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UNIVERSITY OF MIAMI

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Pilot Study Report

Effects of Revity on Dermatophytes Using a Deep Partial Thickness Wound Porcine Model

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INSTITUTIONAL POLICIES AND REGULATIONS

The following experiment was submitted for approval by University of Miami's Animal Use Committee. This study was conducted in compliance of the University of Miami's Department of Dermatology & Cutaneous Surgery's Standard Operating Procedures (SOPs). Animal was monitored daily for any observable signs of pain or discomfort. In order to help minimize possible discomfort, two analgesics (buprenorphine and fentanyl transdermal patches) were used.

OBJECTIVE

The objective of this study was to assess the ability of Revity to reduce dermatophytes in deep partial thickness wounds.

MATERIALS AND METHODS

Experimental Animals

A porcine model was used for our experimental research due to the morphological similarities between swine skin and human skin. [1] One (1) animal was used for this study. The young specific pathogen free (SPF: Looper Farms, North Carolina) pig weighing 35-45 kg was kept in house for at least 5 days prior to initiating the experiment. The animal was fed a basal diet *ad libitum* and was housed individually in our animal facilities (meeting American Association for Accreditation of Laboratory Animal Care [AAALAC] accredited) with controlled temperature (19-21^{o}C) and lighting (12h/12h LD).

Procedure Technique

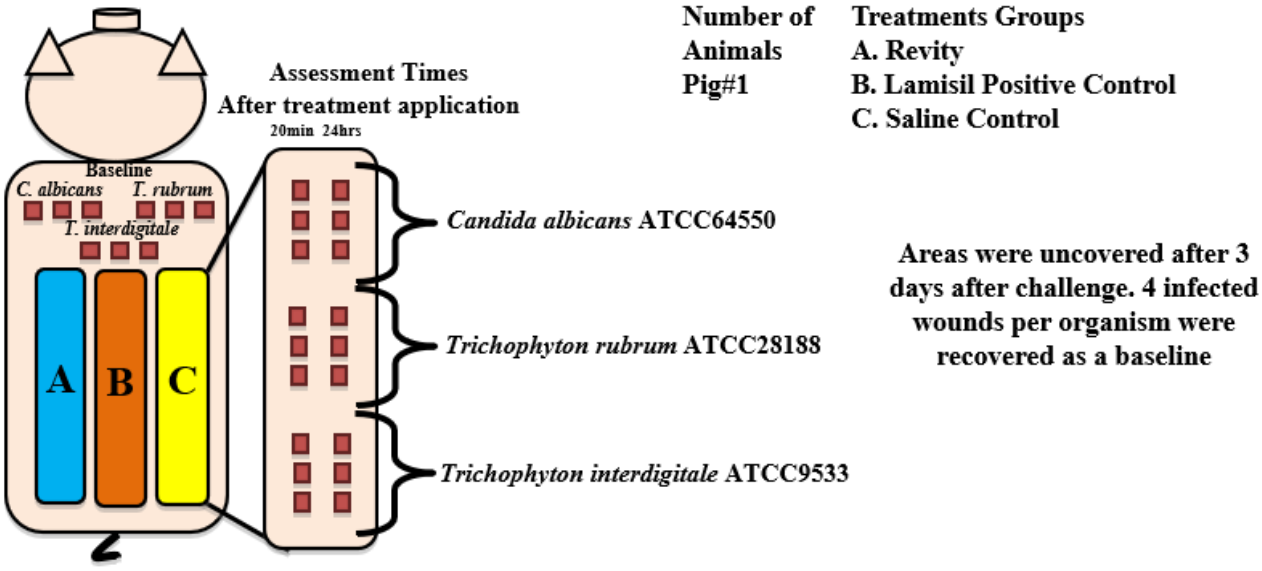
The back of the experimental animal was clipped with standard animal clippers on the day of the experiment. The skin on both sides of the animal was prepared by washing with a non-antibiotic soap (Neutrogena Soap Bar; Johnson and Johnson, Los Angeles, CA) and sterile water. Animal was anesthetized and given analgesics till the end of the study.

Sixty-three (63) deep partial thickness wounds measuring (10 mm x 7 mm x 0.5 mm deep) were made in the paravertebral and thoracic area with a specialized electrokeratome fitted with a 10 mm blade. The wounds were separated from one another by more than 3-5 cm of unwounded skin and individually dressed. Wounds were randomly divided into three (3) groups of eighteen (18) wounds in each group, in each group 3 subgroups of six wounds were infected with different organisms. Three extra wounds were assigned as a baseline for each microorganism (see Figure 1 below).

Wound Inoculation

A fresh culture of *Trichophyton rubrum* ATCC28188 (TR28188), *Trichophyton interdigitale* ATCC9533 (TR9533) and *Candida albicans* ATCC64550 (CA64550) were used for the infected animal. The challenge inoculum suspension was prepared by inoculating a 25 mL bottle of Sabouraud Dextrose Broth with a loop of each organism saved in a cryotube at -80^{o}C stock culture, the bottle was placed to growth in a shaker overnight 600rpm at 30^{o}C. This resulted in a suspension concentration of approximately 10^5 to 10^6 colony forming units/ml (CFU/ml) for the fungus. The inoculum was vortexed and 100 µL of the suspension was inoculated into each wound. In addition, serial dilutions of the suspension were plated onto selective media (see below) and plates were incubated aerobically overnight (2-4 days) at 30^{o}C, to quantify the exact concentration of viable organisms used for this experiment.

Figure 1: Experimental Design

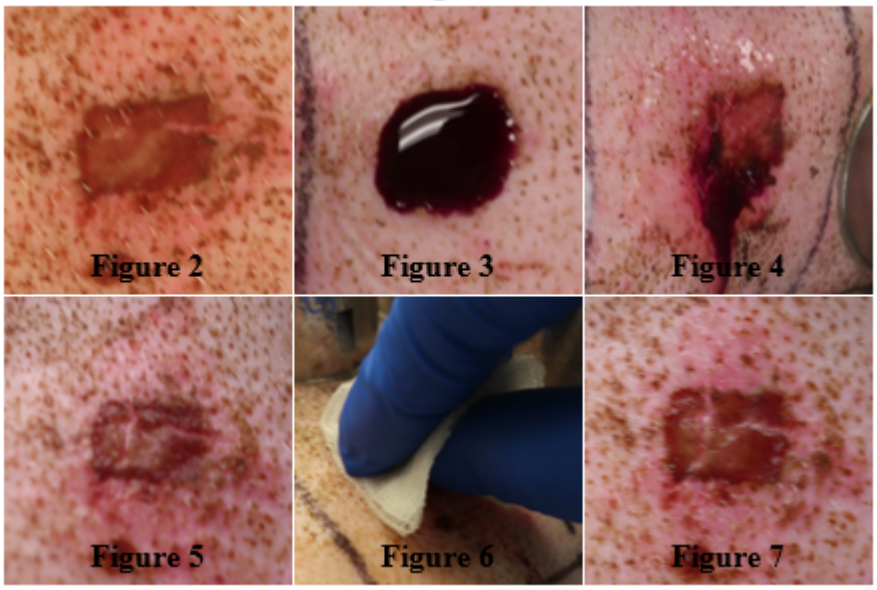


Treatment Regimen

After 72 hours of infection (Day 0 of treatment), the polyurethane film dressings were removed, and 3 infected wounds were recovered as a baseline for each microorganism. The remaining wounds were randomly assigned one of the following treatments groups: A) Revity, B) Positive Control [Lamisil], or C) Saline Control (see Figure 1 above).

The Revity treated area received approximately 200 µL of treatment and was allowed to remain in place for 30 seconds, then was rinsed with a 5mL syringe with sterile saline followed by wiping the area with sterile saturated gauze (see Figures 2 – 7 for example).

All Positive Control wounds received 200mg of Lamisil each and was spread



around the wound with a sterile spatula (see process in Figures 8 – 10).

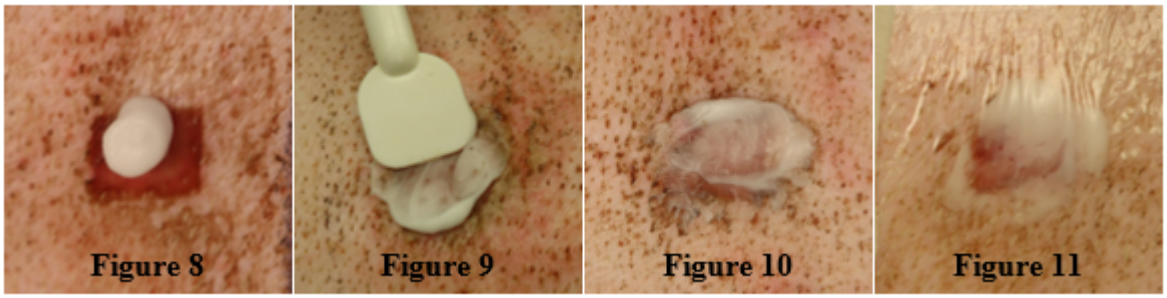


Figure 8 Figure 9 Figure 10 Figure 11

Saline Control wounds each receive a saline moisten sterile gauze placed over for 30 seconds as shown in Figures 12 and 13, then was rinsed with sterile saline as discussed above in Revity procedure.

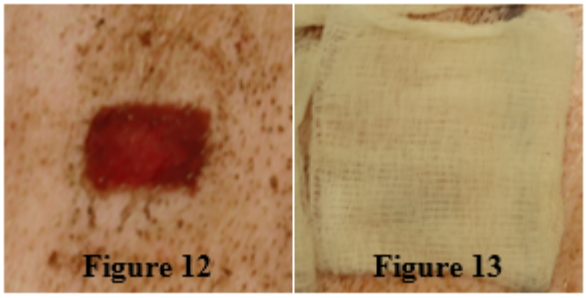


Figure 12 Figure 13

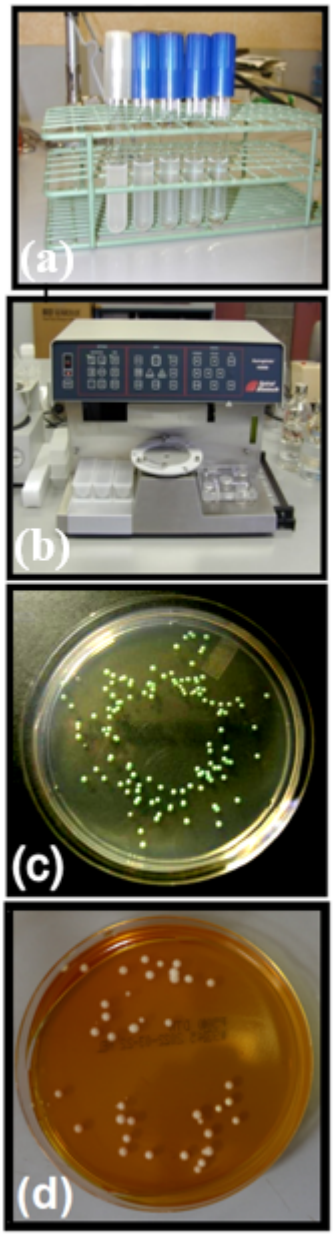
All treatments were applied only once. Within 20 minutes of treatment application, 3 wounds were cultured as described below in "Microbiology Assessment". The remaining wounds were individually covered with a polyurethane film dressing (Tegaderm, 3M, St. Paul MN) as seen in Figure 11 above as example. All dressings were secured in place with tape and covered with Coban wrap (3M, St. Paul MN).

Microbiology Assessment

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 (Figure 14: photo **a**) were made from all culture samples and the extent of microbiological contamination assessed using the Spiral Plater System (Spiral Biotech, Norwood, MA – photo **b**). This system deposits a 50µL aliquot of the scrub fungal suspension over the surface of a rotating agar plate. BBL™ CHROMagar™ Candida was used to isolate CA64550 (Figure 14: photo **c**) and Dermatophyte Test Medium (Figure 14: photo **d**) was used to isolate the other 2 dermatophytes (TR28188 and TI9533). All plates were incubated aerobically (24 hours – 5 days) at 30°C, after which the number of viable colonies were counted.

Figure 14:



(a) Serial Dilutions,
(b) Spiral Platter,
(c) CHROMagar™ Candida
(d) Dermatophyte Test Medium

Clinical Observations

All wounds infected in each treatment group with different organisms were observed visually and for erythema (see photo examples in Appendix 1: Table 1 – 3).

Erythema Measurements

During each assessment time the amount of erythema (redness) around the area were clinically scored.

Erythema – indicative of the amount of inflammation present*
* Score: 1 = absent, 2 = mild, 3 = moderate, 4 = marked, 5 = exuberant

All CA64550 infected wounds exhibited mild erythema on Day 0 before treatment application. No erythema was observed on TR28188 and TI9533 infected wounds on the same day. At 24 hours after treatment applications, none of the wounds infected with pathogens (CA64550, TR28188, and TI9533) had any signs of erythema as shown in Appendix 1, Tables 1-3.

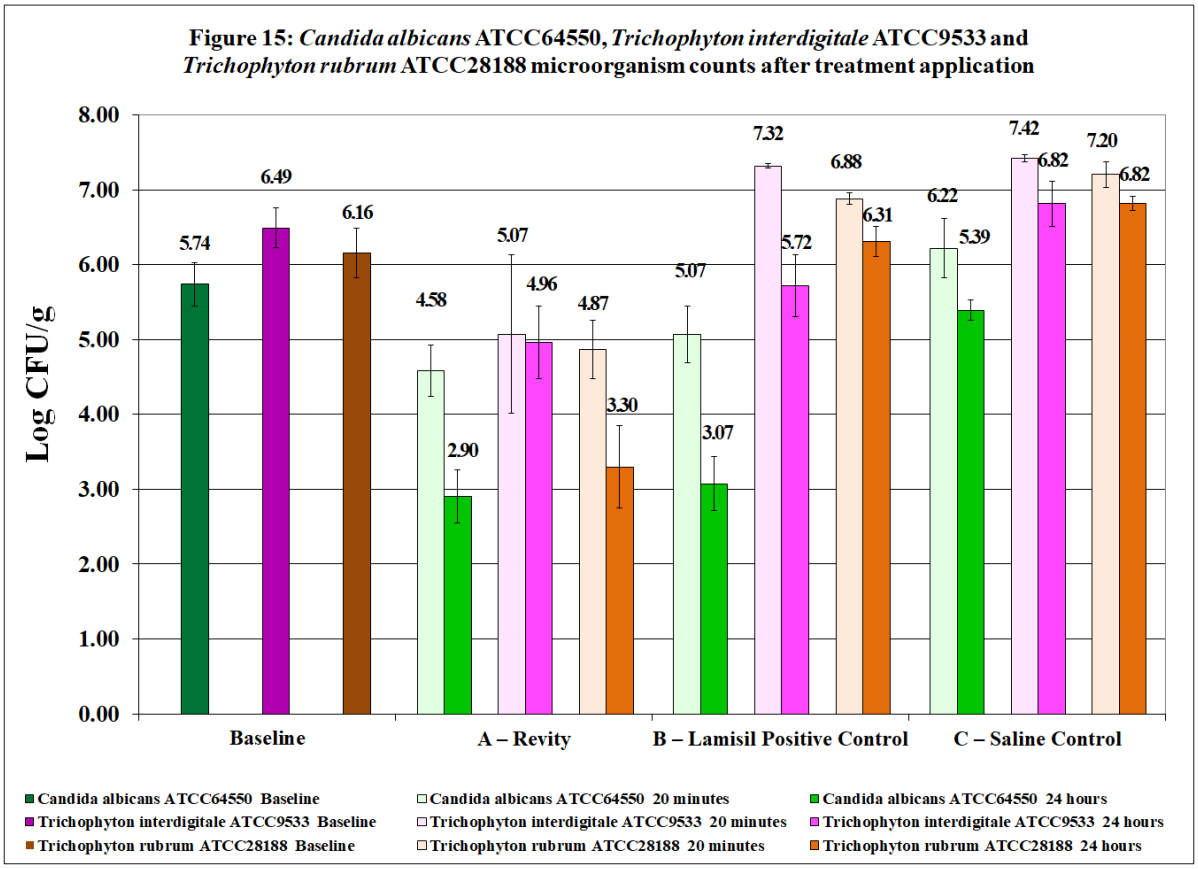
RESULTS

Microbiology Results

After counting the colonies, the data was tabulated and the Log of colony forming units/g (Log CFU/g) was determined. The mean of the Log (CFU/g) were calculated for each time and treatment. Appendix 2 contains the raw data.

When analyzing the data and compare within each groups' results, Figure 15 shows the differences between baseline and counts after 20 minutes and 24 hours after treatment. When comparing baseline wounds results, those wound infected with *Trichophyton interdigitale* ATCC9533 exhibited the highest microorganism counts at 6.49 ± 0.27 Log CFU/g. Both *Candida albicans* ATCC64550 and *Trichophyton rubrum* ATCC28188 exhibited baseline counts of 5.74 ± 0.29 and 6.16 ± 0.33 Log CFU/g, respectively.

Figure 15: *Candida albicans* ATCC64550, *Trichophyton interdigitale* ATCC9533 and *Trichophyton rubrum* ATCC28188 microorganism counts after treatment application



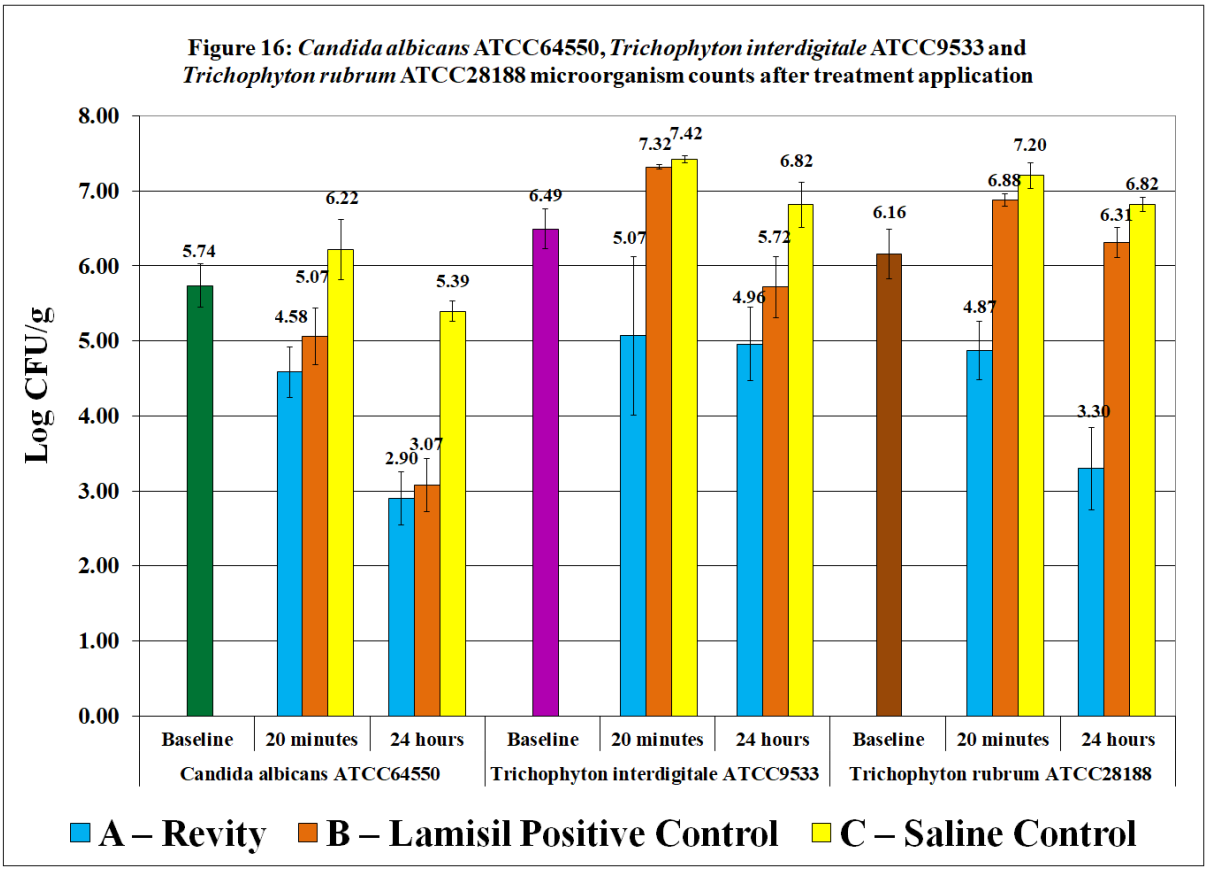
Wounds infected with *Candida albicans* ATCC64550 and *Trichophyton rubrum* ATCC28188 then treated with Revity were able to reduce their fungal counts when comparing 20 minutes and 24 hours after treatment application. While those wounds infected with *Trichophyton interdigitale* ATCC9533 showed fungal counts fairly similar at 20 minutes and 24 hours after treatment at 5.07 ± 1.06 and 4.96 ± 0.49 Log CFU/g, respectively.

All wounds, except those infected with *Candida albicans* ATCC64550, treated with Lamisil Positive Control after 20 minutes reached fungal counts higher than baseline, with those wounds infected with *Trichophyton interdigitale* ATCC9533 which had the highest count at 7.32± 0.03 Log CFU/g. At 24 hours after treatment application, wounds infected with *Trichophyton rubrum* ATCC28188 exhibited the highest fungal count (6.31 ± 0.20 Log CFU/g) among those wounds treated with Lamisil Positive Control. Those wounds infected with *Candida albicans* ATCC64550 showed

substantially lower fungal counts than all other results within this treatment group at 5.07 ± 0.38 Log CFU/g after 20 minutes and then further reducing fungal counts to 3.07 ± 0.36 Log CFU/g.

Those wounds treated with Saline Control exhibited the highest fungal counts among the entire study for every microorganism. The largest reduction from 20 minutes to 24 hours after treatment application for Saline Control was shown by wounds infected with *Candida albicans* ATCC64550 at 6.22 ± 0.40 and 5.39 ± 0.14 Log CFU/g, respectively. Those wounds infected with *Trichophyton interdigitale* ATCC9533 had the highest fungal counts for both 20 minutes and 24 hours at 7.42 ± 0.05 and 6.82 ± 0.30 Log CFU/g, respectively.

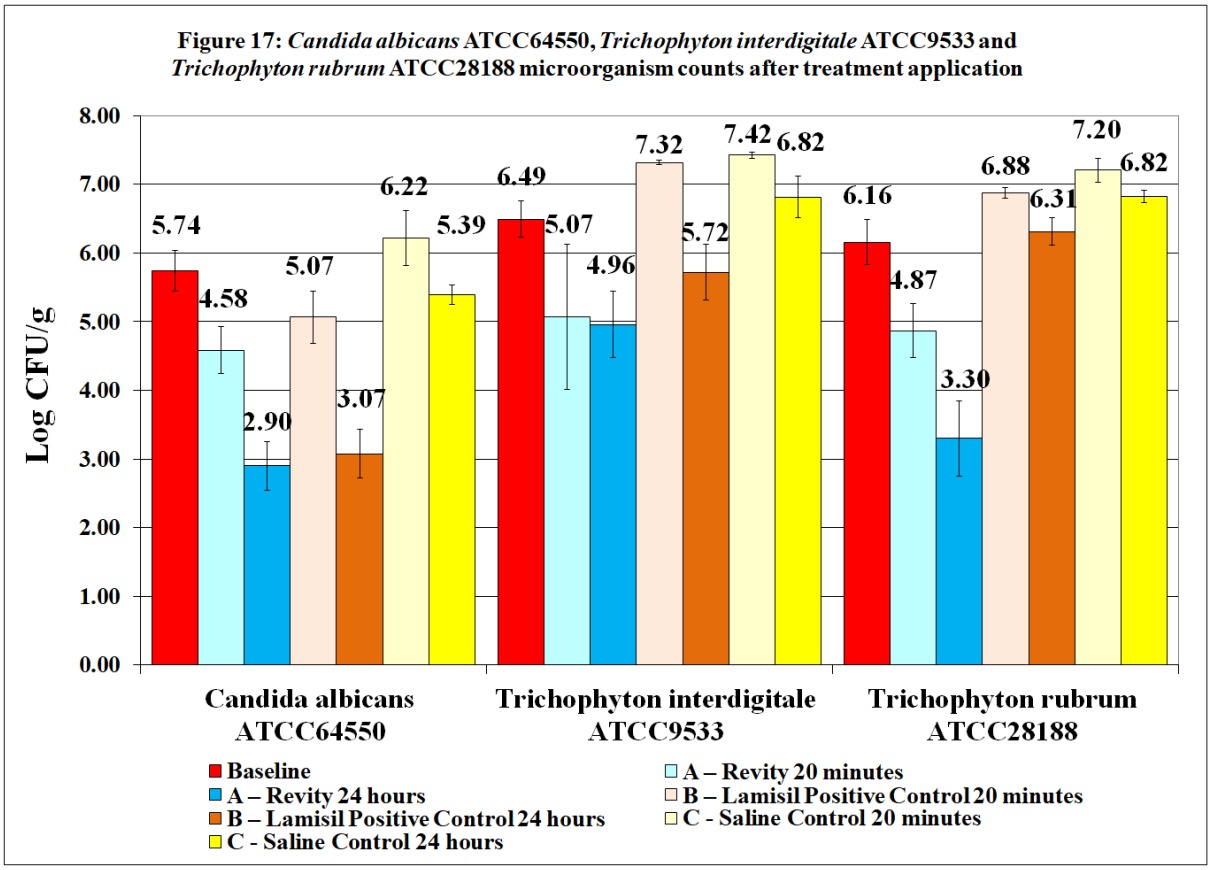
Figure 16: *Candida albicans* ATCC64550, *Trichophyton interdigitale* ATCC9533 and *Trichophyton rubrum* ATCC28188 microorganism counts after treatment application



When comparing all results by time points, Figure 16 shows wounds treated with Revity being the only treatment group that resulted in lower counts than baseline results (both 20 minutes and 24 hours). Wounds infected with *Trichophyton interdigitale* ATCC9533 and *Trichophyton rubrum*

ATCC28188 were not affected by Lamisil Positive Control or Saline Control at 20 minutes and 24 hours since their results increased compared with Baseline wounds. Only those wounds treated with Revity showed a large fungal difference when compared to baseline wounds, having 96.22% and 94.88% reductions against *Trichophyton interdigitale* ATCC9533 and *Trichophyton rubrum* ATCC28188, respectively. Furthermore differences in fungal counts were exhibited after 24 hours with Revity treated wounds showing fungal reductions of 97.06% and 99.86% when compared against *Trichophyton interdigitale* ATCC9533 and *Trichophyton rubrum* ATCC28188, respectively. When challenging wounds treated with *Candida albicans* ATCC64550, both Revity and Lamisil Positive Control exhibited large fungal differences after 24 hours at 99.85% and 99.78%, respectively. Figure 16 also shows Revity treated wounds having lower fungal counts than both Lamisil Positive Control and Saline Control at every time point against each of the three microorganisms.

Wounds infected with *Candida albicans* ATCC64550 showed the lowest fungal counts, with Saline Control having the highest fungal counts. After 24 hours, those wounds treated with Revity had slightly lower fungal counts than wounds treated with Lamisil Positive Control at 2.90 ± 0.36 and 3.07 ± 0.36 Log CFU/g, respectively. Wounds infected with *Trichophyton interdigitale* ATCC9533, having the highest baseline count, showed wounds treated with Revity having substantially lower fungal counts than both Lamisil Positive Control and Saline Control for both 20 minutes and after 24 hours. Wounds infected with *Trichophyton rubrum* ATCC28188 showed a similar trend by having Revity treated wounds with substantially lower fungal counts than all remaining wounds in both 20 minutes and 24 hours.



When comparing all results within each of the three microorganisms, those wounds infected with *Candida albicans* ATCC64550 showed the lowest fungal counts as shown in Figure 17. Revity treated wounds, in both 20 minutes and 24 hours results, exhibited substantially lower fungal counts against *Candida albicans* ATCC64550 and *Trichophyton interdigitale* ATCC9533 when compared against results of wounds infected with *Trichophyton rubrum* ATCC28188. Those wounds infected with *Candida albicans* ATCC64550 and treated with Revity after 24 hours had a fungal difference of 2.49 ± 0.22 Log CFU/g when compared against Saline Control, which yields a fungal reduction percentage of 99.68%. Revity treated wounds after 20 minutes had large fungal differences against both Lamisil Positive Control and Saline Control at 99.44 and 99.56%, respectively, when treating wounds infected with *Trichophyton interdigitale* ATCC9533. When Revity was compared against Saline Control after 24 hours, there was a fungal reduction of 98.60%. For those wounds infected

with *Trichophyton rubrum* ATCC28188 after 20 minutes, Revity treated wounds had substantially lower fungal counts, with fungal reductions of 99.02% and 99.54% when compared against Lamisil Positive Control and Saline Control, respectively. The same comparison after 24 hours shows a larger fungal count difference between Revity and the other groups with fungal reductions of 99.90% and 99.97%, respectively.

CONCLUSIONS

Overall, those wounds treated with Revity showed substantially lower fungal counts against the three microorganisms and in both 20 minutes and 24 hours. Lamisil 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.

Appendix 1. Clinical Observations

Visual and erythema observations of all groups for each organism on both assessment days.

Table 1: CA64550 Infected Wounds

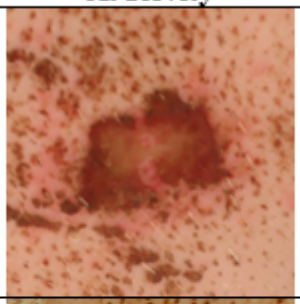
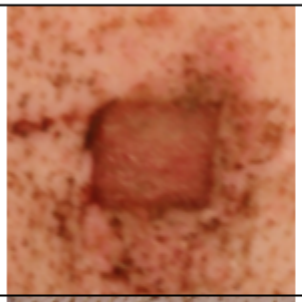
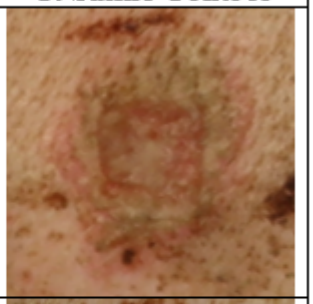
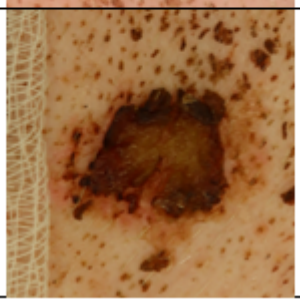
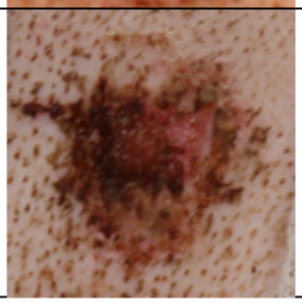
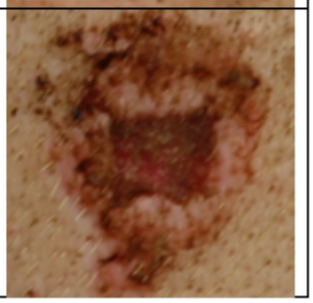
	A. Revity	B. Positive Control	C. Saline Control
Day 0			
Day 1			

Table 2: TR28188 Infected Wounds

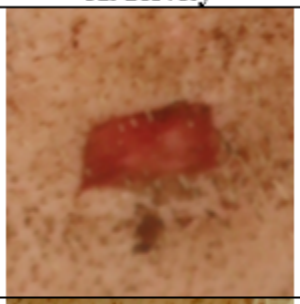
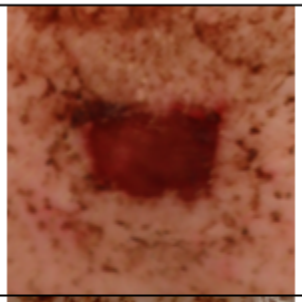
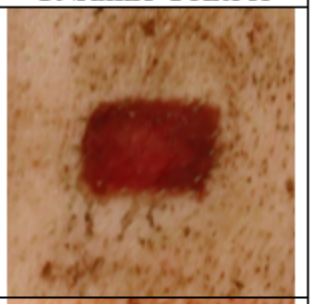
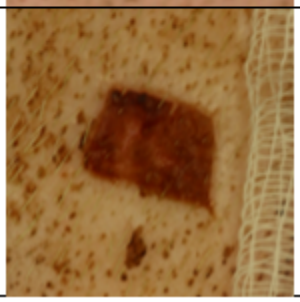
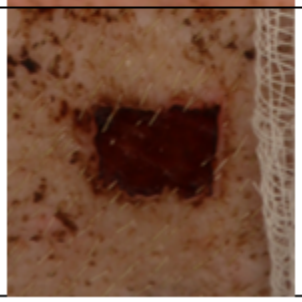
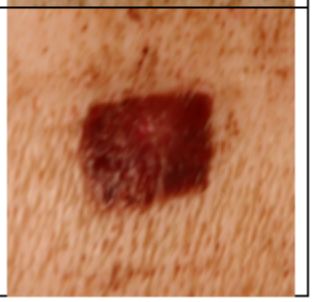
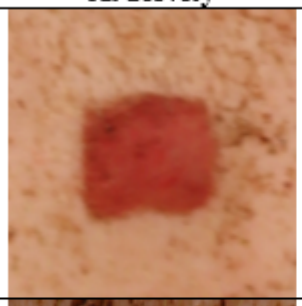
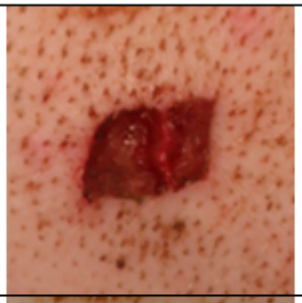
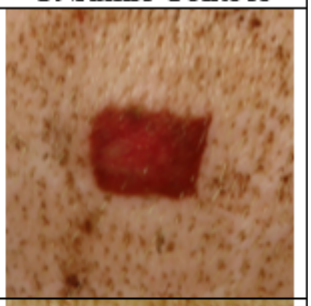
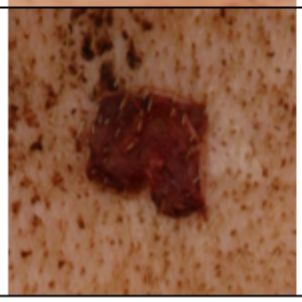
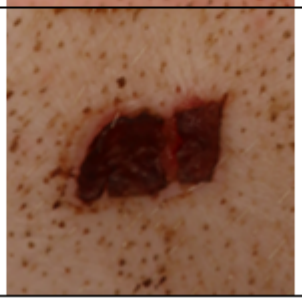
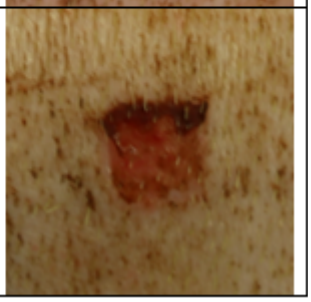
	A. Revity	B. Positive Control	C. Saline Control
Day 0			
Day 1			

Table 3: TI9533 Infected Wounds			
	A. Revity	B. Positive Control	C. Saline Control
Day 0			
Day 1			

Appendix 2. (Raw Data)

Effects of Revity on Dermatophytes Using a Deep Partial Thickness wound Porcine Model

Pig P22-166/24

Inoculum

Strain	**Dilution**	**Count**	**CFU/ml**	**Log CFU/ml**
Candida albicans ATCC64550	**-4**	**76**	**1.52E+07**	**7.18**
Trichophyton interdigitale ATCC9533	**-4**	**49**	**9.79E+06**	**6.99**
Trichophyton rubrum ATCC28188	**-4**	**28**	**5.60E+06**	**6.75**

***Candida albicans* ATCC64550**

Baseline

BBL™ CHROMagar™ Candida count in wounds recovered 72 after wounding and infection

Treatment	Dilution	Count	CFU/ml	Log CFU/ml	
Baseline	-3	121	4.84E+06	6.68	
	-3	38	1.52E+06	6.18	
	-3	21	8.39E+05	5.92	STDV
		Mean	2.40E+06	6.26	0.39

Number of organism per g

Treatment	Number of Colonies (N)	Volume of ALL purpose Neutralizer (V)	Dilution Factor (D)	Weight Biopsy(g) X	CFU/g	Log CFU/g	
Baseline	121	2	1000	0.211	1.15E+06	6.06	
	38	2	1000	0.163	4.66E+05	5.67	
	21	2	1000	0.137	3.07E+05	5.49	STDV
				Mean	6.40E+05	5.74	0.29

20 minutes

BBL™ CHROMagar™ Candida count in wounds recovered 20 minutes after tretament application

Treatment	Dilution	Count	CFU/ml	Log CFU/ml	
A – Revity	-2	31	1.24E+05	5.09	
	-1	186	7.84E+04	4.89	
	-2	94	3.76E+05	5.57	STDV
		Mean	1.93E+05	5.18	0.35

Number of organism per g

Treatment	Number of Colonies (N)	Volume of ALL purpose Neutralizer (V)	Dilution Factor (D)	Weight Biopsy(g) X	CFU/g	Log CFU/g	
A – Revity	31	2	100	0.198	3.13E+04	4.50	
	186	2	10	0.187	1.99E+04	4.30	
	94	2	100	0.206	9.13E+04	4.96	STDV
				Mean	4.75E+04	4.58	0.34

BBL™ CHROMagar™ Candida count in wounds recovered 20 minutes after tretament application

Treatment	Dilution	Count	CFU/ml	Log CFU/ml	
B – Lamisil Positive Control	-3	24	9.59E+05	5.98	
	-2	170	6.80E+05	5.93	
	-2	51	2.04E+05	5.31	STDV
		Mean	6.14E+05	5.74	0.37

Number of organism per g

Treatment	Number of	purpose	Dilution	Weight	CFU/g	Log CFU/g	
B – Lamisil Positive Control	24	2	1000	0.194	2.47E+05	5.39	
	170	2	100	0.239	1.42E+05	5.15	
	51	2	100	0.228	4.47E+04	4.65	STDV
				Mean	1.45E+05	5.07	0.38

BBL™ CHROMagar™ Candida count in wounds recovered 20 minutes after tretament application

Treatment	Dilution	Count	CFU/ml	Log CFU/ml	
C – Saline Control	-3	178	7.12E+06	6.85	
	-4	30	1.20E+07	7.08	
	-3	52	2.08E+06	6.32	STDV
		Mean	7.07E+06	6.75	0.39

Number of organism per g

Treatment	Number of Colonies (N)	Volume of ALL purpose Neutralizer (V)	Dilution Factor (D)	Weight Biopsy(g) X	CFU/g	Log CFU/g	
C – Saline Control	178	2	1000	0.136	2.62E+06	6.42	
	30	2	10000	0.200	3.00E+06	6.48	
	52	2	1000	0.180	5.78E+05	5.76	STDV
				Mean	2.07E+06	6.22	0.40

24 hours

BBL™ CHROMagar™ Candida count in wounds recovered 24 hours after tretament application

Treatment	Dilution	Count	CFU/ml	Log CFU/ml	
A – Revity	0	29	1.16E+03	3.06	
	0	115	4.60E+03	3.66	
	0	146	5.84E+03	3.77	STDV
		Mean	3.87E+03	3.50	0.38

Number of organism per g

Treatment	Number of Colonies (N)	Volume of ALL purpose Neutralizer (V)	Dilution Factor (D)	Weight Biopsy(g) X	CFU/g	Log CFU/g	
A – Revity	29	2	1	0.187	3.10E+02	2.49	
	115	2	1	0.183	1.26E+03	3.10	
	146	2	1	0.223	1.31E+03	3.12	STDV
				Mean	9.59E+02	2.90	0.36

BBL™ CHROMagar™ Candida count in wounds recovered 24 hours after tretament application

Treatment	Dilution	Count	CFU/ml	Log CFU/ml	
B – Lamisil Positive Control	-1	148	5.92E+04	4.77	
	-1	41	1.64E+04	4.21	
	-2	20	8.00E+04	4.90	STDV
		Mean	5.19E+04	4.63	0.37

Number of organism per g

Treatment	Number of	purpose	Dilution	Weight	CFU/g	Log CFU/g	
B – Lamisil Positive Control	148	2	1	0.157	1.89E+03	3.28	
	41	2	1	0.178	4.61E+02	2.66	
	20	2	10	0.207	1.93E+03	3.29	STDV
				Mean	1.43E+03	3.07	0.36

BBL™ CHROMagar™ Candida count in wounds recovered 24 hours after tretament application

Treatment	Dilution	Count	CFU/ml	Log CFU/ml	
C – Saline Control	-2	140	4.60E+05	5.75	
	-3	24	9.59E+05	5.98	
	-2	197	7.88E+05	5.90	STDV
		Mean	7.36E+05	5.88	0.12

Number of organism per g

Treatment	Number of Colonies (N)	Volume of ALL purpose Neutralizer (V)	Dilution Factor (D)	Weight Biopsy(g) X	CFU/g	Log CFU/g	
C – Saline Control	140	2	100	0.144	1.94E+05	5.29	
	24	2	1000	0.136	3.53E+05	5.55	
	197	2	100	0.179	2.20E+05	5.34	STDV
				Mean	2.56E+05	5.39	0.14

Trichophyton interdigitale ATCC9533

Baseline

Dermatophyte Test Medium Trichophyton interdigitale count in wounds recovered 72 after wounding and infection

Treatment	Dilution	Count	CFU/ml	Log CFU/ml	
Baseline	-4	43	1.72E+07	7.24	
	-3	118	4.72E+06	6.67	
	-4	26	1.04E+07	7.02	STDV
		Mean	1.08E+07	6.98	0.29

Number of organism per g

Treatment	Number of Colonies (N)	Volume of ALL purpose Neutralizer (V)	Dilution Factor (D)	Weight Biopsy(g) X	CFU/g	Log CFU/g	
Baseline	43	2	10000	0.158	5.44E+06	6.74	
	118	2	1000	0.146	1.62E+06	6.21	
	26	2	10000	0.153	3.40E+06	6.53	STDV
				Mean	3.49E+06	6.49	0.27

20 minutes

Dermatophyte Test Medium Trichophyton interdigitale count in wounds recovered 20 minutes after tretament application

Treatment	Dilution	Count	CFU/ml	Log CFU/ml	
A – Revity	-2	22	8.79E+04	4.94	
	-2	20	8.00E+05	5.90	
	-3	140	5.60E+05	5.75	STDV
		Mean	4.83E+05	5.53	0.52

Number of organism per g

Treatment	Number of Colonies (N)	Volume of ALL purpose Neutralizer (V)	Dilution Factor (D)	Weight Biopsy(g) X	CFU/g	Log CFU/g	
A – Revity	22	2	100	0.158	2.78E+04	4.44	
	20	2	100	0.135	2.96E+04	4.47	
	140	2	1000	0.143	1.96E+06	6.29	STDV
				Mean	6.72E+05	5.07	1.06

Dermatophyte Test Medium Trichophyton interdigitale count in wounds recovered 20 minutes after tretament application

Treatment	Dilution	Count	CFU/ml	Log CFU/ml	
B – Lamisil Positive Control	-4	171	6.84E+07	7.83	
	-4	196	7.84E+07	7.89	
	-4	183	7.32E+07	7.86	STDV
		Mean	7.33E+07	7.86	0.03

Number of organism per g

Treatment	Number of	purpose	Dilution	Weight	CFU/g	Log CFU/g	
B – Lamisil Positive Control	171	2	10000	0.178	1.92E+07	7.28	
	196	2	10000	0.177	2.21E+07	7.35	
	183	2	10000	0.171	2.14E+07	7.33	STDV
				Mean	2.09E+07	7.32	0.03

Dermatophyte Test Medium Trichophyton interdigitale count in wounds recovered 20 minutes after tretament application

Treatment	Dilution	Count	CFU/ml	Log CFU/ml	
C – Saline Control	-4	192	7.68E+07	7.89	
	-4	199	7.96E+07	7.90	
	-5	23	9.19E+07	7.96	STDV
		Mean	8.28E+07	7.92	0.04

Number of organism per g

Treatment	Number of Colonies (N)	Volume of ALL purpose Neutralizer (V)	Dilution Factor (D)	Weight Biopsy(g) X	CFU/g	Log CFU/g	
C – Saline Control	192	2	10000	0.139	2.76E+07	7.44	
	199	2	10000	0.170	2.34E+07	7.37	
	23	2	100000	0.160	2.88E+07	7.46	STDV
				Mean	2.66E+07	7.42	0.05

24 hours

Dermatophyte Test Medium Trichophyton interdigitale count in wounds recovered 24 hours after tretament application

Treatment	Dilution	Count	CFU/ml	Log CFU/ml	
A – Revity	-2	160	6.40E+05	5.81	
	-2	64	2.56E+05	5.41	
	-1	193	7.72E+04	4.89	STDV
		Mean	3.24E+05	5.37	0.46

Number of organism per g

Treatment	Number of Colonies (N)	Volume of ALL purpose Neutralizer (V)	Dilution Factor (D)	Weight Biopsy(g) X	CFU/g	Log CFU/g	
A – Revity	160	2	100	0.123	2.60E+05	5.42	
	64	2	100	0.123	1.04E+05	5.02	
	193	2	10	0.137	2.82E+04	4.45	STDV
				Mean	1.31E+05	4.96	0.49

Dermatophyte Test Medium Trichophyton interdigitale count in wounds recovered 24 hours after tretament application

Treatment	Dilution	Count	CFU/ml	Log CFU/ml	
B – Lamisil Positive Control	-3	122	4.88E+06	6.69	
	-2	191	7.64E+05	5.88	
	-3	53	2.12E+06	6.33	STDV
		Mean	2.59E+06	6.30	0.41

Number of organism per g

Treatment	Number of	purpose	Dilution	Weight	CFU/g	Log CFU/g	
B – Lamisil Positive Control	122	2	1000	0.182	1.34E+06	6.13	
	191	2	100	0.187	2.04E+05	5.31	
	53	2	1000	0.202	5.25E+05	5.72	STDV
				Mean	6.90E+05	5.72	0.41

Dermatophyte Test Medium Trichophyton interdigitale count in wounds recovered 24 hours after tretament application

Treatment	Dilution	Count	CFU/ml	Log CFU/ml	
C – Saline Control	-4	46	1.84E+07	7.26	
	-4	91	3.64E+07	7.56	
	-4	27	1.08E+07	7.03	STDV
		Mean	2.19E+07	7.28	0.27

Number of organism per g

Treatment	Number of Colonies (N)	Volume of ALL purpose Neutralizer (V)	Dilution Factor (D)	Weight Biopsy(g) X	CFU/g	Log CFU/g	
C – Saline Control	46	2	10000	0.132	6.97E+06	6.84	
	91	2	10000	0.144	1.26E+07	7.10	
	27	2	10000	0.170	3.18E+06	6.50	STDV
				Mean	7.60E+06	6.82	0.30

Trichophyton rubrum ATCC28188

Baseline

Dermatophyte Test Medium Trichophyton rubrum count in wounds recovered 72 after wounding and infection

Treatment	Dilution	Count	CFU/ml	Log CFU/ml	
Baseline	-3	42	1.68E+06	6.23	
	-3	171	6.84E+06	6.83	
	-4	22	8.79E+06	6.94	STDV
		Mean	5.77E+06	6.67	0.38

Number of organism per g

Treatment	Number of Colonies (N)	Volume of ALL purpose Neutralizer (V)	Dilution Factor (D)	Weight Biopsy(g) X	CFU/g	Log CFU/g	
Baseline	42	2	1000	0.141	5.96E+05	5.78	
	171	2	1000	0.160	2.14E+06	6.33	
	22	2	10000	0.186	2.37E+06	6.37	STDV
				Mean	1.70E+06	6.16	0.33

20 minutes

Dermatophyte Test Medium Trichophyton rubrum count in wounds recovered 20 minutes after tretament application

Treatment	Dilution	Count	CFU/ml	Log CFU/ml	
A – Revity	-2	174	6.96E+05	5.84	
	-2	27	1.08E+05	5.03	
	-2	48	1.92E+05	5.28	STDV
		Mean	3.32E+05	5.38	0.41

Number of organism per g

Treatment	Number of Colonies (N)	Volume of ALL purpose Neutralizer (V)	Dilution Factor (D)	Weight Biopsy(g) X	CFU/g	Log CFU/g	
A – Revity	174	2	100	0.175	1.99E+05	5.30	
	27	2	100	0.158	3.42E+04	4.53	
	48	2	100	0.161	5.96E+04	4.78	STDV
				Mean	9.76E+04	4.87	0.39

Dermatophyte Test Medium Trichophyton rubrum count in wounds recovered 20 minutes after tretament application

Treatment	Dilution	Count	CFU/ml	Log CFU/ml	
B – Lamisil Positive Control	-4	52	2.08E+07	7.32	
	-4	66	2.64E+07	7.42	
	-4	89	3.56E+07	7.55	STDV
		Mean	2.76E+07	7.43	0.12

Number of organism per g

Treatment	Number of	purpose	Dilution	Weight	CFU/g	Log CFU/g	
B – Lamisil Positive Control	52	2	10000	0.168	6.19E+06	6.79	
	66	2	10000	0.164	8.05E+06	6.91	
	89	2	10000	0.203	8.77E+06	6.94	STDV
				Mean	7.67E+06	6.88	0.08

Dermatophyte Test Medium Trichophyton rubrum count in wounds recovered 20 minutes after tretament application

Treatment	Dilution	Count	CFU/ml	Log CFU/ml	
C – Saline Control	-4	67	2.68E+07	7.48	
	-4	112	4.48E+07	6.05	
	-4	191	7.64E+07	6.57	STDV
		Mean	4.93E+07	6.70	0.72

Number of organism per g

Treatment	Number of Colonies (N)	Volume of ALL purpose Neutralizer (V)	Dilution Factor (D)	Weight Biopsy(g) X	CFU/g	Log CFU/g	
C – Saline Control	67	2	10000	0.126	1.06E+07	7.03	
	112	2	10000	0.137	1.64E+07	7.21	
	191	2	10000	0.162	2.36E+07	7.37	STDV
				Mean	1.69E+07	7.20	0.17

24 hours

Dermatophyte Test Medium Trichophyton rubrum count in wounds recovered 24 hours after tretament application

Treatment	Dilution	Count	CFU/ml	Log CFU/ml	
A – Revity	-1	61	2.40E+04	4.38	
	0	116	4.64E+03	3.67	
	0	47	1.88E+03	3.27	STDV
		Mean	1.02E+04	3.77	0.56

Number of organism per g

Treatment	Number of Colonies (N)	Volume of ALL purpose Neutralizer (V)	Dilution Factor (D)	Weight Biopsy(g) X	CFU/g	Log CFU/g	
A – Revity	61	2	10	0.159	7.67E+03	3.88	
	116	2	1	0.140	1.66E+03	3.22	
	47	2	1	0.150	6.27E+02	2.80	STDV
				Mean	3.32E+03	3.30	0.55

Dermatophyte Test Medium Trichophyton rubrum count in wounds recovered 24 hours after tretament application

Treatment	Dilution	Count	CFU/ml	Log CFU/ml	
B – Lamisil Positive Control	-3	97	3.88E+06	6.59	
	-4	22	8.79E+06	6.94	
	-3	149	5.96E+06	6.77	STDV
		Mean	6.21E+06	6.77	0.18

Number of organism per g

Treatment	Number of	purpose	Dilution	Weight	CFU/g	Log CFU/g	
B – Lamisil Positive Control	97	2	1000	0.122	1.59E+06	6.20	
	22	2	10000	0.125	3.52E+06	6.55	
	149	2	1000	0.191	1.56E+06	6.19	STDV
			1000	Mean	2.22E+06	6.31	0.20

Dermatophyte Test Medium Trichophyton rubrum count in wounds recovered 24 hours after tretament application

Treatment	Dilution	Count	CFU/ml	Log CFU/ml	
C – Saline Control	-4	54	2.16E+07	7.33	
	-3	49	1.96E+07	7.29	
	-3	37	1.48E+07	7.17	STDV
		Mean	1.87E+07	7.26	0.08

Number of organism per g

Treatment	Number of Colonies (N)	Volume of ALL purpose Neutralizer (V)	Dilution Factor (D)	Weight Biopsy(g) X	CFU/g	Log CFU/g	
C – Saline Control	54	2	10000	0.150	7.20E+06	6.86	
	49	2	10000	0.128	7.66E+06	6.88	
	37	2	10000	0.142	5.21E+06	6.72	STDV
				Mean	6.69E+06	6.82	0.09

REFERENCES

[1] Sullivan TP, Eaglstein WH, Davis SC, and Mertz PM. The pig as a model for human wound healing. Wound Repair and Regeneration 9, 2, 2001, 66-76