Antibiofilm Treatment for Onychomycosis and Chronic Fungal Infections

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Abstract

Onychomycosis is a difficult-to-treat chronic fungal infection of the nail. The chronic nature of onychomycosis, with relevance to current treatment practices, could be attributed to host anergy, development of increased virulence in causal agents (multidrug resistance efflux pump), and biofilms. Biofilms must be disrupted prior to antifungal treatment suggesting the necessity of combination treatment. Once the biofilm has been disrupted, further techniques in addition to antifungal usage are suggested to ensure a positive prognosis including use of antimicrobial photodynamic therapy or low-frequency surface acoustic waves. Overall, with continued success in developing antibiofilm treatment for bacterial and yeast pathogens, therapy can be more quickly expanded to dermatophytes. With a rise in predisposing factors, it is important to preemptively address treatment for this disease with continued investigation into antibiofilm therapy including optimal treatment combinations and dosages targeted specifically at dermatophytes.

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Introduction

Despite their ubiquitous presence, fungal pathogens rarely lead to disease in immunocompetent individuals. Moreover, in the event of successful infection, many are superficial with effective therapy available. In contrast, dermatophyte infection of the nail, onychomycosis, is difficult to treat and often leads to recurrences or relapse [1]. As onychomycosis is believed to affect 7–14% of North Americans [2–5] and contributes to 50% of nail disease [6], possible reasons for the chronic nature of the disease with relevance to current treatment practices were investigated.

Chronic Disease

Dermatophyte pathogens are normally susceptible to antifungal treatment; thus, it is unclear why infection of the nail or onychomycosis often results in chronic disease. Drug malabsorption and degradation by microsomal enzymes has been proposed as a contributing factor [7]. Additional explanations for the discrepancy that have been explored include host anergy and development of increased virulence in causal agents.

Fig. 1. Planktonic growth versus biofilm formation. As microbial numbers reach a minimum density, biofilm formation begins with surface attachment and development of the protective extracellular matrix (ECM) resulting in differential phenotypic characteristics that lead to resistance to both antimicrobial agents (including antifungals) and the host immune system.

Evidence of anergy, or lack of immune response to specific fungal antigens, has been observed in patients with chronic (greater than 5 years) dermatophytosis [8]. The constitutive presence of dermatophytes and residual fungal elements is believed to induce selective immune tolerance, suggesting the importance of early treatment (within 5 years of infection) to prognosis [8]. Alternatively, complete elimination of fungal presence such as that achieved with oral terbinafine treatment can restore cell-mediated immunity [8]. Immune restoration is not observed in all patients while some require additional treatments, allowing for recurrence or chronic disease to continue. It is hypothesized that refractoriness to immunological conversion may be genetically determined [8].

In combination with anergy, pathogen virulence is also believed to influence disease progression and treatment recalcitrance. For instance, a proposed multidrug resistance efflux pump has been identified in dermatophytes that leads to resistance against both griseofulvin and tioconazole [9]. It is expressed in response to diverse toxic compounds including ethidium bromide, ketoconazole, cycloheximide, fluconazole, griseofulvin, imazalil, and itraconazole [9], suggesting a mechanism for strains carrying the corresponding gene to escape diverse

antifungal treatments. Further investigation, however, has revealed that acquisition of genetic resistance to antifungals is not likely the reason for treatment recalcitrance. Genetically identical isolates of *Trichophyton rubrum* leading to chronic nail disease remain susceptible to antifungals such as griseofulvin, ketoconazole, itraconazole, and fluconazole when grown in vitro [10]. Therefore unknown contributing factors of the nail environment remain to be elucidated.

Biofilms and Onychomycosis

Another explanation for the chronic nature of onychomycosis is the presence of biofilms. Biofilm formation is known to lead to chronic infection with recalcitrance to traditional treatment [11, 12], similar to what has been described for onychomycosis. Biofilms are also known to lead to similar symptoms including persistent inflammation and tissue damage [11]. Moreover a significant amount of microbial infections are known to be a result of biofilm formation [13].

The presence of biofilms can also explain the difference in treatment response as phenotypic characteristics

of the same organism change (including growth rate and gene activation) when converting from planktonic to biofilm lifestyles [14]. For example, microbes can form communities insulated from the environment within biofilms. In addition, increased communication, increased virulence, improved metabolic cooperation, and cooperative regulation of gene expression have been observed [15] leading to increased resistance to antimicrobial agents and increased protection from host defenses (Fig. 1) [13].

To further build on this theory, dermatophytes such as *T. rubrum* are known to be able to form biofilms on nails [14]. This can be established in part through quorum sensing. Quorum sensing, a method of communication between microbial cells used to establish population density, can act to coordinate a group response including biofilm formation and virulence. When a minimum density of bacteria/fungi accumulate, genes involved in biofilm formation are induced, thus increasing the odds of successfully establishing a mature biofilm prior to immune detection [16–18].

Antibiofilm Treatments

Through the process of biofilm formation, microbes have the ability to bind irreversibly to a surface. Therefore, once formed, surgical removal of a mature biofilm may be necessary and is used in the case of implanted devices or live tissue [19]. Similarly, microbes encased in a mature biofilm are not as accessible to the immune system, which can lead to tissue damage without clearing of the infection [20, 21]. This is believed to be accomplished through the development of a protective layer, the extracellular matrix (ECM). Presence of the ECM also limits the efficacy of antimicrobial agents such as antifungal treatment, either through physically inhibiting contact with microbes or slowing the penetration rate [22]. As a result, increased resistance up to 1,000 fold is observed [12, 23].

Therefore, biofilms must be disrupted prior to antifungal treatment suggesting the necessity of combination treatment. Components of the ECM which could act as targets for treatment include polysaccharides, proteins, and extracellular microbial DNA [24]. Use of enzymes including deoxyribonuclease I (DNase I), α -amylase, and lyase as well as lactic acid, chitosan, terpinen-4-ol-loaded lipid nanoparticles, and povidone-iodine (PVP-I) have all been investigated for an ability to disrupt biofilms.

In response to bacteria, DNase I degraded 50% of common biofilms, which increased efficacy of the antibiotics

azithromycin, rifampin, levofloxacin, ampicillin, and cefotaxime [25], while up to 95% reduction in biofilm was observed in a second study [26]. Use of α -amylase was effective in detaching biofilms for some bacterial species (*Staphylococcus aureus*) but not others (*S. epidermidis*) [27]. Lyase in combination with gentamycin reduced viable counts of *Pseudomonas aeruginosa* by 2–3 $\log_{10}$ units [28], while lactonase increased penetration of ciprofloxacin and gentamycin [29]. A combination of lactic acid and cetrime eliminated *Enterococcus faecalis* and *E. duran* biofilms [30]. Chitosan reduced biofilms of *Streptococcus mutans*, *Listeria monocytogens*, *Bacillus cereus*, *S. aureus*, *Salmonella enterica*, and *P. fluorescens* [31, 32]. Finally, PVP-I demonstrated antibiofilm activity against *Porphyromonas gingivalis* and *Fusobacterium nucleatum* with a 4–6 $\log_{10}$ reduction in viable counts [33].

In response to *Candida albicans* biofilm, use of DNase I improved amphotericin B treatment by a 1–5 $\log_{10}$ reduction in viability depending on dosage but did not improve caspofungin and fluconazole efficacy [34]. Likewise terpinen-4-ol-loaded lipid nanoparticles were observed to eradicate *C. albicans* biofilms [35].

Once the biofilm has been disrupted, further techniques in addition to antifungal usage are suggested to ensure a positive prognosis including use of antimicrobial photodynamic therapy (PDT) [24]. Use of PDT for bacterial biofilms led to an almost complete disruption and clearance of *P. aeruginosa* [36], a reduction of greater than 6–7 $\log_{10}$ units depending on treatment protocol for *P. aeruginosa* and methicillin-resistant *S. aureus* [37, 38], and a reduction of 1.9 $\log_{10}$ in the viable counts of *E. faecalis* [39]. Use of PDT for *C. albicans* biofilms led to a reduction of 0.33–0.85 $\log_{10}$ [40]. Similarly, in mouse models, PDT has also been successful in reducing bacterial density [41–43] and *C. albicans* biofilms [44]. For best results, an increase in light dose is recommended when treating a biofilm which can help maintain efficacy against this resilient target without raising photosensitizer concentration, which could increase toxicity to patients [24].

A second option includes the use of low-frequency surface acoustic waves (SAW). SAW were previously shown to reduce bacterial biofilms (*Escherichia coli*, *S. epidermidis*, and *P. aeruginosa*) formed on catheters by greater than 85% when used in combination with antibiotics [45].

Overall, with continued success in developing antibiofilm treatment for bacterial and yeast pathogens, therapy can be more quickly expanded to dermatophytes.

Conclusion

Observational evidence suggests that the abnormally difficult-to-treat nature of onychomycosis may be caused by the formation of biofilms. The phenotypic changes, differential gene regulation, and increased virulence of sessile microbes in comparison to planktonic growth correlates with genetically identical dermatophyte strains observed to be recalcitrant to treatment in patients with nail disease but susceptible to antifungals when grown in vitro. To address the possible role of biofilms in onychomycosis, antibiofilm treatment is suggested in combination with traditional antifungals. This includes disruption of the biofilm, possible through use of enzymes and exog-

enous treatments along with additional therapy such as PDT or SAW. With a rise in predisposing factors (diabetes, immunosuppression, obesity, smoking, and advancing age [46–50]) it is important to preemptively address treatment for this disease with continued investigation into antibiofilm therapy including optimal treatment combinations and dosages targeted specifically at dermatophytes.

Disclosure Statement

The authors have no conflicts of interest. There were no funding sources for this work.

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