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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:

Prevalence of candida and dermatophyte infections is increasing.[1,2] Trichophyton rubrum is one of the most prominent human pathogenic dermatophytes, and accounts for almost approximately 70% of chronic dermatophytosis in humans. Candidiasis is mostly caused by *Candida albicans* and produce major complications in burn wounds.[3] Candida and dermatophyte species cannot be overlooked as an infectious entity in chronic wounds and clinicians should be focused as well as bacteria biofilms. Burn wound infections remain the most important factor limiting survival in burn intensive care units. Large wounds, impaired immune systems and broad-spectrum antibiotic therapy contribute to the growth of opportunistic fungal species.[4] The aim of this study was examine the effects of a novel debridement method to remove necrotic tissue and dermatophytes using a porcine model.[5,6] Deep partial-thickness wounds (63) were created and inoculated with either *Trichophyton rubrum* ATCC28188(TR), *Trichophyton interdigitale* ATCC9533(TI) or *Candida albicans* ATCC64550(CA). Colonization was allowed by 72 hours then baseline wounds (3) were assessed prior apply treatments: 1) Regenerative Debridement Technology [RDT*], 2)Clotrimazole 1% Positive Control+, or 3)Gauze with sterile saline. Wounds were treated (30seconds) and then rinsed with 5ml of saline. Sterile gauze was used to remove slough. Biopsies (6mm) were taken 20minutes and 24hours for microbiology assessments. RDT treated wounds showed the lowest CA64550, TI9533 and TR28188 counts at 20 minutes and 24 hours as compared to positive and untreated controls. When challenging wounds treated with CA64550, both RDT and Clotrimazole 1% exhibited large fungal differences after 24 hours at 99.85% and 99.78%, respectively. Only those wounds treated with RDT showed a large fungal difference when compared to baseline wounds, having 96.22% and 94.88% reductions against TI9533 or TR28188, respectively. At 24 hours when comparing RDT to untreated control there was a 2.5 LogCFU/g, 2.3 LogCFU/g and 3.5 Log CFU/g reduction with CA, TI and TR, respectively. Overall, wounds treated with RDT showed lower fungal counts against the three microorganisms at both 20minutes and 24hours. Comparing the Clotrimazole 1% to untreated, we observed a good reduction in fungal counts when treating wounds infected with *Candida albicans* ATCC64550. However, Clotrimazole 1% did not appear as effective in wounds infected with either TI9533 or TR28188. Additional animals are currently planned to substantiate these findings which may have important clinical implications in the acute and chronic wound care therapies.

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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), *Trichophyton interdigitale* ATCC9533(TI) or *Candida albicans* ATCC64550(CA).

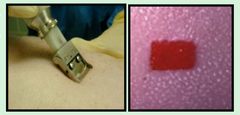
Materials and Methods:

1. Experimental Animals:

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

2. Wounding Technique:

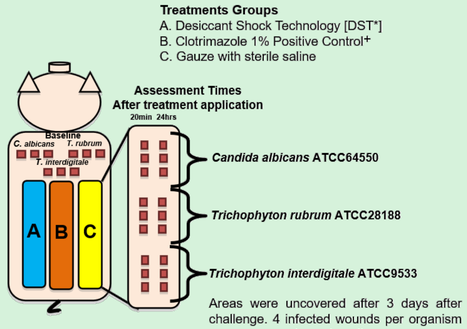
A specialized electrokeratome was used to create Sixty-three (63) deep partial thickness wounds measuring (10 mm x 7 mm x 0.5 mm deep) on the paravertebral and thoracic area.



3. Inoculation:

After creation of wounds, 100µl of *Trichophyton rubrum* ATCC28188 (TR28188), *Trichophyton interdigitale* ATCC9533 (TR9533) 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).
Nine (18) 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).

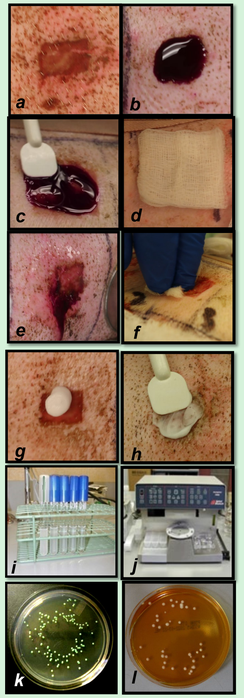
4. Experimental Design:



Areas were uncovered after 3 days after challenge. 4 infected wounds per organism were recovered as a baseline

5. Treatment Regimen:

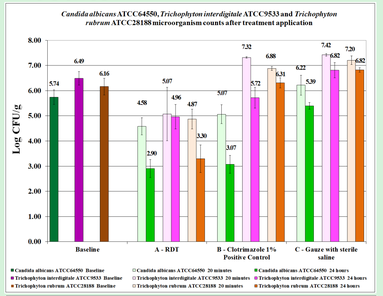
a. After 72 hours, all wounds were treated.
b. Wounds treated with RDT received 200ul.
c. RDT treatment was spread with spatula and allowed to stay in place for 30 seconds
d. Saline Irrigation wounds each had a premoisten gauze (200 µL of sterile saline) placed over the wound which was allowed to stay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

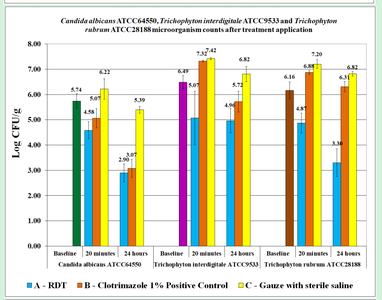


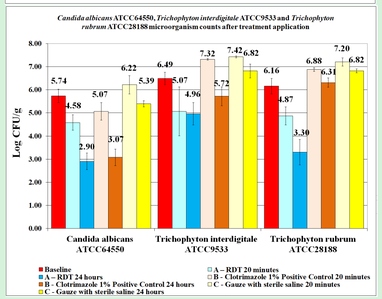
6. Wound Recovery:

- 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 the other 2 dermatophytes (TR28188 and TI9533). All plates were incubated aerobically (24 hours – 5 days) at 30oC, after which the number of viable colonies were counted.

Results:







- When comparing baseline wounds results, those wound infected TI9533 exhibited the highest microorganism counts at 6.49 ± 0.27 Log CFU/g. Both *CA*64550 and *TR*28188 exhibited baseline counts of 5.74 ± 0.29 and 6.16 ± 0.33 Log CFU/g, respectively.
- Those wounds treated with Gauze with Sterile Saline exhibited the highest fungal counts among the entire study for every microorganism.
- Wounds infected with CA64550 and TR28188 then treated with RDT were able to reduce their fungal counts when comparing 20 minutes and 24 hours after treatment application.
- RDT was the only treatment group that resulted in lower counts than baseline results (both 20 minutes and 24 hours).
- Fungal counts were exhibited after 24 hours with RDT treated wounds showing fungal reductions of 97.06% and 99.86% when compared against TI9533 and TR28188, respectively. When challenging wounds treated with CA64550, both RDT and Clotrimazole 1% Positive Control exhibited large fungal differences after 24 hours at 99.85% and 99.78%, respectively.
- For CA64550, after 24 hours, those wounds treated with RDT had slightly lower fungal counts than wounds treated with Clotrimazole 1% Lamisil Positive Control at 2.90 ± 0.36 and 3.07 ± 0.36 Log CFU/g, respectively.
- RDT treated wounds, in both 20 minutes and 24 hours results, exhibited substantially lower fungal counts against CA64550 and TI9533 when compared against results of wounds infected with TR28188.
- RDT showed compared against Gauze with Saline Control after 24 hours a fungal reduction of 98.60%. For those wounds infected with TR28188 after 20 minutes, RDT treated wounds had substantially lower fungal counts, with fungal reductions of 99.02% and 99.54% when compared against Positive Control and Gauze with Saline.

Conclusions

- Overall, those wounds treated with RDT showed substantially lower fungal counts against the three microorganisms and 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 either *Trichophyton interdigitale* ATCC9533 or *Trichophyton rubrum* ATCC28188. Additional samples would be required to substantiate these claims.

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