Dermatophytoma: Recalcitrance to treatment because of existence of fungal biofilm

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Biofilms are populations of microorganisms that adhere to surfaces and produce an extracellular polysaccharide, increasing resistance to standard antimicrobials. This microbiologic principle may be beneficial in understanding refractory dermatophyte infections, such as dermatophytomas of the nail. In this condition, circumscribed dense white fungal masses live within and under the nail plate. Biofilm research could lead to finding new targets for antifungal therapy, including agents that reduce attachment of microorganisms, alter microorganisms' ability to synthesize extracellular matrix, and better penetrate the extracellular matrix to kill the organism contained with the biofilm. (J Am Acad Dermatol 2002;47:629-31.)

Dermatophytomas are a form of onychomycosis often refractory to standard oral antifungal therapies, which may require surgical removal of the diseased nail plate.[1] Clinically, dermatophytomas present as circumscribed dense white masses within the nail (Fig 1). They may be round or longitudinal (in which case they can be linear or conical). On removing the overlying nail plate, a thick hyperkeratotic mass is noted.[2] Histologically, the loculated hyperkeratotic mass lying within and under the central nail plate exhibits clumps of fungal elements, which have been described as thick-walled and somewhat abnormal in appearance.[2] The actively growing portion of dermatophytomas is tenaciously adherent to the surrounding nail plate.

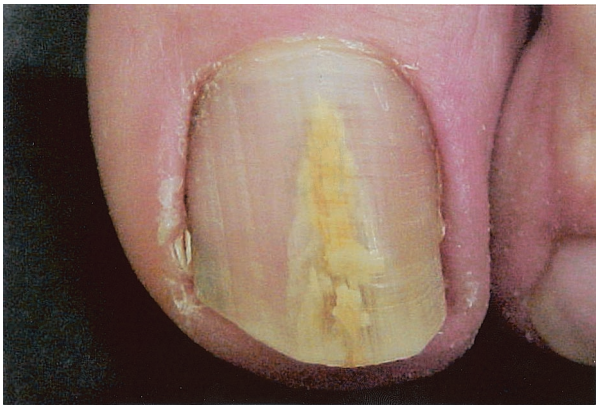

Fig 1. Clinical case of dermatophytoma demonstrating circumscribed white mass of fungus within nail plate that proves resistant to simple topical and oral antifungal therapy.

Most microorganisms exist in nature not like plankton, or free-floating microorganisms in suspension, but as biofilms.[3,4] In this article we introduce the concept of biofilms in dermatophyte infections. Biofilms are populations or communities of microorganisms that adhere to surfaces. An extracellular polysaccharide usually encases this aggregation of microorganisms, which is synthesized by microbial constituents of the biofilm. The extracellular matrix usually composes two thirds of the biofilm mass. The matrix components include microbial cells, polysaccharides, water, and excreted cellular products.[4] Bacterial organisms encased in biofilms are reported to be 50 to 500 times more resistant than planktonic microorganisms to chemotherapy. Resistance is related to reduced penetration of antimicrobial agents into the biofilm polysaccharide matrix, slow growth rate of organisms within the biofilm, ability of microorganisms to express properties distinct from planktonic cells, and other physiologic changes brought about by interaction of the organisms with surfaces.[5,6] Classic examples of biofilms include dental plaques, infections in urinary cathe-

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ters and implanted prosthetic devices, and slippery coating on rocks. In fact, most microorganisms exist in nature as biofilms.

Biofilms are a dynamic state of homeostasis in which the component microorganisms are optimally organized to make use of all available nutrients.[3] The biofilm matrix reveals great microheterogeneity, thereby allowing numerous microenvironments to exist within one biofilm.[3] The nature of the matrix is dependent on factors that are both intrinsic (genetic profile of the component microbial cells) and extrinsic (physicochemical environment, solute transport, and solute diffusion gradients).[3] Thus, for example, one fungal element may be actively producing keratinases, proteolytic and other enzymes, and actively reproducing, whereas other members of the same species may lay dormant within the same biofilm. The hyphal element with low activity would be very recalcitrant to standard antifungal therapies that attack actively synthesizing organisms.

Although bacteria are often associated, pure fungal biofilms are a recognized entity.[7,8] Yeasts have great genetic ability to initiate surface adherence, and their function can be altered by various microenvironmental factors including the surrounding bacterial flora and temperature.[8,9] In terms of dermatophytes, one of the fungal cell surface glycoproteins, namely Flo11p, is directly involved in adherence to surfaces.[7] As with dental plaques, certain species of microorganisms have more genetic potential to adhere to surfaces. At present, the various dermatophyte organisms have not been adequately studied for their functional abilities to adhere to nail tissue.

The interaction of the environment, the nail plate, and surrounding microorganisms is complex. A suitable environment on a nail must be present for a fungal biofilm to develop. Factors increasing the ability for surface adherence would include nail trauma, dryness of the nail from aging, increase in nail hydration by occlusion with a nonporous material, removal of nail cuticle leading to a moisture defect, interference of normal nail barrier function, removal of protective lipids by soaking, and external damage of the nail by mycotoxins.[10] In addition, fungal biofilms may form when there is a minor defect in the normal nail architecture. The nail has recently been shown by synchroton x-ray microdiffraction to contain 3 distinct layers characterized by different orientations of the keratin molecules separated by lipid bilayers which are 49 Å thick.[11] Of note, fungal elements have been localized between these nail laminae by in vivo confocal microscopy.[12] Thus a structural defect of nail formation would facilitate fungal attachment. Any fungal growth within the nail further disrupts the keratin structure of nails.[11]

In the specific case of dermatophytoma, the concept of biofilms explains many of its peculiar characteristics. Biofilms, by nature, prove considerably more resistant to traditional therapies, as seen in dermatophytomas. All forms of onychomycosis can be host to more than one fungal species as well as bacterial "contamination," as clearly demonstrated with immunohistochemistry and flow cytometry studies.[13,14] The presence of biofilms explains the previously reported abnormal-appearing hyphal elements in the keratinous waste portion of a dermatophytoma.[2] Living fungal elements with normal-appearing histologic features would reside within the active biofilm, strongly adherent to the nail plate and difficult to remove surgically. Inasmuch as microorganisms may exist in a somewhat dormant state within biofilms,[3] this explains the histologic findings of resting chlamydospores and arthroconidia within infected nail plates.[15,16] The concept of biofilms supports the theory behind boosted oral antifungal treatment for dermatophytomas.[15,16] Microbiology may offer other avenues of therapy to attack fungal biofilms.

Thus biofilm research may lead to finding new targets for antifungal therapy. Such therapies could include application of a molecule that reduces the attachment of microorganisms that would otherwise initiate biofilm production. Another angle might be directed at altering the bacterial and fungal ability to synthesize the extracellular polysaccharide. In addition, one could disrupt specific components of the biofilm matrix. Effective treatment would also dictate that the agent should adequately penetrate the biofilm to kill the microorganism contained within it. Further research is warranted to examine the wide range of biologic, chemical, and physical factors that affect the microenvironment of biofilms in dermatophyte infections. Moreover, the transformation of planktonic fungus to a biofilm is a complex and highly regulated process that needs further study.

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