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המעבדה לחקר מחלות זיהומיות
The Infectious Diseases Research lab

http://eng.sheba.co.il/Research_and_Development/Infectious_Diseases_Research_Lab/

July 20, 2015

The Effectiveness of Radiancy's Light & Heat Energy (LHE) Device on the Viability of the Dermatophyte Fungus *Trichophyton Rubrum*.

In the course of July 2015, the Infectious Diseases Laboratory at Sheba Medical Center, Israel has examined the effect of Radiancy Ltd's Light & Heat Energy (LHE) device on the viability of the dermatophyte fungus *Trichophyton rubrum*. All experiments were done by Dr. Ohad Gal-Mor and Ms. Shalhevet Azriel in collaboration with Dr. Daniele Perl-Treves

For that purpose, a clinical isolate of *T. rubrum* (isolate number 1778,isolated from a patient in Sheba Medical Center) was grown to pure culture on Potato Dextrose Agar (PDA) plates (Difco; catalog # 213400) supplemented with Penicillin-Streptomycin (Biological Industries; catalog # 03031-13) for 6 days at 28°C.

At day 6, *T. rubrum* colonies were scraped aseptically from the PDA plates in a biological hood and suspended in 5 ml of sterile YM broth (Difco; catalog # 27120). or in Saline (experiment dependent as specified below).

One day before the experiment, the experimental system was placed in the hood and tested with a thermal camera by a Radiancy scientist (Dr. Daniele Perl-Treves) to verify that the temperature reached meets the required conditions
At the day of the experiment, 100 µl of *T. rubrum* suspension were pipetted in the center of a 50 mm sterile petri dish that was placed on a Thermoblock adjusted to 37°C or used at room temperature, according to the experimental setting. The *T. rubrum* suspension was then exposed to the LHE treatment of the Radiancy device, according to a protocol determined by Radiancy. A second equivalent suspension of 100 µl of *T. rubrum* served as the untreaded control – it was handled and placed in similar conditions but was not exposed to the LHE treatment.

Experimental setup

T. rubrum suspensions (LHE treated or untreated samples) were suspended in 3 ml of YM broth (or saline if stated) and transferred to 15 ml tubes.

Serial dilutions were prepared and 100 µl were plated on PDA plates supplemented with Penicillin-Streptomycin for CFU count.

After 4 to 5 days of incubation at 28°C, *T. rubrum* CFU were counted.

The experiment results are summarized below:

Experiment 1 (01.07.15). Effect of Temperature on *T. rubrum* viability.

An independent experiment was designed to test the effect of temperature on *T. rubrum* viability. 100µl of a 1.7 $\times 10^4$/ ml culture suspended in saline were incubated for 90 minutes at room-temperature, 37°C, and 40-41°C (Thermoblock). Every 30 minutes, 15 µl of ddH2O were added to cultures, to account for evaporation. At 90 minutes, cultures were suspended with 3 ml of saline and serial dilutions were plated on PDA plates. CFU were counted after 5 days of incubation at 28°C:

CFU counts after 5 days of incubation at 28°C:

Temperature	CFU count - undiluted
RT °C	43
37 °C	41
40-41 °C	18

This experiment showed more than 50% drop in the viability of *T. rubrum* following a 90 minute incubation at 40-41°C. No drop in viability was observed at 37°C.

Experiment 2: Effect of LHE treatment on *T.rubrum* viability (08.07.15)

Two dilutions of *T. rubrum* culture (1:1 and 1:5) were suspended in YM, placed on a Thermoblock at room temperature and subjected to LHE treatment using the Radiancy device. The protocol used consists of 14 treatments (2x10second pulse series), separated by at least 5 minute intervals. 15 μl of ddH2O were added every second treatment to account for evaporation. Identical suspensions, which were not submitted to LHE treatment, were used as control.

Summary of CFU counts: LHE Treated and control samples

T. rubrum dilution 1:1 (4.5×10^5 CFU)

	CFU count		
Dilution	undiluted	10^{-1}	10^{-2}
C1 (control)	-	289	46
C2	-	260	43
C3	-	291	26
Average	**-**	**280**	**38**
T1(treatment)	-	240	29
T2	-	162	18
T3	-	78	10
Average		**160**	**19**
Ratio T/C		**0.57**	**0.5**

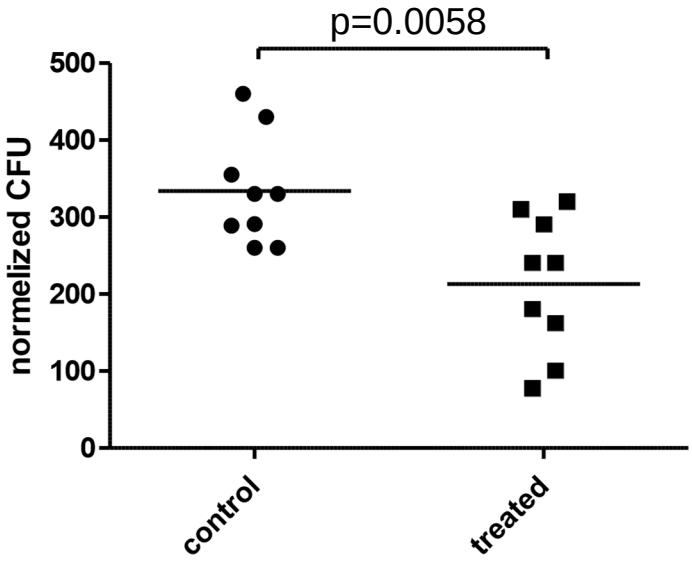
T. rubrum dilution 1:5 (1.5×10^5 CFU)

	CFU count		
Dilution		10^{-1}	
C1 (control)	-	71	
C2	-	66	
C3	-	66	
Average	**-**	**68**	
T1(treatment)	-	62	
T2	-	48	
T3	-	64	
Average		**58**	
Ratio T/C		**0.85**	

Statistical analysis of the normalized CFU count (values multiplied by the dilution factor):

Table Analyzed	Data 1
Column A	control
vs	vs
Column B	treated

Unpaired t test	
P value	0.0058
P value summary	**
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=3.182 df=16
How big is the difference?	
Mean ± SEM of column A	333.9 ± 23.69 N=9
Mean ± SEM of column B	213.3 ± 29.56 N=9
Difference between means	120.6 ± 37.88
95% confidence interval	40.25 to 200.9
R square	0.3876



Statistical analysis of the difference in CFU: LHE treated vs. control samples:

Number of values	6
Minimum	4.000
25% Percentile	10.75
Median	22.50
75% Percentile	47.00
Maximum	74.00
Mean	29.00
Std. Deviation	25.03
Std. Error	10.22

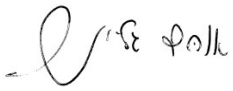
Lower 95% CI of mean	2.735
Upper 95% CI of mean	55.27
One sample t test	
Theoretical mean	0.0
Actual mean	29.00
Discrepancy	-29.00
95% CI of discrepancy	2.730 to 55.27
t, df	t=2.838 df=5
P value (two tailed)	0.0363
Significant (alpha=0.05)?	Yes
Sum	174.0

This experiment showed a statistically significant reduction of 29% in *T. rubrum* CFU count in the LHE treated sample compared to the control sample (P =0.0363).

Summary and conclusions

1. These experiments show that Light and Heat Energy (LHE) delivered by the Radiancy device decreases *T. rubrum* viability.
2. An antifungal activity of 15 to 50% against *T. rubrum* (average 29% p=0.0363) was observed.
3. In addition, exposure to a temperature of 40-41°C for 90 minutes reduces *T. rubrum* viability by more than 50%.

Dr. Ohad Gal-Mor,



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