

Sanitization of Contaminated Footwear from Onychomycosis Patients Using Ozone Gas: A Novel Adjunct Therapy for Treating Onychomycosis and Tinea Pedis?

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Background: Ozone gas possesses antimicrobial properties against bacteria, viruses, and yeasts. Previously, we demonstrated the efficacy of ozone in killing ATCC strains of the dermatophyte fungi *Trichophyton rubrum* and *Trichophyton mentagrophytes*.

Objective: To test the efficacy of ozone gas in sanitizing onychomycosis patient footwear contaminated with fungal material as a means of minimizing the risk of reinfection.

Methods: Swabs of footwear from onychomycosis patients were cultured prior to and after ozone exposure to test the ability of ozone to sanitize these items.

Results: We identified contamination of footwear from most onychomycosis patients, a potential source of reinfection in these individuals. Furthermore, ozone gas was effective in sanitizing contaminated footwear.

Conclusion: Ozone gas is effective in sanitizing footwear and represents a novel adjunct therapy to be used in conjunction with antifungal medications and/or devices to better treat onychomycosis and tinea pedis patients in both the short and the long term.

Contexte: L'ozone est un gaz qui a des propriétés antimicrobiennes contre les bactéries, les virus, et les levures. Nous avons déjà démontré que l'ozone était efficace pour tuer des souches ATCC de champignons dermatophytes, plus précisément *Trichophyton rubrum* et *Trichophyton mentagrophytes*.

Objectif: L'étude visait à vérifier l'efficacité de l'ozone dans le nettoyage antiseptique des chaussures contaminées aux champignons, chez les personnes atteintes d'onychomycose, comme moyen de réduction du risque de réinfection.

Méthodes: Des prélèvements ont été effectués sur des chaussures de patients atteints d'onychomycose, suivis de culture, avant et après l'exposition à l'ozone afin que soit vérifié le pouvoir antiseptique de l'ozone dans ces parties de vêtement.

Résultats: Nous avons identifié les agents en cause dans la contamination des chaussures chez la plupart des patients atteints d'onychomycose, source possible de réinfection chez ces personnes. De plus, l'ozone s'est montré efficace pour désinfecter les chaussures contaminées.

Conclusion: L'ozone est efficace pour désinfecter les chaussures et représente une nouvelle forme de traitement d'appoint, à utiliser en association avec des médicaments ou des dispositifs antifongiques afin de mieux traiter l'onychomycose et le pied d'athlète, à court et à long terme.

TRADITIONAL TOPICAL or oral antifungal drugs have a poor prognosis for long-term cure in onychomycosis patients achieving complete cure,¹ with

some patients never achieving complete cure after treatment.² Recently, device-based therapies have been developed to treat onychomycosis, but it is too early to assess the potential success of this type of therapy.

One hypothesis to account for the difficulty in successfully treating onychomycosis patients involves reinfection through exposure to viable conidia within contaminated footwear.³⁻⁶ One suggested but expensive means of minimizing the risk of reinfection involves replacing footwear once cure is achieved.⁷ A more realistic and cost-effective alternative involves sanitization of footwear as a complement to ongoing therapy.

Previously, to develop a means of sanitizing footwear, we performed in vitro studies demonstrating the efficacy of

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gaseous ozone to kill dermatophyte fungi,⁸ adding to the literature documenting the range of microorganisms susceptible to this compound.^{9–12} We then applied ozone to the complex issue of effectively treating onychomycosis. We demonstrated that footwear from the majority of onychomycosis patients is contaminated with various combinations of fungi, molds, and yeasts, all of which are known to cause onychomycosis. We also tested and adapted existing ozone technology to safely and economically sanitize contaminated footwear from onychomycosis patients within the time frame of a patient clinic visit, reducing the risk of reinfection from chronic exposure to infecting organisms.

Materials and Methods

Fungal Material

All experiments were performed using fungal material obtained by swabbing the interior of footwear from patients clinically diagnosed with onychomycosis attending a private dermatology practice in London, Ontario. Footwear was composed of a combination of natural and synthetic materials and were of a closed-toe design (shoes/boots), with one exception (open sandals were tested from one patient). All footwear appeared to have been worn repeatedly over an extended period of time and repeatedly exposed to potential contamination by shedding of fungal material during active disease.

The region of the footwear in contact with the toes during wearing was swabbed prior to and following ozone exposure. In pilot experiments, footwear was sampled using sterile swabs (Puritan, Guilford ME), which subsequently were used to inoculate solid media followed by liquid media. To minimize the potential for intraexperimental error, we subsequently altered the sampling process, substituting sterile swabs with sterile gauze (American Fiber and Finishing, Albemarle, NC). Once the appropriate area of the interior of the footwear had been swabbed, the gauze was dissected to ensure equal inoculation of both solid and liquid cultures.

Fungal Cultures

All cultures were performed in duplicate for up to 7 days at 30°C in ambient air using Sabouraud's dextrose agar or broth (Oxoid, Nepean, ON) in 50 mL culture flasks (Corning, Guelph, ON) supplemented with cycloheximide, chloramphenicol, and gentamicin (Sigma-Aldrich, Oakville, ON). The use of both agar and broth ensured that a

quantitative and a qualitative readout of ozone sanitization could be assessed, and as traditional mycologic assessment occurs on agar, it was important to directly compare our data to the gold standard.

Sanitization of Footwear

Following swabbing, footwear were placed into a custom ozone delivery device (Sani Sport) and exposed to either a standard or an adapted cycle of ozone exposure. The standard ozone exposure cycle consisted of a defined period of ambient air evacuation and replacement by gaseous ozone to a minimum part per million (ppm) ratio followed by a defined time period of exposure to ozone at a minimum ppm ratio and, finally, a defined period of ozone neutralization. All aspects of this process are currently under patent protection by the manufacturer. In contrast, the adapted cycle of ozone exposure involved experimental alteration of timing for each stage of the process (\pm consecutive heating and ozone exposure) to maximize the sanitizing effect of ozone exposure within the time frame of a clinical appointment. Ozone exposure not channeled into the interior of the footwear was termed "passive," whereas consecutive heating and ozone exposure channeled into the footwear was termed "directed."

Statistical Analysis

Chi-square analysis to compare clinical observations to contamination of footwear was performed using *GraphPad* online (<<http://www.graphpad.com/quickcalcs/Contingency1.cfm>>). Online Student *t*-test (College of Saint Benedict and St. John's University) was used to assess ozone treatment efficacy (<<http://www.physics.csbsju.edu/stats/t-test>>).

Results

Testing for Contamination of Patient Footwear

Our original experiments involved testing to confirm the common hypothesis that footwear from onychomycosis patients becomes contaminated during repeated use.

Of the 10 pairs of footwear tested, based on morphologic presentation, we were able to culture dermatophyte fungi, nondermatophyte molds, yeasts, or a combination of multiple organisms from at least one of the patients' two items of footwear in 80% of cases tested (representative data are presented in Figure 1). The dermatophyte fungi were further identified as either *Trichophyton rubrum* or *Trichophyton*

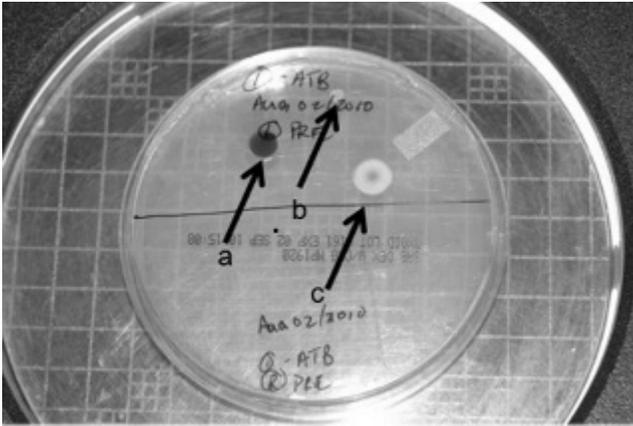


Figure 1. Patient footwear contains viable organisms known to cause onychomycosis. The upper half of the plate represents a 7-day culture of a swab from the left shoe of a patient. This culture produced growth of a single colony of a nondermatophyte mold (a), yeast (b), and a dermatophyte fungus (c). The lower half of the plate represents a culture from the corresponding right shoe that did not produce any colony growth.

mentagrophytes, the two most commonly isolated pathogens from onychomycosis and tinea pedis patients.

Of those patients whose footwear was contaminated, five (62.5%) had bilateral contamination of footwear. This observation did not strongly correlate with a clinical diagnosis of active bilateral onychomycosis, keeping with the hypothesis that repeated exposure to contaminated footwear is a risk factor for disease relapse over time. Of note is the fact that all contaminated footwear was of the closed-toe shoe/boot variety, once again supporting the hypothesis of chronic exposure to viable pathogenic organisms shed into the interior of the footwear.

Sanitization of Footwear through a Standard Cycle of Passive Ozone Exposure

We next tested the capacity of a standard cycle of ozone exposure to sanitize footwear from three onychomycosis

patients. Cultures from footwear showed delayed growth kinetics of 2 to 3 days, possibly due to a decrease in the number of viable fungi present after ozone exposure (data not shown). As ozone appeared capable of decreasing the number of viable organisms, we adapted our experiments with the aim of reducing the fungal burden in footwear further.

Sanitization of Footwear through Adaptation of Parameters of Passive Ozone Exposure

Our experiments involved sanitization of a porous material (lining of footwear) within the enclosed space of the shoe itself, and perhaps a standard cycle of passive ozone exposure was insufficient to produce maximum results. Therefore, we adapted the parameters of ozone exposure. We observed a significant improvement in the effectiveness of ozone sanitization in footwear from a further two onychomycosis patients (representative data are presented in Figure 2). The quantitative data from agar culture were replicated qualitatively in broth culture (data not shown). The adapted ozone exposure parameters enhanced the fungicidal effect observed from passive exposure; therefore, we adapted the experimental system to direct ozone into the interior of the footwear and consecutive heating/drying of the footwear.

Sanitization of Footwear through Directed Ozone Exposure

Further adaptation was performed to include directed ozone exposure into the interior of footwear in parallel with a heating/drying cycle to improve upon the sanitizing effects obtained. Data produced from these experiments were inconsistent when conducted on footwear from three onychomycosis patients. Sanitization of footwear from two patients produced data demonstrating that ozone is highly effective in reducing fungal burden

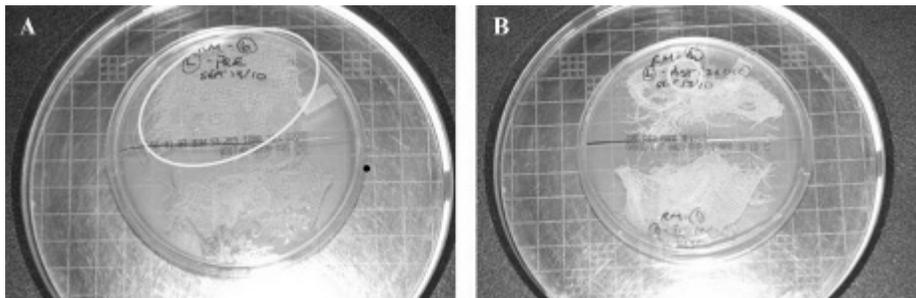


Figure 2. A novel ozone exposure cycle effectively sanitizes contaminated footwear. Prior to ozone exposure, approximately 25 colonies grew in culture from the left shoe of a patient clinically diagnosed with onychomycosis (A). In comparison, after adapting the ozone exposure parameters, passive ozone exposure resulted in a > 95% reduction in the number of viable organisms (B).

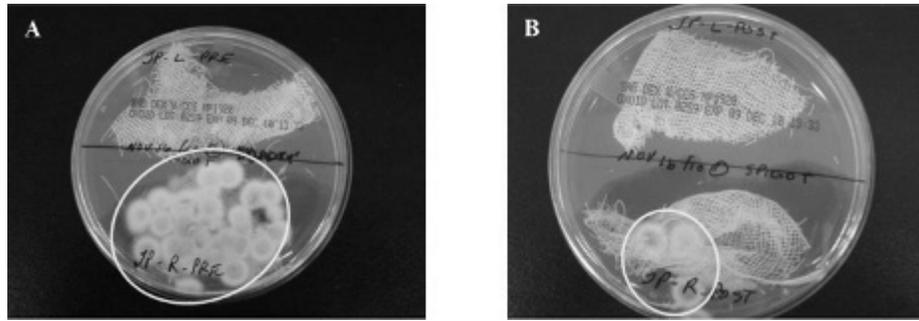


Figure 3. Consecutive heating and directed ozone exposure sanitizes some samples. Prior to ozone exposure, approximately 26 colonies grew in culture from a swab of the shoe of a patient clinically diagnosed with onychomycosis (A). In comparison, after further adaptation of ozone exposure parameters to incorporate consecutive heating and directed ozone exposure, a significant reduction was observed in the number of viable organisms (B).

when directed into the interior of the shoe in conjunction with a drying cycle (representative images are presented in Figure 3). From the third experiment, we observed enhanced growth representative of increased numbers of viable organisms in the postexposure sample compared to that of the preexposure sample (data not shown). We consistently demonstrated in the majority (87.5%) of our experiments a reduction of viable organisms through exposure to ozone.

Statistical Analysis

Chi-square analysis was used to compare bilateral versus unilateral onychomycosis with (1) the presence or absence of footwear contamination and (2) bilateral versus unilateral footwear contamination. In both instances, no significant difference was observed between the clinical presentation of onychomycosis at the time of testing and either the presence of footwear contamination or the contamination of one versus both pieces of footwear (data not presented).

The Student *t*-test examined the null hypothesis that there was no significant difference in the number of colonies isolated from contaminated footwear regardless of ozone exposure. Due to the limited sample size, all eight articles of footwear quantitatively assessed for colony numbers with and without ozone exposure from five onychomycosis patients were analyzed collectively. Data analysis demonstrated a significant difference between a mean colony count (\pm SD [range]) of 16.1 ± 7.0 (3–26) of cultures prior to ozone exposure versus 4.09 ± 5.93 (1–15) of cultures following ozone exposure ($p = .001$). These data are further strengthened by the fact that one of these items of footwear demonstrated increased colony counts following ozone exposure.

Discussion

The two objectives of these experiments were (1) to test the theory that during active disease, onychomycosis patients shed viable fungal material from the site of infection and contaminate their footwear, and (2) to test the ability of ozone gas to sanitize patient footwear as a theoretical means of minimizing the risk of relapse/reinfection posttherapy.

To address our first objective, we sampled footwear from 10 onychomycosis patients and isolated viable fungi from at least one shoe from the majority (80%) of these patients. These data support the theory that footwear becomes contaminated during repeated use during active infection. Furthermore, in most (62.5%) of the experiments identifying footwear contamination, it was found to be bilateral in nature. These data did not correlate well with the patients' disease presentation at the time of testing, providing further evidence to support the theory that long-term cure may be impacted through repeated exposure via contact with contaminated footwear.

To address our second objective, we performed a series of experiments exposing patient footwear to either a standard or an adapted cycle of ozone gas through either passive or directed means in the absence or presence of concurrent heating, respectively, and demonstrated a stepwise increase in the fungicidal capacity of ozone, theoretically decreasing the patients' risk of reinfection through exposure to contaminated footwear.

In one experiment, we observed an increased number of colonies being isolated after ozone exposure, possibly due to the heating/drying component of the ozone exposure cycle. We have yet to examine if certain conidia are thermophilic, which was not within the scope of the aims of our study; however, representative examples of these types of fungi have been discussed in the literature.^{13–17} In certain

circumstances, heating may activate specific organisms from a dormant to a viable state. During the initial recovery stage, when specific proteins such as heat shock proteins are transiently active, ozone exposure may be less effective in its ability to kill these specific organisms. In addition, humidity may be involved in the efficacy of ozone sanitization.^{10,12,18,19} and this combination of ozone and humidity may be necessary to effectively kill specific organisms that otherwise are available to become thermally activated from a dormant state.

Data in support of our findings of contamination of footwear from onychomycosis patients over time are lacking in many respects. For example, despite numerous studies being conducted into therapeutics to treat onychomycosis, rarely has a study endeavored to sample footwear in an attempt to isolate organisms, much less investigate variables within the footwear, such as the numbers and types of organisms present, the viability of these contaminants over time, and the critical mass of organisms required to affect cure rates.

Articles published in English allude to infected footwear as a risk factor for high relapse rates in patients after cessation of therapy for onychomycosis. For example, a report from a workshop targeting onychomycosis advocated discarding old footwear as a means of minimizing the risk of recurrence.⁷ Other publications also mention that infected footwear is a risk factor for disease recurrence.³⁻⁶

Some groups have begun to study the correlation between long-term repeated exposure to potentially infected materials and successful long-term cure in onychomycosis and tinea pedis. Although these studies are few in number and only preliminary investigations, their findings, discussed below, strengthen the argument in support of this being a risk factor for poor long-term cure rates in onychomycosis.

An early article indicated that a small number of studies were able to isolate dermatophytes from footwear.²⁰ Broughton investigated hosiery from at least 100 tinea pedis patients and normal controls and identified viable organisms in socks from 33.6% of the patients versus 5% of controls. A more recent study investigated (1) various parameters to demonstrate the ability of dermatophytes to contaminate footwear, (2) means by which this can be minimized or prevented, and (3) how best to overcome the infection of footwear or hosiery.²¹ Although the study involved only one patient diagnosed with tinea pedis due to a *T. rubrum* infection, the findings demonstrated that short-term exposure of infected skin to footwear can contaminate footwear and hosiery. Furthermore, only boiling water was guaranteed to neutralize the contaminants from the footwear after the fact, although ozone was not tested. Finally, of particular

interest was their observation that infected hosiery, when sampled immediately after contamination, contained significantly less viable organisms than if sampled 24 hours after removal. Collectively, these data provide strong evidence in support of the theory that through normal use, patient footwear and/or hosiery becomes infected and that if not attended to regularly, the number of viable organisms increases over time.

In keeping with the findings of Tanaka and colleagues,²¹ the use of footwear has also been implicated as a risk factor for the development of foot diseases, including onychomycosis, in Thailand²² and Colombia.²³ In these instances, one can extrapolate how repeated intimate exposure to a reservoir of viable organisms in footwear, as demonstrated by Tanaka and colleagues,²¹ in turn decreases the potential for successful therapy and increases the risk of relapse/reinfection after completion of therapy.

In contrast, a Mexican study in a unique region of very high rainfall/humidity implicated open (sandal type) footwear as a risk factor for developing fungal infections of the feet more so than closed footwear due to the potential for exposure to the soil, where infecting organisms are known to exist endemically.²⁴ This finding is in contrast to many other references to wearing closed footwear, presumably due to perspiration causing high levels of humidity in the environment that the foot is exposed to chronically.^{3-7,22,23} Despite the difference in style of footwear implicated in the Mexican study, the premise of chronic exposure to disease-causing organisms is the hypothesis for the development or perpetuation of disease.

The literature demonstrates the risk of relapse/reinfection in onychomycosis being elevated through repeated exposure to infected material; however, it is of interest to review the means of negating the risk through "decontamination," the second and primary objective of this article. As previously mentioned, Tanaka and colleagues found that to ensure the sterility of the footwear after contamination, only treatment with boiling water was 100% effective²¹; however, this is not practical or economical.

Several devices have had patents registered that use ozone alone or in combination with other means to sanitize footwear; however, the vast majority of these devices appear to have never been marketed. Those that have gone to market, primarily in the Far East, are not currently marketed in North America. Furthermore, their design is open rather than closed, as in the device used in our experiments. Given the toxic characteristics of ozone gas and associated safety concerns, these open-style devices are likely to produce very low and ineffective levels of



ozone based on the findings from our studies involving adaptation of ozone exposure to maximize efficacy.

Conclusion

We previously demonstrated in vitro that ozone gas is effective in obtaining > 99% killing of fungal conidia.⁸ In our current studies, we have built on this observation and show that the majority of patients clinically diagnosed with onychomycosis have contaminated footwear that does not necessarily correlate with their current disease state. Through repeated use of contaminated footwear, they may be increasing their risk of relapse/reinfection after cessation of therapy. Variables such as number, species, and viability of organisms, as well as duration of exposure to these organisms, although of profound importance from a clinical perspective, are not within the scope of this study, which is a proof-of-principle testing in the first instance the hypothesis that footwear becomes contaminated over time. If further studies are permitted given safety and ethical considerations, only then will data become available testing the correlation between chronic exposure to this reservoir of organisms and the effect this has on successful cure in onychomycosis.

However, including testing variables into the study design, such as contamination and the potential pathogenicity of the organisms isolated from the footwear, as well as sanitization of contaminated footwear and the risks associated with these variables or, for that matter, the impact these variables have on the efficacy of specific antifungal agents, is theoretically feasible. One means to minimize the risk associated with chronic exposure to pathogenic organisms contaminating footwear, which was the second aim of this study, is through sanitization of footwear via exposure to ozone gas, a technology that we have demonstrated to have a significant ability to kill contaminating organisms.

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