

Optimization of an Infected Shoe Model for the Evaluation of an Ultraviolet Shoe Sanitizer Device

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Background: Onychomycosis and tinea pedis (athlete's foot) are infections of the nails and skin caused by pathogenic fungi collectively known as dermatophytes. These infections are difficult to treat, and patients often relapse; it is thought that a patient's footwear becomes infected with these fungal organisms and, thus, is an important reservoir for reinfection. Therefore, it is important to find an effective means for killing the dermatophytes that may have colonized the inner surface of the shoes of patients with superficial fungal infections. In this study, we developed an in vitro model for culturing dermatophytes in footwear and used this model to evaluate the effectiveness of a commercial ultraviolet shoe sanitizer in eradicating the fungal elements residing in shoes.

Methods: Leather and athletic shoes (24 pairs) were inoculated with either *Trichophyton rubrum* or *Trichophyton mentagrophytes* (10^7 colony-forming units/mL) strains and were placed at 35°C for 4 to 5 days. Next, we compared the ability of swabbing versus scraping to collect microorganisms from infected shoes. Following the optimized method, shoes were infected and were irradiated with one to three cycles of radiation. The inner surfaces of the shoes were swabbed or scraped, and the specimens were cultured for dermatophyte colony-forming units.

Results: Leather and canvas shoes were infected to the same extent. Moreover, scraping with a scalpel was overall more effective than was swabbing with a cotton-tipped applicator in recovering viable fungal elements. Irradiation of shoes with one, two, or three cycles resulted in reduction of fungal colonization to the same extent.

Conclusions: The developed infected shoe model is useful for assessing the effectiveness of ultraviolet shoe sanitizers. Also, ultraviolet treatment of shoes with a commercial ultraviolet C sanitizing device was effective in reducing the fungal burden in shoes. These findings have implications regarding breaking foot infection cycles. (J Am Podiatr Med Assoc 102(4): 309-313, 2012)

The prevalence of superficial fungal infections, most often caused by dermatophytes belonging to one of three genera, *Trichophyton*, *Microsporum*, and *Epidermophyton*, has been estimated to be as high as 25% in the worldwide population.¹ Dermatophytes are so named because they can metabolize the keratin in skin, nails, and hair, and most infections involve onychomycosis (nail infection) and tinea pedis (athlete's foot).¹ Tinea pedis is often found in persons with onychomycosis and may be a precursor to the development of nail infection.²

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Dermatophyte propagules are transmitted from person to person or by contact with contaminated surfaces, such as shower stalls and dressing rooms at communal gyms.^{3,4}

Footwear has been shown to be an important reservoir for harboring organisms that cause infections.⁵⁻⁷ The presence of fungi in the shoes has been postulated as a major reason for reinfection. Thus, eliminating the infecting fungal spores from the shoes will likely break the cycle of reinfection. For the treatment of footwear, Tanaka et al⁸ found that rinsing athletic shoes with either boiling or cold water was effective in removing dermatophytes but that neither method was effective in boots. Other suggested methods to disinfect footwear of fungal elements have included treatment with chemical

disinfectants, such as chlorine, phenol, sodium dodecyl sulfate, and quaternary ammonium salts.⁹ These methods are not shoe friendly, and, clearly, a less toxic and less destructive method for disinfecting shoes is needed.

Commercial ultraviolet shoe sanitizers have been developed as a means to irradiate the inner surfaces of any type of shoe. One such device uses an ultraviolet C lamp with a peak frequency of 253.7 nm, which has been used as a germicide for decades because of its ability to destroy microorganisms.¹⁰ Ultraviolet C light has been shown to reduce bacterial and fungal surface contamination, and it is an integral component of the biosafety cabinets used by commercial and research laboratories to sterilize work spaces heavily contaminated with microorganisms, including dermatophytes.^{11,12} However, to demonstrate the efficacy of the ultraviolet shoe sanitizer device in reducing the number of organisms residing in the shoe, a model for infecting shoes with dermatophytes and recovering the fungal conidia in shoes needed to be optimized. A previous model for recovery of dermatophytes from shoes¹³ using adhesive tape for spore collection had the disadvantage of providing qualitative rather than quantitative results. In this study, we developed a model for infecting shoes and recovering dermatophytes, and we used this model to determine the efficacy of ultraviolet C irradiation to decrease fungal load.

Development of the Infected Shoe Model

Materials

Isolates. One clinical strain each of *Trichophyton rubrum* and *Trichophyton mentagrophytes*, taken from the culture collection at the Center for Medical Mycology (Case Western Reserve University, Cleveland, Ohio), was used in this study. Isolates were removed from frozen stock (-80°C) and were subcultured to potato dextrose agar or cereal agar (*T. rubrum* strain) and were incubated at 35°C until good conidiation was achieved.

Substrate. Newly purchased pairs each of leather and athletic shoes were used in this study.

Methods

Infection of Shoe Material. Dermatophyte strains were inoculated into leather and athletic shoes to assess their ability to grow on different substrate surfaces. Initially, inoculum was prepared by harvesting conidia to sterile saline and adjusting

to a concentration of 10^3 colony-forming units (CFUs)/mL using a hemacytometer. Inoculum was then applied by sterile cotton-tipped applicator to the inner surfaces of one trial pair of athletic shoes and was allowed to dry.

Recovery of Dermatophyte Conidia From Infected Shoes. The insoles of the infected shoes were sampled by two different collection methods: scraping with a scalpel blade or swabbing with a sterile cotton-tipped applicator. Subsequently, the tops of the shoes were removed from the soles and were sampled in the same manner. Swabs and scalpel blades were used to inoculate the surface of potato dextrose agar (Difco Laboratories Inc, Detroit, Michigan) plates, which were then struck for isolation. After 4 to 5 days of incubation at 30°C , colonies were counted and CFUs were recorded for each culturing method. This method resulted in the recovery of few colonies by either swabbing or scraping with a scalpel, and, thus, the inoculum size was increased to 10^7 conidia/mL for the remainder of the experiments with canvas and leather shoes.

Results

Test Material. Figure 1 compares the difference in fungal growth of untreated control shoes between canvas and leather substrates. For *T. mentagrophytes*, the mean \pm SD log CFUs for canvas and leather were 2.79 ± 0.42 and 3.04 ± 0.40 , respectively. There was no significant difference between canvas and leather ($P = .305$). For *T. rubrum*, the mean \pm SD log CFUs for canvas and leather were

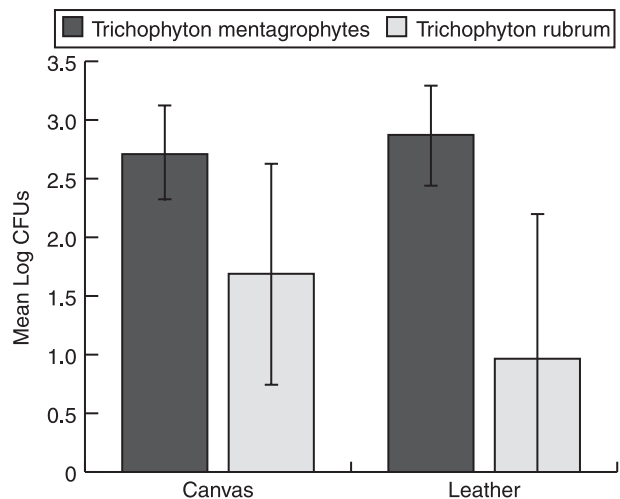


Figure 1. Comparison of the ability of shoe material to support dermatophyte growth. Error bars represent SD. CFU indicates colony-forming unit.

1.14 ± 1.06 and 1.85 ± 1.09, respectively. There was no significant difference between canvas and leather ($P = .281$).

Culturing Method. Table 1 and Figure 2 compare the difference in fungal growth between scraping the infected surfaces with a scalpel versus swabbing the infected surfaces with a cotton-tipped applicator. In the growth controls of *T mentagrophytes*, scraping the test material with a scalpel blade resulted in a total colony count nearly 2.5 times greater than that obtained by swabbing with a sterile cotton-tipped applicator. The mean ± SD log CFUs for scraping and swabbing were 3.06 ± 0.47 and 2.77 ± 0.33, respectively. There was no significant difference between the scraping and swabbing methods ($P = .255$).

For *T rubrum* growth controls, scraping the test material with a scalpel blade resulted in a total colony count nearly eight times greater than that obtained by swabbing with a sterile cotton-tipped applicator. The mean ± SD log CFUs for scraping and swabbing were 2.21 ± 0.60 and 0.78 ± 1.02, respectively. The scraping method yielded significantly greater CFUs than the swabbing method ($P = .015$).

The previous studies showed that the use of an inoculum containing 10⁷ conidia/mL subsequently harvested by scraping with a scalpel blade were conditions for a successful model of dermatophyte infection of canvas and leather shoes.

Determination of the Irradiation Schedule

Once the parameters for shoe infection and recovery of fungal elements were determined, we proceeded to use the method to optimize the irradiation treatment schedule for infected shoes.

Methods

Two pairs each of leather and canvas shoes were infected with *T mentagrophytes* or *T rubrum* and

were subsequently used to determine optimum irradiation schedules. One shoe of each pair was treated by exposure to the sanitizer (SteriShoe; Shoe Care Innovations Inc, Redwood City, California) for one, two, or three cycles of irradiation (a cycle is 45 min). The other shoe of each pair acted as a growth control and was not exposed to the ultraviolet C radiation. The device was placed firmly against the toe of the shoe and was locked into place. The shoe with the inserted device was then enclosed in a black cloth bag and left undisturbed for the entire irradiation cycle(s).

Results

Efficacy of Ultraviolet C Shoe Sanitizer.

Figure 3 shows the fungal burden for treated and growth control shoes. After irradiation, the percentages of fungal reduction for shoes treated with one, two, and three cycles compared with the growth controls were 83.9%, 77.6%, and 85.4%, respectively, when testing *T mentagrophytes*. As expected, the growth controls showed the highest mean ± SD fungal burden at 2.91 ± 0.41. The mean (SD) log CFUs for one, two, and three cycles were 2.04 ± 0.46, 2.12 ± 0.14, and 2.44 ± 0.19, respectively. Treatment with one and two cycles significantly reduced the fungal burden compared with the growth control ($P = .003$ and $.008$, respectively).

When testing *T rubrum*, percentage efficacies for shoes treated with one, two, and three cycles compared with growth controls were 88.8%, 75.6%, and 68.3%, respectively. Growth controls showed the highest mean ± SD fungal burden at 1.49 ± 1.09. The mean ± SD log CFUs for one, two, and three cycles were 0.55 ± 0.64, 1.29 ± 1.23, and 0.70 ± 0.82, respectively. There was no significant difference in CFUs between treated and growth control shoes ($P > .05$) due to the standard deviation, but there was a mean *T rubrum* CFU reduction in the treated shoes of 76.28%.

Table 1. Total CFUs Obtained by Two Sampling Methods in Growth Control and Treated Shoes

Culture Method	Growth Control (CFU)	Treated (CFU)	Reduction from Control (%)
<i>Trichophyton mentagrophytes</i>			
Scalpel	10,442	1,501	85.63
Swab	4,246	903	78.73
<i>Trichophyton rubrum</i>			
Scalpel	2,651	626	76.39
Swab	287	71	75.26

Abbreviation: CFU, colony-forming unit.

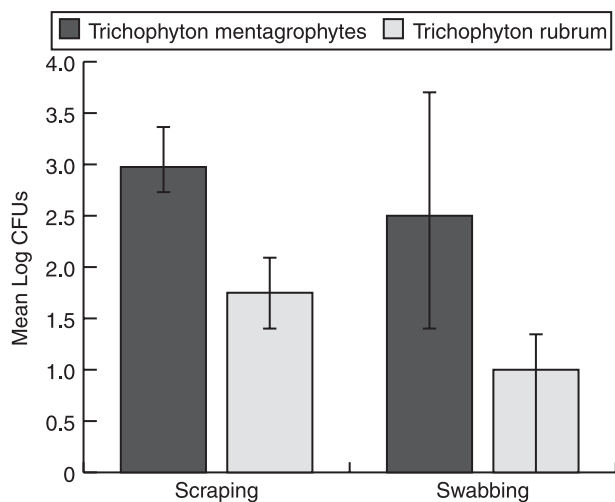


Figure 2. Comparison of dermatophyte recovery methods from growth control shoes. Error bars represent SD. CFU indicates colony-forming unit.

Discussion

Ultraviolet C irradiation has been used for many years in various settings to disinfect environmental surfaces, among them being hospital isolation rooms and laboratory biosafety cabinets.^{11,14} More recently, the use of this technology has been investigated for the reduction of bacterial biofilms on indwelling catheters¹⁵ and even for the decontamination of carpet surfaces.¹⁶ To our knowledge, this is the first published method for establishing a dermatophyte-infected shoe model to study the effectiveness of ultraviolet C irradiation.

First, we identified the necessary parameters for a successful shoe model. The data show that we could recover *T mentagrophytes* and *T rubrum* from leather and canvas inoculated substrates and that there was no significant difference in the colony counts retrieved from the two types of shoes. However, we had more success retrieving *T mentagrophytes* than *T rubrum* from shoes, as indicated by the higher colony counts yielded by *T mentagrophytes*-inoculated shoes. This finding may be attributable to the propensity toward greater conidia production in *T mentagrophytes* strains, although this is unlikely. More likely is a possible differential in the growth rate between the two species, as visible colonies of *T mentagrophytes* from clinical specimens tend to be produced more quickly on culture media than do those of *T rubrum*. Furthermore, we demonstrated that scraping the interior surface of the shoe with a scalpel blade was significantly more effective than was swabbing for the retrieval of dermatophyte conidia.

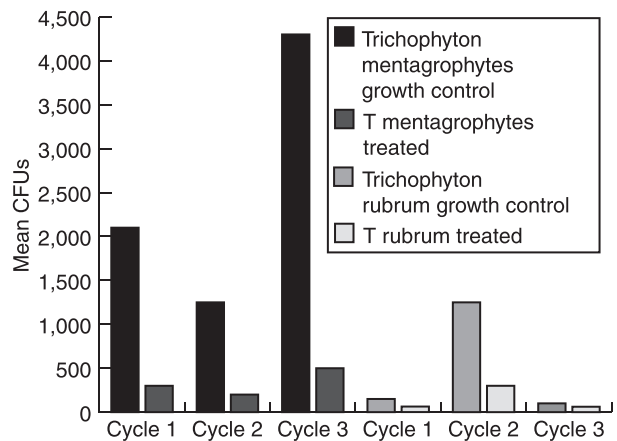


Figure 3. Comparison of the efficacy of one, two, and three cycles of irradiation. CFU indicates colony-forming unit.

Based on the previous findings, we conclude that the following parameters are necessary for successful shoe inoculation and retrieval of fungi from infected shoes: use of an inoculum containing 10^7 conidia/mL and harvesting by scraping with a scalpel blade.

Once the parameters for a successful infected shoe model were identified, we used this model to evaluate the commercial ultraviolet C shoe sanitizer device. These data demonstrate that one, two, and three treatment cycles with the shoe sanitizer reduced the dermatophyte fungal load in shoes. This reduction was significant by treatment with one and two cycles of irradiation. Treatment with two and three cycles did not result in significant additional reduction of fungal growth of either *T mentagrophytes* or *T rubrum* compared with treatment with one cycle. Thus, this lack of reduction in fungal growth with additional exposure time indicates that one cycle should be satisfactory.

Conclusions

This study evaluated several parameters for establishing a dermatophyte-infected shoe model intended to evaluate an ultraviolet C shoe sanitizer, including type of shoe material, inoculum size, and methods for dermatophyte recovery. Overall, this infected shoe model has been demonstrated to be a successful method by which the efficacy of ultraviolet C irradiation in reducing dermatophyte contamination of footwear can be determined. Furthermore, by using this model, the SteriShoe ultraviolet shoe sanitizer was shown to be effective in reducing the fungal burden in shoes. These findings have

implications regarding the ability to address the footwear environment as a means of breaking the foot infection cycle.

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Conflict of Interest: Due to a conflict of interest, JAPMA editor, Warren Joseph, DPM, was not involved in the review or decision-making process of this paper. Dr. Ghannoum has no conflicts to report.

References

1. HAVLICKOVA A, CZAICA VA, FRIEDRICH M: Epidemiological trends in skin mycoses worldwide. *Mycoses* **51** (suppl 4): 2, 2008.
2. PEREA S, RAMOS MJ, GARAU M, ET AL: Prevalence and risk factors of tinea unguis and tinea pedis in the general population in Spain. *J Clin Microbiol* **35**: 3226, 2000.
3. ALY R: Ecology and epidemiology of dermatophyte infections. *J Am Acad Dermatol* **31**: S21, 1994.
4. ALTUNAY ZT, ILKIT M, DENLI Y: Investigation of tinea pedis and toenail onychomycosis prevalence in patients with psoriasis. *Mikrobiyol Bul* **43**: 439, 2009.
5. BROUGHTON R: Reinfection from socks and shoes in tinea pedis. *Br J Dermatol* **67**: 249, 1955.
6. UNGPAKORN R, LOHAPRATHAN S, REANGCHAINAM S: Prevalence of foot diseases in outpatients attending the Institute of Dermatology, Bangkok, Thailand. *Clin Exp Dermatol* **29**: 87, 2004.
7. AJELLO L, GETZ ME: Recovery of dermatophytes from shoes and shower stalls. *J Invest Dermatol* **22**: 17, 1954.
8. TANAKA K, KATO T, IRIMAJIRI J, ET AL: Preventive effects of various types of footwear and cleaning methods on dermatophyte adhesion. *J Dermatol* **33**: 528, 2006.
9. GUPTA AK, AHMAD I, SUMMERBELL RC: Comparative efficacies of commonly used disinfectants and antifungal pharmaceutical spray preparations against dermatophytic fungi. *Med Mycol* **39**: 321, 2001.
10. NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH. NIOSH eNews **5**: 12, 2008.
11. KATARA G, HEMVANI N, CHITNIS S, ET AL: Surface disinfection by exposure to germicidal UV light. *Ind J Med Microbiol* **26**: 241, 2008.
12. KRISHNAMURTHY K, TEWARI JC, IRUDAYARA J, ET AL: Microscopic and spectroscopic evaluation of inactivation of *Staphylococcus aureus* by pulsed UV light. *Food Bioprocess Technol* **3**: 93, 2010.
13. KNUDSEN EA: Isolation of dermatophytes from footwear with adhesive tape strips. *J Med Vet Mycol* **25**: 59, 1986.
14. ANDERSEN BM, BANRUD H, BOE E, ET AL: Comparison of UV C light and chemicals for disinfection of surfaces in hospital isolation units. *Infect Control Hosp Epidemiol* **27**: 729, 2006.
15. BAK J, JORGENSEN TM, HELFMANN J, ET AL: Potential in vivo UVC disinfection of catheter lumens: estimation of the doses received by the blood flow outside the catheter tip hole. *Photochem Photobiol* **87**: 350, 2011.
16. LUTZ EA, SHARMA S, CASTO B, ET AL: Effectiveness of UV-C equipped vacuum at reducing culturable surface-bound microorganisms on carpets. *Environ Sci Technol* **44**: 9451, 2010.