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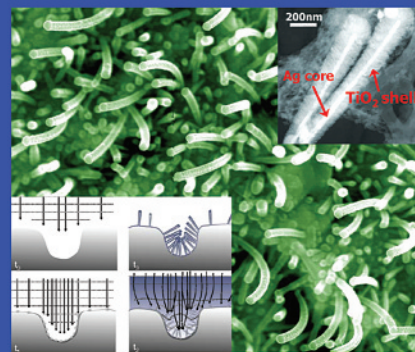
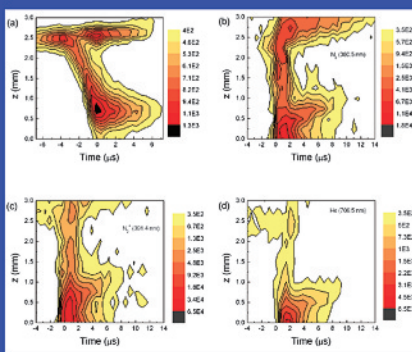
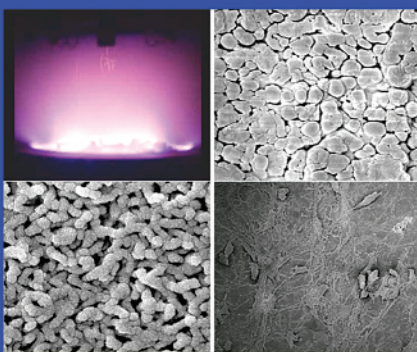
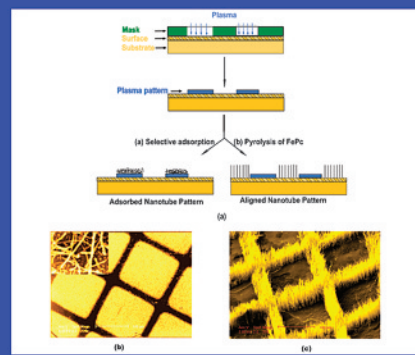
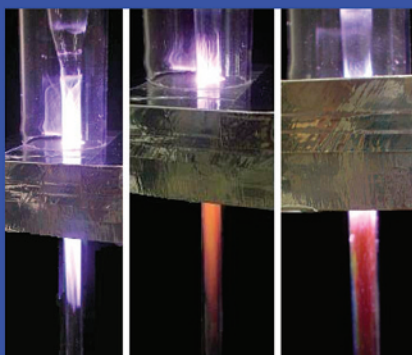
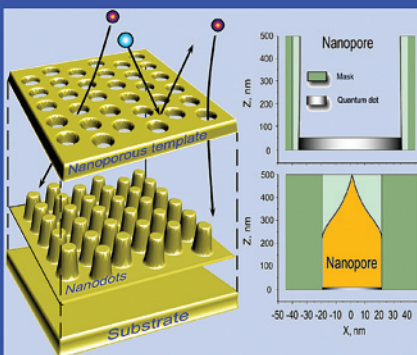
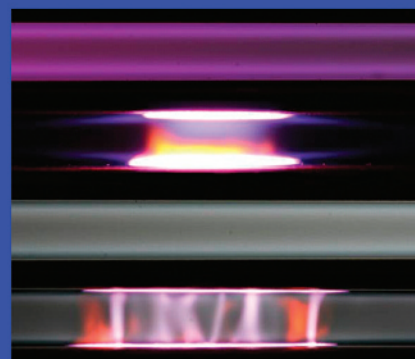
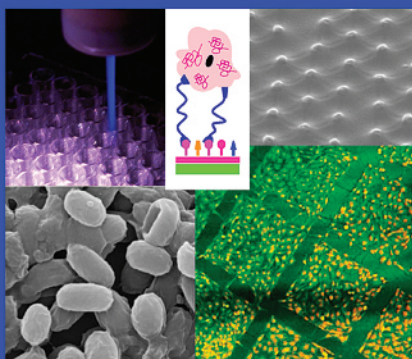
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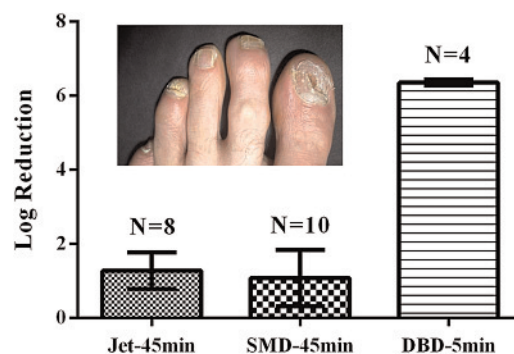
Reprints

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Plasma Treatment of Onychomycosis

Zilan Xiong, Jeffrey Roe, Timothy C. Grammer, David B. Graves*

We report on the use of three “cold” atmospheric plasma (CAP) devices in treating model nails that have been coated on the backside of the nail with either *Escherichia coli* (*E. coli*) bacteria or *Trichophyton rubrum* (*T. rubrum*) fungus. We tested a helium plasma jet; a surface microdischarge plasma device; and a floating electrode dielectric barrier discharge. All of the devices, acting through the model nail, showed significant log reductions of bacterial and fungal targets in several tens of minutes. CAP technology appears to offer promise as a safe, effective, and inexpensive therapy for fungal nail infection treatment.



1. Introduction

Onychomycosis or fungal nail is a common fungal infection of the toenail or fingernail with significant barriers to successful treatment.^[1] An image of human toe nails with onychomycosis is shown in Figure 1(a). It is the most commonly diagnosed and treated disease by podiatrists.^[2] The prevalence of onychomycosis is estimated to affect 10% of the world's adult population and as many as 38 million people in the United States.^[1] The incidence of onychomycosis has been increasing, particularly among the elderly and patients with compromised immune systems.^[3] It is estimated that ~30% of diabetics and ~50% of people over 70 have onychomycosis.

Typical symptoms of onychomycosis include nail discoloration, thickening, brittleness, and in later stages, possibly nail detachment. These are mainly cosmetic problems, but there are more serious consequences as well, including pain and a reduction in the patient's mobility. These symptoms can have a significant adverse effect on quality of life. Patients with other diseases run additional risks if they contract onychomycosis. For example, effective

treatment of onychomycosis can be important for diabetics. In these patients, fungal infections can lead to foot ulcers and secondary bacterial infections, possibly leading to lower limb amputation.^[4] Finally, the growing problems associated with antimicrobial resistance have begun to limit the effectiveness of some drugs used for infectious disease control, prompting the search for medical device alternatives.^[5]

Among fungal strains associated with onychomycosis, *Trichophyton rubrum* (*T. rubrum*) represents the most clinically important species. It accounts for ~80% of all dermatophytoses in humans.^[6–8] Current onychomycosis treatment options include various home remedies, topical creams, oral drugs, and laser treatments. These conventional approaches, however, are characterized by limited success, unsafe side effects, high costs, and prolonged time course required for treatment.

Treatment of onychomycosis is challenging because the infection is embedded underneath or within the nail and is difficult to reach with the above-listed methods.^[9] Physical techniques, including surgical removal, nail abrasion, or thinning are often performed in conjunction with topical treatment. However, these physical techniques can result in considerable patient discomfort, increased costs, and may result in subsequent infection.

Systemic oral therapy is the mainstay of treatment due to the generally poor permeability of the nail plate to topically applied drugs. Systemic orals yield cure rates ranging from 17 to 38%,^[10] but require frequent blood tests for hepatic monitoring and are frequently associated with

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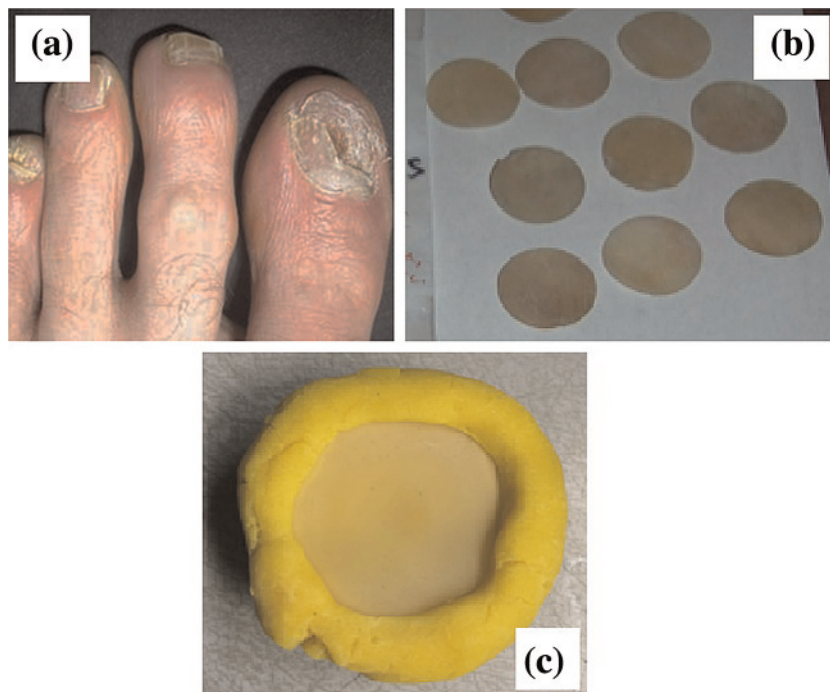


Figure 1. (a) Photograph of human nail with typical fungal discoloration; (b) Set of bovine hoof slices; (c) Bovine hoof with clay sealing at edges, suitable for plasma exposure testing.

various adverse reactions, drug–drug interactions and toxicities due to the doses required to concentrate therapy at the actual site of infection.

Topical therapy of nail disease is desirable to avoid the side effects associated with systemic therapy, to increase patient compliance and to reduce the cost of treatment. However, current topical drugs require daily applications over the course of a year, and have a 17% or less chance of success. Until the recent introduction of better performing topical creams, FDA-approved topical treatment yields historically had cure rates of only 5.5–8.5% after a year of treatment.^[11–13]

Laser treatment is relatively costly and currently lacks adequate clinical data to determine efficacy rates. The US Food and Drug Administration (FDA) has allowed the devices to be marketed for “temporary increase in clear nail” only. A published study using a Nd:YAG 1064 nm laser showed some fungicidal effects in suspension, but an *in vivo* treatment of toenails showed no improvement in Onychomycosis Severity Index Scores.^[14]

It would be advantageous to have a medical device that delivers topical therapy directly to the site of infection. In this article, we report on the use of three non-thermal or “cold” atmospheric plasma (CAP) devices in treating model nails with either *E. coli* bacteria or *T. rubrum* fungus applied to the nail surface. The primary goal of this study is to demonstrate the effectiveness of various plasma devices in treating fungal infections

of the nail, and the model fungal organisms we chose to study, namely *T. rubrum*, is known to be the primary cause (~80%) of human fungal nail infections. Experiments with *E. coli* served to help connect the results of the present experiments with previous plasma disinfection studies that employed bacterial targets. Plasma exposure was tested with microbial contamination on both the nail backside as well as the topside (i.e., directly exposed to plasma) in order to show most clearly the effects of the nail barrier on antimicrobial kinetics of the plasma devices. The three plasma devices tested are operated in a room air environment: Helium (He) plasma jet; surface microdischarge (SMD); and floating electrode dielectric barrier discharge (FE-DBD). After summarizing recent progress in plasma biomedicine, we describe the devices and model nail treatment protocols. Then we summarize the effectiveness of each of these approaches and conclude with recom-

mendations for future studies and a comment on the future prospects of this technology.

2. Plasma Treatment of Onychomycosis

The use of CAP for various biomedical applications is now well established, and several devices have been tested in animal and even human trials.^[15–18] Numerous examples have been reported, including treating dental infections; various dermatological applications; promoting wound sterilization, healing and bleeding cessation; and cancer treatment, among others.^[19–23] Von Woedtke et al. report that three clinical trials of CAP devices have been conducted in Germany and two CAP devices received CE marking in Europe (equivalent to FDA device approval in the USA) in 2013, both for treating skin wounds and ulcers.^[17]

Traditional plasma-based electrosurgical “ablation” devices have been used commercially for several decades for tissue cutting, coagulation, dessication, and cauterization.^[24–27] Plasma ablation devices operate mainly via tissue heating; the effects are therefore primarily thermal. CAP, also referred to as “low temperature plasma,” by contrast, transfers little heat and the effects are primarily non-thermal.

CAP devices generally use either rare gas (He, Ne, or Ar) jets in air or some type of dielectric barrier discharge in air. CAP-generating devices have been shown to readily kill bacteria, viruses, and fungi both on surfaces and in aqueous

solution. The microbial killing mechanisms are not fully understood, but are generally thought to be associated with reactive chemical species; photons; electric fields; and electrical charge. Recent studies reporting the use of CAP for skin treatments, skin disinfection, and related applications.^[28–33] Reactive plasma species, at the moderate concentrations and doses found in non-thermal plasma streams, appear to cause little to no permanent damage to living tissue, while being capable of efficiently destroying microbial cells. This ability of plasma to destroy microbes without significantly damaging tissue is central to the success of plasma applications in medicine for both decontamination and disinfection. There is currently one report of a clinical trial investigating plasma treatment of onychomycosis.^[34] However, to the authors' knowledge, this is the first report on the application of CAP to treat onychomycosis in the current scientific literature.

3. Experimental System and Procedures

Human nails are relatively expensive and are not well suited for proper experimental testing of plasma devices. For example, human cadaver nails come in different sizes, curvatures, and thicknesses that limit the ability to compare instrument modification results from experiment to experiment. Therefore, we used bovine hoof slices as a surrogate model for the human toe nail. Bovine hoof is a well-recognized and tested surrogate for human nails.^[35] In addition to being cost effective, bovine hoof has the advantage that it can be sliced to a consistent size and thickness. Bovine hooves were obtained from a local butcher and soaked in water over 24 h to facilitate their cutting (via microtome) into thin slices. Slices ranged in thickness from 0.2–0.3 mm for the anti-microbial experiments. Slices were punched out ($\varnothing = 29$ mm) and dried at room temperature before being stored (Figure 1b). The hoof slice thickness matched the lower range of healthy human nail plates, which typically vary from 0.5 to 1.0 mm at their distal edge. The individual hoof slice samples are typically sealed at the edges using clay in order to eliminate the possibility of gas leakage around the edge (*cf.* Figure 1c).

Figure 2(a) shows a modified Franz cell, a one-chamber diffusion chamber commonly used in topical drug experiments for nails, with a mounted hoof slice. When the hoof

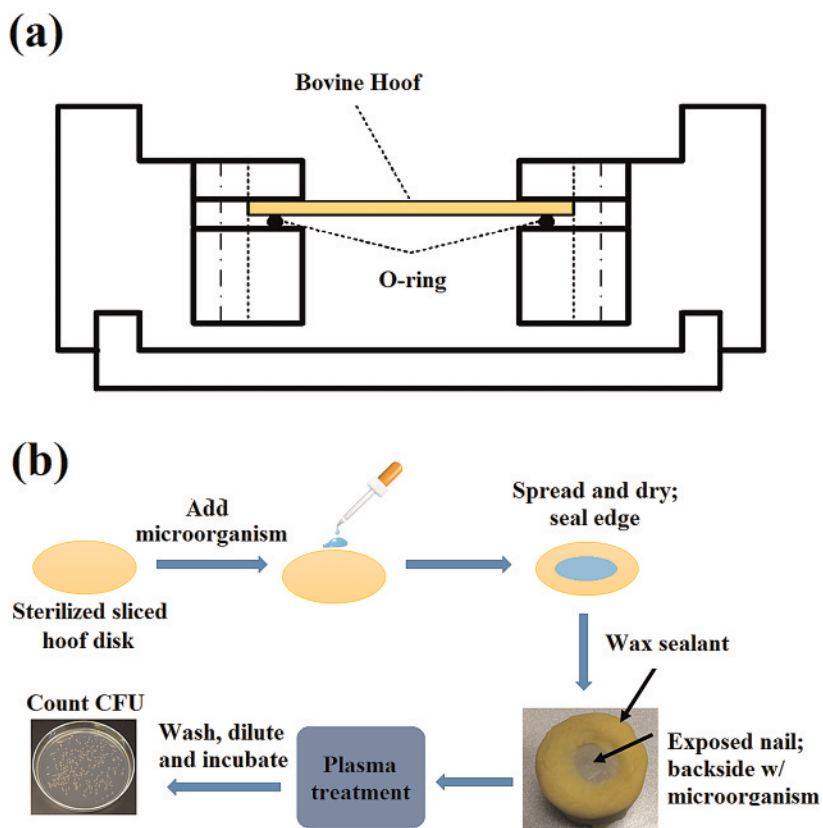


Figure 2. (a) Schematic of a modified Franz cell with a mounted hoof disc, in this case sealed with an o-ring at the edge; (b) Summary of the processes of preparation and treatment.

slice is mounted, the top hoof surface is open to the air and the bottom surface resides within an O-ring sealed chamber. In other experiments, we sealed the edges of the hoof disc with clay, as noted above and as shown in Figure 1(c). For anti-microbial experiments, viable microorganisms were spread on the bottom surface of the hoof, which was enclosed in the sealed chamber within the holder. Plasma in various forms was applied to the exposed top of the bovine hoof slice with edges fully sealed. Consequently, the antifungal plasma had to penetrate through the keratin barrier to access and kill the microorganism. In some cases, experiments were performed with microorganisms spread on the top of the hoof disc as well. It is well known that plasma can kill microorganisms that are on surfaces of various materials. The major result of the present paper is to describe the effects of plasma acting through the nail material and so we emphasize the through-nail results here.

The flow chart of the treatment protocol is shown in Figure 2(b). Details of the experimental procedure is given in the following paragraphs.

The bacterial experiments used the common *Escherichia coli* bacterial species *E. coli* K12, which was cultured

and prepared as described previously.^[36] Bacteria were grown in lysogeny broth (LB) medium to $OD_{600} = 1$ corresponding to approximately 5×10^8 colony-forming units per ml ($cfu\ ml^{-1}$). Suspensions of *E. coli* were pelleted in $150\ \mu\text{l}$ aliquots by centrifugation at 5000 rpm. *E. coli* were dried on hoof discs in preparation for exposure to plasma. The bacteria were spread to a diameter of approximately 1 cm in the center of the disc. Prior to adding bacteria to the discs, the discs were washed with sterile water that was allowed to evaporate completely from the surface of the disc. To seed the discs with bacteria, pellets of *E. coli* were spread with a plastic inoculating loop. The discs were allowed to dry in a sterile environment until the surface of the discs was no longer visibly wet and additional drying time did not result in any change in the appearance of the surface. This drying procedure did not significantly affect the bacteria viability. Once the surface was dry, the hoof discs were ready for plasma treatment.

Fungal experiments used *T. rubrum* as noted above. The source of *T. rubrum* was American Type Culture Collection (Manassas, VA) listed as *T. rubrum* (Castellani) Sabouraud, anamorph (ATCC[®] MYA-3108). To obtain a living culture for our studies, *T. rubrum* fungus was grown on petri dishes containing Emmon's modified Sabouraud medium agar (Spectrum Labs) and grown at $28\ ^\circ\text{C}$. Growth continued for 7–10 d until the dishes were confluent. The fungal cells (conidia and mycelia) were harvested by scraping and homogenously re-suspending in sterile phosphate buffered saline (PBS). For each bovine hoof slice tested, $100\ \mu\text{l}$ aliquots of fungal culture were withdrawn and pipetted onto one side of the bovine hoof slice surface and stored in a sterile environment until dry (about 2 h). As with the bacterial inoculation, the fungus was spread to a diameter of approximately 1 cm at the center of the disc.

The direct killing experiments involved placing the fungal (or bacterial) contaminated hoof within 5 mm of the plasma efflux, directly exposing the contaminated side.

To quantitate fungal (*T. rubrum*) killing after plasma treatment, each hoof was placed in a container with 10 ml PBS and vortexed for 10 min to wash the fungal cells from the hoof surface. The resulting wash solution was serially diluted (five $10\times$ dilutions) into sterile PBS. To culture and colony count, $10\ \mu\text{l}$ of each diluted solution was plated onto media agar plates (Emmons' modification of Sabouraud's agar, ATCC[®] Medium 28) and placed into a $28\ ^\circ\text{C}$ incubator. Each plating was done in triplicate.

The count and calculation of treatment results was performed after typically 7–10 d incubation. Bacterial (*E. coli*) experiments were completed in the same way with the exceptions that cultures were grown at $37\ ^\circ\text{C}$ overnight and LB media and agar plates were used.

Counting colony-forming units (CFU) quantified fungal and bacterial reduction by revealing the number of viable cells remaining after plasma treatment. We measure disinfection (the anti-microbial effect of each treatment) by calculating both the % reduction and the logarithmic (log) reduction factor (RF) using the following formula: $RF = \log_{10}(\text{CFU}_{\text{untreated plate}}) - \log_{10}(\text{CFU}_{\text{treated plate}})$. A 1 log reduction means that 90% of the cells were inactivated and a 2 log reduction corresponds to a 99% kill.

Three different plasma devices were tested in this study. The first device is a Helium (He) plasma jet operated in He with 0.5% O_2 , as illustrated in Figure 3. Figure 3(a) is a photograph of the device showing the plasma plume contacting a human finger nail and Figure 3(b) shows a schematic of the treatment using sliced hoof disc. The gas enters a syringe with a quartz tube inside. The quartz tube is 4 mm in diameter. Inside the tube is the electrode (copper wire 1 mm in diameter), powered by a function generator (Protek 9301) with a high voltage amplifier (Trek 10/40A). The peak voltage was 8 kV, approximately sinusoidal, at a frequency of 4 kHz. The gas flow is $3\ \text{L}\ \text{min}^{-1}$, the separation between the tip of the syringe and the hoof surface is 5 mm. The spatial extent of the antibacterial effects of the He plasma jet is significantly larger than the 1 cm diameter of the microbial samples used here.^[37]

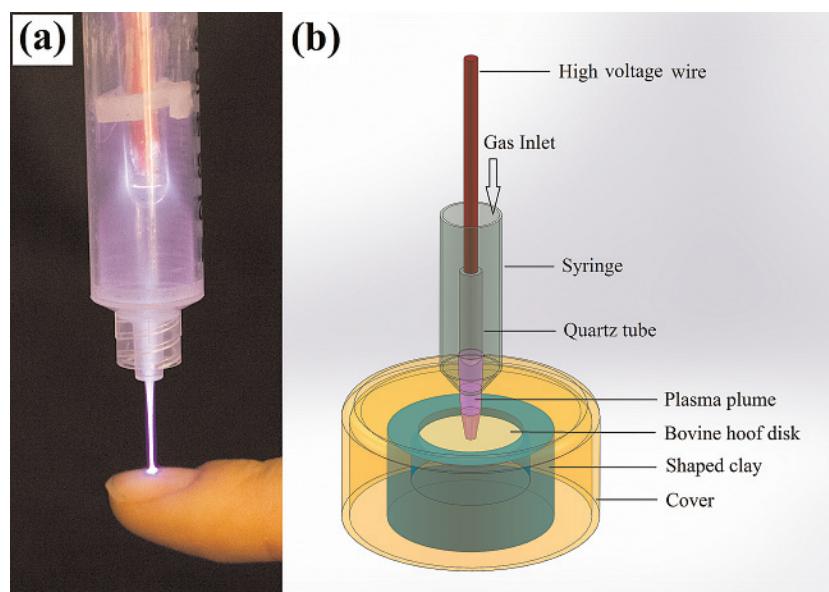


Figure 3. (a) Photograph of He plasma jet device applied to human nail; the same jet device was applied to bovine hoof model nail; (b) Schematic of He plasma jet device applied to bovine hoof disc mounted in holder.

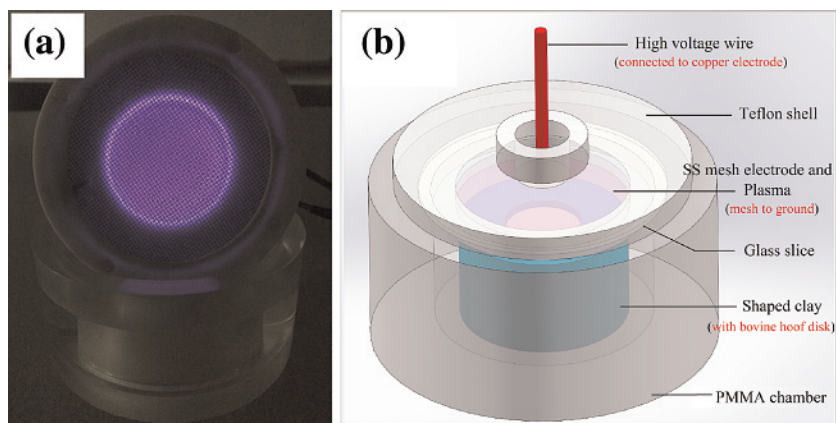


Figure 4. (a) Photograph of SMD device operated in air; (b) Schematic of device applied to bovine hoof disc mounted in holder.

The second device tested is the surface microdischarge (SMD) plasma, shown in Figure 4(a) (photograph) and 4(b) (schematic). The power supply and conditions are similar to those described above for the plasma jet (2.5 kV; ~25 kHz; and 5 mm separation between grounded mesh and hoof surface). This device is operated in room air. More details about this device can be found elsewhere.^[36,38]

The third device is similar to the SMD, but it is operated in the floating electrode-DBD mode by eliminating the grounded mesh and allowing microfilament discharges to directly contact the hoof disc. The power supply was operated at about 6 kV at a frequency of about 4 kHz, with a separation of about 1 mm in room air. In this case, a discharge will not be established unless the backside of the hoof disc is in contact with a grounded surface. We employed a metal plate with the diameter of 1.5 cm for this

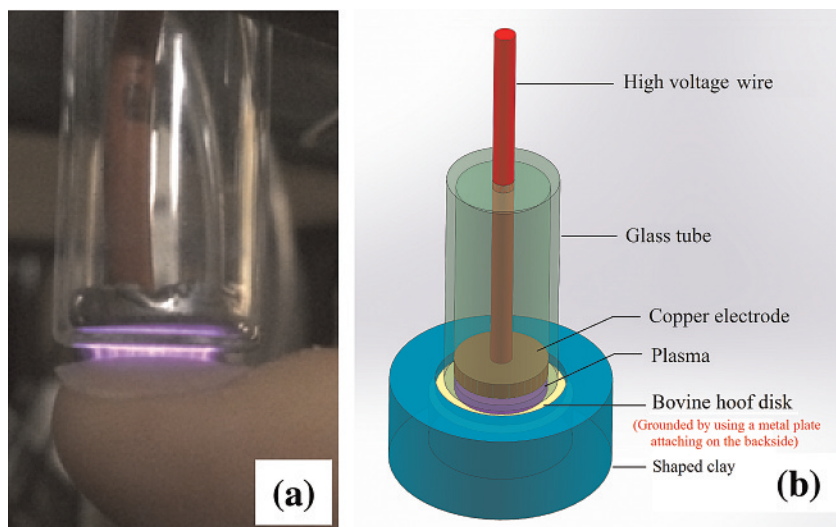


Figure 5. (a) Photograph of FE-DBD device contacting fingernail; (b) Schematic of similar device applied to bovine hoof disc mounted in holder.

purpose. Of course, the bacteria or fungus on the backside of the disc will be in contact with the ground plate and this could affect the measured antimicrobial actions of the plasma. The metal plate and hoof disc were therefore washed together after FE-DBD treatment for both the treated and the control cases. Figure 5(a) is a photograph of a FE-DBD device contacting a fingernail and Figure 5(b) is the corresponding schematic diagram for the hoof disc treatment.

4. Results

Figure 6 shows results of *E. coli* killing on the backside of a 0.2–0.3 mm thick hoof disc for 5, 10, and 20 min exposures by the He jet device. The log reduction at 5 and 10 min was about 1 and at 20 min of exposure, this increased to a log reduction of about 2. By contrast, about 3 log reduction was observed with the jet in direct contact with the bacteria on the topside of hoof disc (facing the jet) for this 20 min exposure.

Figure 7 shows bacterial kill rates using the SMD device for exposures of 5, 10, and 20 min. These results are approximately the same as for the He jet case showed in Figure 6. The SMD device appeared to be somewhat more effective than the He plasma jet in topside killing. For higher exposure times (10 or 20 min), virtually all the bacteria were killed when the bacteria were on the topside of the hoof near the SMD electrode. These topside results on keratin surfaces are consistent with previous work,^[36] and are of course much faster than the anti-microbial results on the backside.

Figure 8 shows results for FE-DBD exposure, similar to Figures 6 and 7 for the other two devices. The approximately 4 log reductions for 5 and 10 min exposures and about 6 log reduction for 20 min exposure are clearly higher than the results for the He jet or the SMD device. We also tested the topside antibacterial effect of FE-DBD and these are similar to the helium jet and SMD device results for topside disinfection.

Figure 9 shows the log reductions for SMD applied to the hoof discs with *T. rubrum* fungus targets on the back of the hoof disc. The trends are similar to the results observed against an *E. coli* target, but the observed kill rates are slower. For example, Figure 7 shows SMD

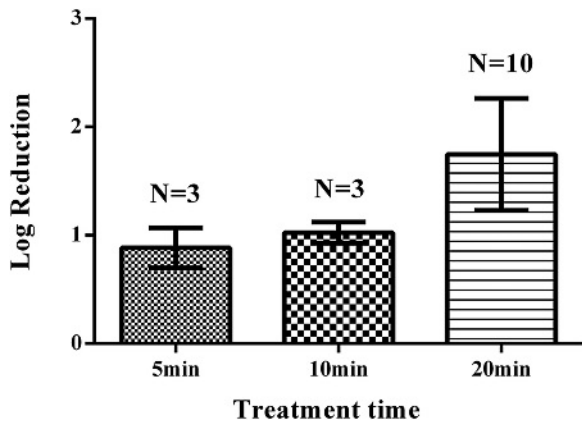


Figure 6. Results from He plasma jet acting on *E. coli* for various times.

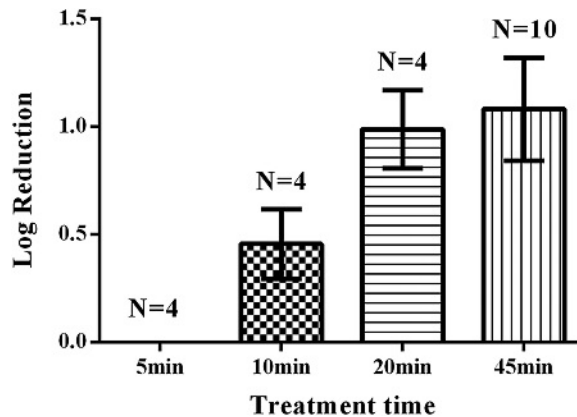


Figure 9. Results from the SMD plasma acting on *T. rubrum* for various times.

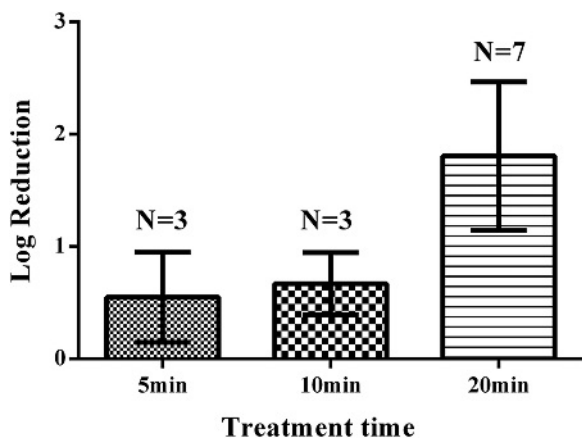


Figure 7. Results from the SMD plasma acting on *E. coli* for various times.

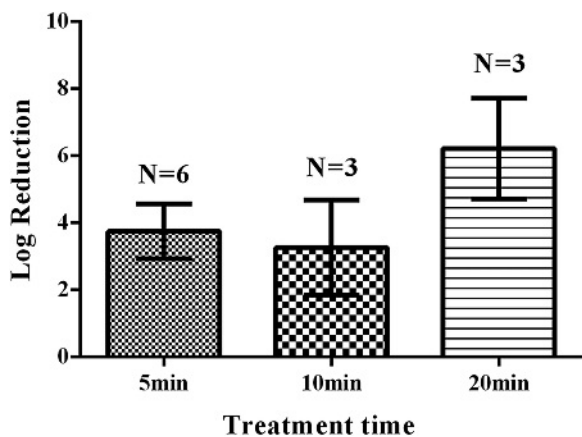


Figure 8. Results from the FE-DBD plasma acting on *E. coli* for various times. In some cases, the FE-DBD damaged the hoof disc; these data were not included in the statistics shown here (see text).

effectiveness against *E. coli* is ~2 log reduction after 20 min exposure whereas it is about one log reduction against *T. rubrum* for the same time of exposure. This was also observed in the topside killing results: a log reduction of about 2 was observed against *T. rubrum* using the SMD for 10 min for fungus on the exposed (top) surface. However, the corresponding result for *E. coli* showed greater than 7 log reduction. Figure 10 shows the effects of the FE-DBD device for 5, 10, and 20 min on *T. rubrum* on the backside of the hoof disc. The reason for the apparently lower log reduction for the intermediate exposure time of 10 min is not known, but some statistical variation was always observed in the log reduction numbers. Once again, the FE-DBD results showed significantly higher kill rates for the *T. rubrum*, as it did for the *E. coli* targets.

Figure 11(a) and (b) summarize the log reductions of *E. coli* and *T. rubrum* corresponding to the three devices, respectively. About 2 log reduction was observed for He jet and SMD and about 6 log reduction for the FE-DBD against bacteria (*E. coli*). The 5 min FE-DBD treatment

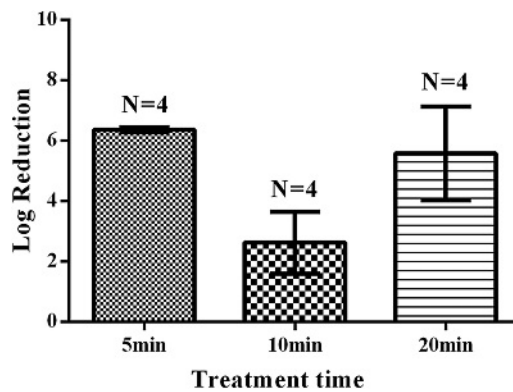


Figure 10. Results from the FE-DBD plasma acting on *T. rubrum* for various times.

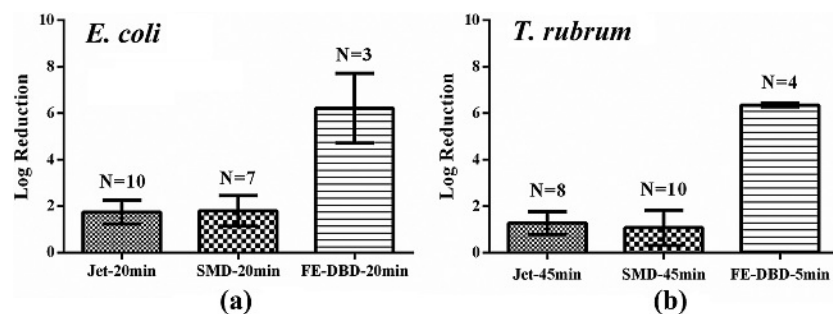


Figure 11. Comparison between the He jet, SMD and FE-DBD applied against (a) *E. coli* and (b) *T. rubrum*, for various times.

against fungus (*T. rubrum*) is clearly superior to the other two with up to 45 min exposure. These results show that FE-DBD can clearly be more effective than the other two devices, but we note there are several limitations and issues with operating the FE-DBD device. These are discussed in the following section.

5. Discussion

The results presented here show that all three of the plasma devices tested: He plasma jet, the SMD device and the air-operated FE-DBD device were all capable of significant rates of killing both the bacterial target and the fungal target on the bovine hoof discs used as a keratin barrier model for a human nail. The observations of killing rates for the bacterial target and the fungal target were qualitatively similar, but the bacterial targets were generally killed faster. All of the tests showed significant statistical variations, but this general trend was consistent over the entire study.

Table 1 lists some published surface disinfection results on exposed surfaces using air SMD as reported by other groups and from our previous work. The model keratin

surface is plasma-disinfected at rates similar to other, non-skin surfaces.

It is apparent from a number of the sets of results we present that there were sometimes variations between results that were not internally consistent. For example, Figure 10 shows, counterintuitively, that the 10-min FE-DBD exposure of the hoof disc with *T. rubrum* present showed less killing than the 5-min exposure. We note that direct killing experiments (not shown here) did not show inconsistencies like this. We concluded that the most likely explanation

is variation between hoof discs. We made significant efforts to obtain and process the model hoof discs (e.g., slicing and sanding to obtain the same thicknesses) as consistently and repeatedly as possible. However, these natural materials are inherently variable and it proved difficult to make the samples sufficiently identical that the killing rates showed all of the expected trends. This no doubt reflects the nature of human nail material as well, so perhaps this characteristic of inherent material variability is to be expected and must be dealt with in any future human patient applications as well.

The SMD and He jet were approximately equally effective in antimicrobial action even though the devices are significantly different in the way they operate. The He jet is known to involve charged species, currents, and fields at the treated surface but the SMD device does not – only neutral reactive species diffuse from the SMD plasma zone to the treated surface. This result is surprising unless the anti-microbial action for each device is solely due to reactive species that diffuse through the nail material to attack the microorganism on the backside of the nail barrier. The floating electrode dielectric barrier discharge device (FE-DBD) showed significantly better results compared to the other devices, for the cases we report here.

Table 1. Antimicrobial results using air SMD on different surfaces.

Surface type	Microorganism	Treatment time	Log reduction	Ref.
Polypropylene	<i>E. coli</i>	24 s	5	[39]
Paper	<i>B. subtilis</i>	10 min	4–5	[40]
Stainless steel	<i>E. coli</i>	5 min	4–5	[36]
Silicone rubber	<i>E. coli</i>	5 min	4–5	[36]
Silicon surface	<i>E. coli</i>	5 min	4–5	[36]
Pig skin	<i>E. coli</i>	5 min	2	[36]
Pig skin	<i>E. coli</i>	6 min	4	[29]
Bovine hoof	<i>E. coli</i>	5 min	~4	This paper
Bovine hoof	<i>T. rubrum</i>	5 min	2	This paper

For example, Figure 11 demonstrates the measured log reductions using all three devices against *E. coli* (bacteria) and *T. rubrum* (fungus) on the backside of the discs. The FE-DBD device showed an average of about 6 log reduction against *E. coli* whereas the other two devices showed only 2 log reductions on average. The corresponding comparison for *T. rubrum* treatment is even more striking. For the cases we show here, the FE-DBD device achieved an average of 6 log reductions after only 5 min exposure while the other two devices achieved an average of under 2 logs after 45 min. One possible explanation for the difference in effectiveness compared to the other devices is that the FE-DBD probably delivers to the surface more reactive species, charges, currents, and a stronger electric field in the filamentary discharges that characterize the FE-DBD. More definitive mechanistic studies will need to be conducted to clearly identify why the different devices show the effectiveness patterns that are observed.

However, regardless of the mechanisms involved, there are several important caveats that should be kept in mind when interpreting these data. Considerable efforts were made to be sure the samples were prepared as consistently as possible, but in some cases, results showed that the hoof disc in question was somehow damaged during sample preparation, so the sample was rejected. For example, in Figure 11, the data denoted $N=10$ and showing a standard deviation of about 1 log reduction as indicated by the magnitude of the error bar, represents in fact 30 experiments. Each set of data represents three measurements for each of the N sets reported. It should also be noted that problems with sealing around the edges of the discs sometimes occurred but was easily detected because the log reductions would be much greater in those cases and not at all consistent with other measurements from the same set of hoof discs. We conclude that the data reported here are generally reliable but clearly more needs to be done to more completely characterize these model nail materials in future studies. For example, we did not systematically image the disc materials with microscopy before use, and the spectroscopic measurements we made were only preliminary.

The second point that is important to keep in mind when comparing results from the different devices is that approximately 50% of the samples that were treated with our FE-DBD device were damaged sufficiently during processing that the samples had to be discarded. The results we present in this paper were the successful tests for which no significant damage was observed. We note that separate tests of sample temperature rise during FE-DBD treatment showed that even after the longest exposures, temperature rise was within 5 °C. Separate experiments (not shown) demonstrate that this small rise in temperature did not cause the change in killing rate. We conclude

that temperature rise is therefore not responsible for the higher killing rates observed with the FE-DBD device.

We tentatively attribute the problems observed with some of the FE-DBD exposure experiments to several characteristics of our FE-DBD device applied to nail material. First, we note that nail material is not flat, and therefore the distance between the flat dielectric surface of the device and the nail surface varies from point to point. This appears to sometimes affect the distribution of current-carrying filaments that originate on the DBD surface and travel to and through the nail. Using the FE-DBD, current flows through the nail to a backing conducting plate and the individual filamentary currents cannot be easily controlled with the plasma device and power supply we used in these experiments. It was sometimes observed that a small hole would appear at some point on the disc during these damaging FE-DBD treatment, leading to a focusing of the current in that location and subsequent macroscopic hole formation. From the point of view of therapeutic applications of plasma devices for treating onychomycosis especially on inherently non-smooth nail surfaces, the FE-DBD devices need to be better controlled to avoid damaging the target nail. Nanosecond pulsed power supplies (e.g., a pulsed-DC configuration) might be beneficial for better FE-DBD control. Damage to the discs was never observed when using either the He jet or the SMD device. They were generally less effective but much safer.

Finally, there is the question of the possible mechanisms leading to backside killing of bacteria or fungus using the plasma devices. We note that the electrical field created at the nail surface by the FE-DBD device, and possibly some plasma jets, may contribute to their antimicrobial effect. A separate study is underway to develop a mathematical model for the effects of the SMD device for which there are no direct plasma (or current) effects occurring during treatment. In the case of the SMD, the killing mechanism seems likely to be due to diffusion of reactive species created in the plasma through the porous nail material to attack the underlying microorganisms. FTIR measurements suggest that nitrogen oxide species (e.g., NO_2) are present in significant concentrations under the conditions we used, and the well known antimicrobial effects of these species may be playing a key role in the effects we observed in the present study. These results will be presented separately, but briefly, the analysis suggests that it is indeed likely that diffusion of known plasma-generated reactive species such as NO_2 are diffusing through the nail material to reach the backside of the target. We note that the complexity of the natural keratin nail material coupled with complex plasma chemistry makes this analysis difficult and we expect that considerable future research will be needed to

significantly advance our theoretical understanding of plasma treatment of onychomycosis.

6. Conclusion

Atmospheric pressure plasma devices have been shown to be effective in killing both *E. coli* bacteria and *T. rubrum* fungus acting through model nail material made from sliced bovine hoofs. A He plasma jet, a surface microdischarge device (SMD) operating in air, and a floating electrode – dielectric barrier discharge (FE-DBD) device operated in air were compared in the study. The bacterial target was killed considerably faster than the fungal target, as expected. The He plasma jet and air SMD device were comparably effective in killing both bacteria and fungus through the model nail material. The FE-DBD device proved considerably more effective than the other two devices but it was more difficult to control. The difficulty to control the FE-DBD device led to numerous instances of damaged nails and suggests that better-engineered FE-DBD devices and possibly improved power supplies are needed. Temperature measurements of the FE-DBD treated samples showed that the higher kill rates with FE-DBD were not due to plasma-induced heating.

The prospects for future clinical applications of plasma devices to treat human onychomycosis seem promising. Three different plasma devices, operating in open air, were shown to be reasonably effective. Plasma devices are relatively safe, simple, inexpensive, and fast acting as compared to the alternative therapies for onychomycosis. The present results suggest that there is a likely future for the use of plasma to treat this serious and widespread affliction.

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