The role of biofilms in onychomycosis

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Onychomycosis is a fungal infection of nails primarily caused by dermatophyte fungi. Fungi are traditionally understood as existing in the environment as planktonic organisms; however, recent advancements in microbiology suggest that fungi form biofilms—complex sessile microbial communities irreversibly attached to epithelial surfaces by means of an extracellular matrix. The extracellular matrix also acts as a protective barrier to the organisms within the biofilm. The biofilm is surprisingly resistant to injury and may act as a persistent source of infection possibly accounting for antifungal resistance in onychomycosis. (J Am Acad Dermatol 2016;74:1241-6.)

Key words: antifungal; biofilm; dermatophyte fungi; extracellular matrix; nail; onychomycosis.

Onychomycosis is a fungal infection of the nails that can be resistant to antifungal treatment, and is associated with persistent infection and/or relapse despite the fact that identical therapy is highly effective in skin infections.[1] Complete cure may take 12 months or longer,[2] and up to 18 months in slower-growing toe nails.[3-7] Nail plate involvement can be so severe that in some cases, nail plate removal is required.[8]

Fungi such as those implicated in onychomycosis are traditionally understood as existing in the environment as planktonic (ie, free floating, suspended, and individually acting).[9] Yet recent advancements suggest that, like bacteria, fungi alternate between planktonic and surface-attached multicellular communities called "biofilms," which offer specific advantages to the organisms they contain,[10] and are an effective adaptation for the evasion of stressful conditions.[11] Benefits include increased resistance to antimicrobial agents,[12] protection from host defenses,[13,14] increased virulence and communication,[15] metabolic cooperation, and community-based differential gene expression.[16-19]

The existence of fungal biofilms has now been observed and documented[20,21] as has use of the nail as an in vitro substrate for biofilm formation.[22] In fact, most naturally occurring microbes have been found to exist in a biofilm.[10,23,24] Moreover, the transition from planktonic to sessile growth has been correlated with pathogenesis,[25,26] which is corroborated by the fact that the interaction and adhesion to host tissues directly impacts disease severity.[27]

Previously observed medically important biofilms have been documented on dental plaques, urinary catheters, and implanted prosthetic devices.[28] Reminiscent of the hard-to-treat nature of onychomycosis, microbial biofilms on artificial joints or osteosynthetic material are often resistant to therapy[29] and require complete removal.[30,31] Their persistence is extreme in that even physical disruption in combination with chemical rinse on dental appliances[32,33] or periodic removal of devices in combination with antifungal treatment has not provided long-term resolution.[10]

Biofilms have also been found to exist on epithelial surfaces.[34] Epithelial biofilms have been associated with several dermatologic diseases including acne,[35,36] rosacea, atopic dermatitis,[37-39] and impetigo.[40] In addition, distinctive dense white masses along with abnormal fungal elements have been observed within diseased nails.[28,41] Therefore, fungal biofilms in the nails may act as a persistent

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source of infection and account for antifungal resistance in onychomycosis.

FUNGAL BIOFILMS

Microbial biofilm communities possess distinctive morphologies compared with their planktonic counterparts, and function as cooperative, organized consortia, in some ways mimicking the behavior of multicellular organisms.[42-44] Fungal biofilms are formed when an adherence to a foreign substrate is developed. An extracellular matrix (ECM) is secreted and encases the entire microbial community. The ECM is essential in protecting against physical disruption of the structure and host immune factors while conferring antifungal resistance.[10] For instance, staphylococcal biofilms deficient in proper matrix formation have an increased susceptibility to phagocytosis.[45]

CAPSULE SUMMARY

- Fungi can form biofilms, complex communities protected by an extracellular matrix.
- Evidence is presented for the idea that the antifungal resistance and high rate of recurrence and relapse of onychomycosis is a result of biofilms.
- Accessible methods for both the diagnosis and treatment of biofilms are required.

A variety of fungi have demonstrated the ability to form biofilms (Table I). Many of which have also been identified as causal agents of onychomycosis.[46-50] The first study of the ability of dermatophytes to form biofilms was conducted in vitro using *Trichophyton rubrum* and *T mentagrophytes*.[51] Results showed that after 72 hours, both species were able to form biofilms with initial formation occurring within 3 hours and a significant increase in fungal mass observed at 48 hours.[51]

Interestingly, cells detaching from the biofilm contain greater cytotoxicity in comparison with planktonic cells as demonstrated through increased mortality in a murine model.[52] Thus the rapid ability of fungi to colonize the nail plate and form a biofilm is likely to play a significant role in the pathogenesis of onychomycosis.

TREATMENT OF ONYCHOMYCOSIS AND ANTIBIOFILM THERAPY

Success rates for onychomycosis therapy are less than optimal with the achievement of disease-free nails at less than 50%.[53] This prognosis is further compounded by high rates of recurrence and reinfection.[54-56] Considering current options, systemic therapy has been the most successful, as topical treatments are limited by their ability to penetrate the keratin of the nail[57] resulting in reduced disease cure and increased risk of reinfection and relapse.[3,58-60] As biofilm formation may be responsible for the recalcitrant nature of onychomycosis to treatment,[28] research into new therapy that targets sessile growth may prove invaluable. For instance, once a mature bacterial biofilm is established, the high antibiotic dosage necessary for effective treatment can be inhibitive[61] as a decreased susceptibility of up to 1000-fold has been documented.[62,63] Many theories have been suggested for the source of the super resistance, although the idea of contributions from multiple synergistic adaptations has generally been proposed. Previously discussed mechanisms include difficult penetration and the presence of drug efflux pumps.

Similar to a fortified community, the presence of the ECM has been credited with reduced penetration of antimicrobial agents.[64,65] Additional evidence correlates rates of higher metabolic activity with resistance in mature (vs developing) biofilms, suggesting that it is the developmental stage that plays the key role in resistance.[66] As was discussed above, communication, metabolism, and horizontal gene transfer are all streamlined within biofilms. Thus this evidence makes it tempting to speculate that as the biofilm matures, it is able to more efficiently synchronize these processes, resulting in the observed increased resistance. Also correlated with a mature biofilm, a higher cell density has been implicated with the decreased efficacy of antifungals including azoles.[67] Finally, the common presence of drug efflux pumps in biofilms has been confirmed and is linked to azole resistance.[68,69] Exploiting a broad-spectrum mechanism, drug efflux pumps are able to confer multifactorial resistance resulting in multidrug-resistant phenotypes.[70-75]

Previous treatments that have shown some efficacy in clearing biofilms include amphotericin B[76-81] and its liposomal formulation,[82] and echinocandins.[76-81] Echinocandins, however, are not completely effective[83] as caspofungin has been shown to be active against some but not all species.[14,78-81] Antifungal lock therapy including amphotericin B[84,85] and its liposomal formulation,[86,87] caspofungin,[88] and ethanol[89] has been used successfully to navigate the issue of high concentration requirements in biofilm treatments. Some success has also been observed with mucolytic agents such as acetylcycteine[90] and ambroxol, which increased

Table I. Fungi with demonstrated biofilm formation ability

Fungal species implicated in biofilm formation	Reference
Trichophyton rubrum	51
Trichophyton mentagrophytes	51
Candida albicans	10
Cryptococcus neoformans	92
Cryptococcus gattii	110
Rhodotorula species	111
Aspergillus fumigatus	112
Malassezia pachydermatis	113
Histoplasma capsulatum	114
Paracoccidioides brasiliensis	115
Pneumocystis species	114,116
Coccidioides immitis	117
Fusarium species	118
Saccharomyces cerevisiae	20
Trichosporon asahii	62
Mucorales	119
Blastoschizomyces capitatus	120

sensitivity to voriconazole.[91] In addition, both cationic antimicrobial peptides[92] and antibody-guided alpha radiation[93] have been shown to be effective biofilm treatments. Adapting or combining biofilm-targeted therapy with current onychomycosis treatments may lead to improvements in success rates.

INVESTIGATIVE ANTIBIOFILM THERAPY

Additional strategies still undergoing investigation include agents that reduce attachment and synthesis of the ECM or increase penetration[28] or persistence of drugs, as the drug must accumulate in the nail plate for extended periods of time.[94,95] Glycoproteins on the cell surface are a coveted target of treatment as they are involved in attachment.[20] Likewise, enzymes have been exploited to facilitate the degradation of the ECM.[96] For instance, DNAse treatments targeted at extracellular DNA were used to weaken the ECM, which successfully increased activity of subsequent polyene and echinocandin therapy by up to 15-fold[96] whereas gentian violet also successfully improved penetration of bacterial biofilms.[97] Antibody-mediated inhibition of matrix polysaccharides is another mechanism shown to inhibit biofilm formation in *Cryptococcus neoformans*.[92] Similar observations were made with the innate immune component lactoferrin in some, but not all, instances.[92] Alternatively, quorum-sensing molecules have been successful in reducing developing *Candida albicans* biofilms but not mature biofilms[98] as inhibitors are expressed by the sessile cells.[99] Other interest has been shown to PacC, the *T rubrum* transcription factor necessary for proper secretion of keratinolytic proteases[100] and sulphite transporter virulence factors.[101] Natural agents that have shown promise include chitosan, which prevents biofilm formation when used as a pre-coater,[102] a *Streptococcus thermophilus* biosurfactant,[103] and capric acid isolated from *Saccharomyces boulardii*.[104] Combination therapy may also be a viable option in the case of mature biofilms. For example, treatment of *Staphylococcus aureus* biofilms with both farnesol and xylitol provided positive results.[39]

Conclusion

Taken together, the evidence suggests the less than ideal treatment success rate for onychomycosis may be explained in part by the formation of biofilms. Moreover, although biofilms are notoriously difficult to treat, progress has been made in the search for efficient antibiofilm therapy. To start, as first proposed by Pierce et al,[105] antifungal agents should be tested against biofilms and not planktonic cells for susceptibility. This is especially true in the case of onychomycosis as in vitro susceptibility analysis of strains cultured from recalcitrant clinical onychomycosis cases produced results inconsistent with clinical observations.[106] As such, the need for a diagnostic test to detect biofilms has been suggested.[107] With the acknowledgment that an improvement in both efficacy and duration of treatment would be beneficial, the search for antibiofilm therapy is anticipated to be an important path in future onychomycosis research. This need is further amplified by increasing reports of terbinafine resistance, the current therapy of choice.[2,108] As a second consideration, with age as a major risk factor,[109] it can be reasonably predicted that instances will increase in both quantity and severity as the population ages. Thus we suggest that the root cause of treatment resistance observed in onychomycosis is a result of the formation of biofilms and future therapy should include treatment specifically designed for degradation of biofilms.

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