatment regimens (2.11 ±0.17 Log CFU/g).
- Significant ($p \leq 0.05$) percentage of reduction was observed in wounds treated with RDT and recovered 24 hours compared to Clotrimazole1% Positive Control and Gauze with sterile saline (97.96% and 99.96%, respectively).



- RDT was the only treatment group that resulted in a significant ($p \leq 0.05$) lower counts than baseline results (both 20 minutes and 24 hours). Baseline wounds showed TR28188 counts of 6.05±0.41 Log CFU/g.
- Wounds treated with Gauze with Sterile Saline by 30 seconds exhibited the highest fungal counts among the entire study (7.07±0.39 Log CFU/g). Similar no significant results were observed in wounds treated with Clotrimazole1% Positive Control and Gauze with sterile saline in all assessment times.
- RDT treated wounds had the lowest fungal ($p \leq 0.05$) recovery showing a 2, 3 or 4 Log CFU/g reduction compared to Clotrimazole1% Positive Control and Gauze with sterile saline in all assessment times.

Conclusions

- Overall, those wounds treated with RDT showed substantially lower fungal counts against both microorganisms in both 20 minutes and 24 hours. Clotrimazole 1% Positive Control had lower fungal counts when treating wounds infected with *Candida albicans* ATCC64550, however it did not appear as effective in wounds infected with *Trichophyton rubrum* ATCC28188. These results showed that other no conventional treatments could be important strategies to take in consideration in the treatment of fungal infection. These preliminary results suggest that REVITY® may have significant benefits when treating wounds, however additional studies are needed to substantiate these findings using different fungal organisms.

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