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Research Article

Nail Histomycology, Onychochromobiology, and Fungal Thigmatropism

Gérald E. Piérard[1] and Sébastien L. Piérard

[1]*Laboratory of Skin Bioingineering and Imaging (LABIC), Department of Clinical Science, University of Liège, 4000 Liège, Belgium*
[2]*Telecommunications and Imaging Laboratory INTELSIG, University of Liège, Belgium*

Corresponding Author: Gérald E. Piérard; email: gerald.pierard@ulg.ac.be

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Abstract. *Background:* Thigmotropism is a biologic feature coping with the directional growth of cells following topographical guidance cues. This mechanism is involved in the invasive phase of pathogen and opportunistic fungi. It was shown experimentally with fungal hyphae of both dermatophytes and nondermatophyte molds, as well as with the mycelial phase of the dimorphic yeast Candida albicans. *Objective:* To revisit histomycology in onychomycoses of a diversity of fungal origins. *Method:* Histopathological section of nails plates were oriented parallel to the nail direction of growth. *Result:* Thigmotropism in part explains the patterns of orientations and shapes of fungi invading nail plates. It is probably influenced by onychochronobiology (speed of growth of the affected nails), and it governs various clinical presentations of onychomycoses.

Keywords: contact-sensing; Histomycology; Hyphal growth; Fungus; Onychomycosis; Onychochronobiology; Fungal nail infection

1. Introduction

The diverse clinical presentations of onychomycoses are in part correlated with the location and distribution of fungal cells inside the nail plates, and with the nail growth as well [1, 2].

A broad range of eukaryotic cells are able to point up their direction of growth in relation to some physical and topographical features of the surrounding environment [3]. In physiological conditions, and in pathology as well, the ability to sense and respond to physical characteristics of the surrounding environment affects a large number of tip growing cells living on and within solid tissues and other materials. Such thigmotropism also known as contact-sensing growth, touch-sensitive response, or contour guidance represents a tropism behaviour. A representative example is given by plants that reversibly rotate the root apex for the circumnavigation of some obstacles present in soil [3–5]. Similar features occur in the guided growth of embryo axonal and various dendritic processes growing within solid embryonic or regenerating tissues to achieve the innervation of specific sites. Tumoral skin pathobiology is possibly influenced by neoplastic cell thigmotropism [6].

Many fungi are multicellular organisms representing a separate group alongside plants and animals. They represent a group of nonmotile eukariotic cell organisms. They are generally divided into saprophytes and parasites. Some fungi form mycelia or hyphae which are in close contact and adhere to organic surface. Ringworm is the most common origin of onychomycosis. Infection begins under the leading edge of the nail or along the lateral borders, and it continues until the entire nail and its bed are infected. Characteristic features are accumulation of subungual debris and an opaque, chalky or yellowish, thickened nail. Loosening of the distal nail commonly takes place, but loss of the nail is unusual. A variant, called superficial white onychomycosis, causes a circumscribed white patch on the surface of a toenail. Inflammatory changes of the skin around the nail, including paronychia, are not seen with most onychomycoses.

Thigmotropism is recognized in tissue penetration stages of some fungi. In particular, filamentous forms of dermatophytes, nondermatophyte molds (NDM) and dimorphic yeasts such as Candida albicans exhibit in vitro thigmotropism [7–11]. Such finding suggests that a set of clinically important fungi use thigmotropism allowing host invasion through wounds, surface invaginations or any other area of weakened surface integrity [1, 12]. Further evidence for the clinical relevance of fungal thigmotropism is supported by ultrastructural investigations performed on pseudomembranous oral candidosis [13]. In such condition, Candida pseudohyphae penetrate into the epithelium through intercellular epithelial spacing before crossing the basement membrane and invading the connective tissues.

The aim of the present study was to revisit, using histomycology, the involvement of thigmotropism in the fungal invasion in onychomycosis.

2. Material and method

Histomycology samplings from proven untreated onychomycoses were retrieved from our laboratory files. Samples corresponded to 160 cases of dermatophyte infections, 30 cases of yeast infections showing both yeast-like cells and pseudohyphae, and 10 NDM infections. The sampling method and the histomycological technique had been previously described in detail [14, 15]. Tissue sections were stained with the PAS method, and observations were performed under both regular and polarized light. In some cases, fluorescence microscopy was used with antibodies directed to specific fungi. The overall orientation of the hyphae was scrutinized inside structures of the nail apparatus.

Any suspected mixed infections combining the presence of distinct fungi inside the nails were discarded in the present study.

3. Results

The nail plate structure invaded by fungi was frequently uneven with regard to the nail bed cornified layer. In the distal and lateral subungual patterns of onychomycoses, the pathogen dermatophyte hyphae predominantly run parallel to the wavy pattern of the hyperkeratosis beneath the nail plate (Fig 1a). When the fungi invaded the nail plate from beneath, the main orientation of the hyphae changed to run almost parallel to the nail surface without any evidence for nail plate dystrophy (Figure 1b and 1c). A similar parallel configuration was present in superficial white onychomycoses and in the mid-plate onychomycoses (Figure 2) as well. These patterns were lost when the nail plate was dystrophic. In such instance, the fungi twisted among the disturbed onychocyte orientations in an haphazard way. Trichophyton rubrum was the most frequent dermatophyte isolated at culture.

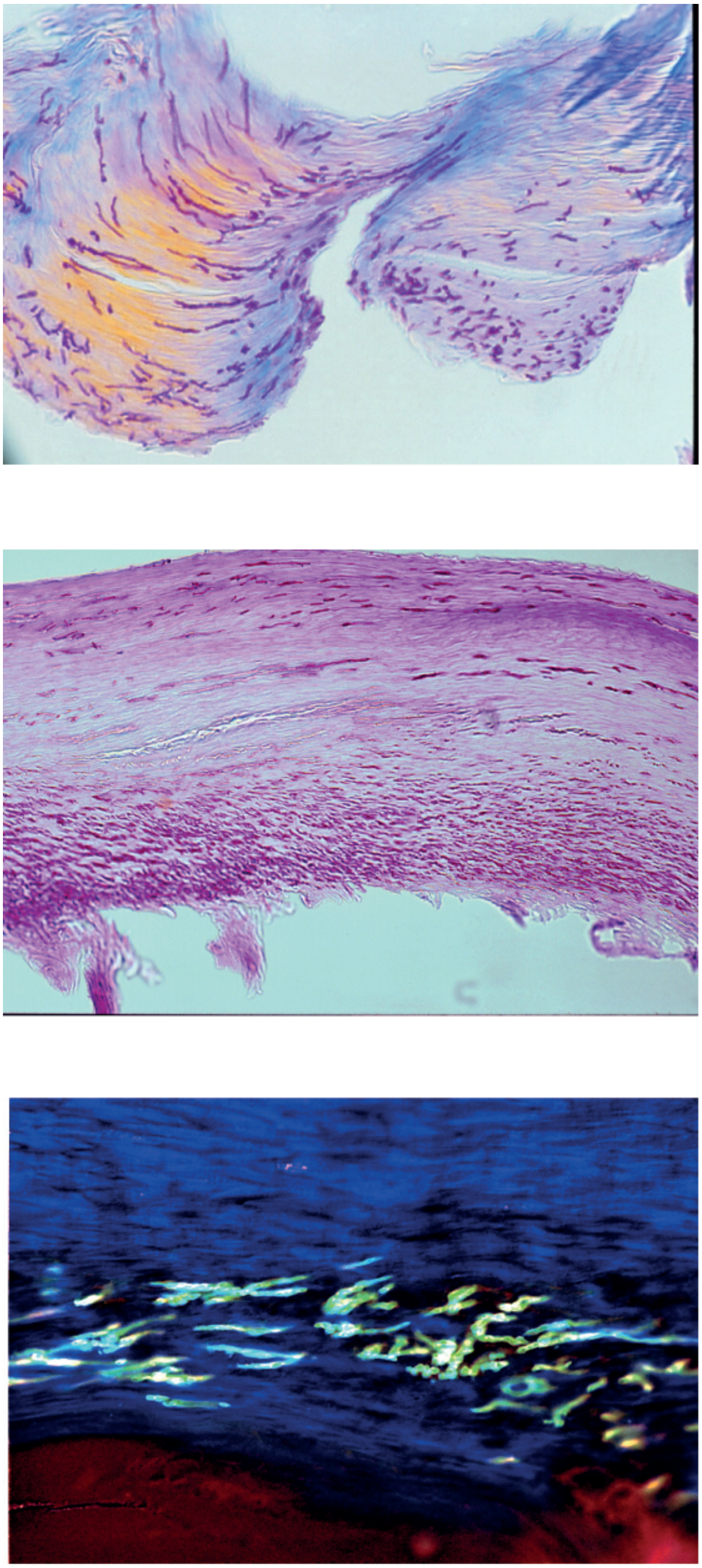
The yeast mycelium of C. albicans commonly showed a unique pattern of infiltration inside the nail. The individual pseudohyphae exhibited helical or twisted growth patterns originating from clumps of yeast-like cells (Figure 3). The growth of pseudohyphae of yeasts appeared multidirectional inside the nail, constrasting with the more linear parallel oriented pattern of dermatophyte invasion. C. parapsilosis was isolated in one case with discrete paronychia.

Basically, the NDM filamentous forms followed patterns of orientation almost similar to dermatophytes, but usually they showed many branchings. A haphazard or wavy pattern predominated in a number of cases. The nail structure frequently appeared dystrophic under polarized light. Aspergillus spp predominated among NDD with rare implications of Scopulariopsis brevicaulis.

The thigmotropic patterns of fungal growth was apparently not influenced by acknowledged predisposing factors for onychomycosis including old age, deficient peripheral circulation and long-standing dermatomycoses. By contrast, repeated nail trauma responsible for onychodystrophy appeared to alter the distribution of hyphae.

4. Discussion

Hyphae elongate by apical growth, although most parts of these organisms have potential for growth. A mass of hyphae is called a mycelium and usually has a distinctly fuzzy appearance on macroscopic examination. The thigmotropic behaviour of some fungi is well acknowledged [1,16–18]. We observed hyphae of pathogenic and saprophytic fungi as well exhibited thigmotropism inside nails. This feature is perceived on sharp histological sections, but obviously, it is lost on rough nail scrapings. It is not present in sporodochium [19]. The fungal mycelium usually produces a circular colony on agar medium and a globose colony in an aerated liquid culture. This is related to the tendency of mycelium to grow similarly in any direction from a central origin. The growth of each hypha occurs at its tip.



(c)

Figure 1: Onychomycosis caused by dermatophytes: (a) thigmotropism in the deep portion of the nail plate (PAS staining, polarized light), (b) peculiar thigmotropism in a total nail invasion by a dermatophyte (PAS staining), (c) thigmotropism in the mid portion of the nail plate (immunostaining).

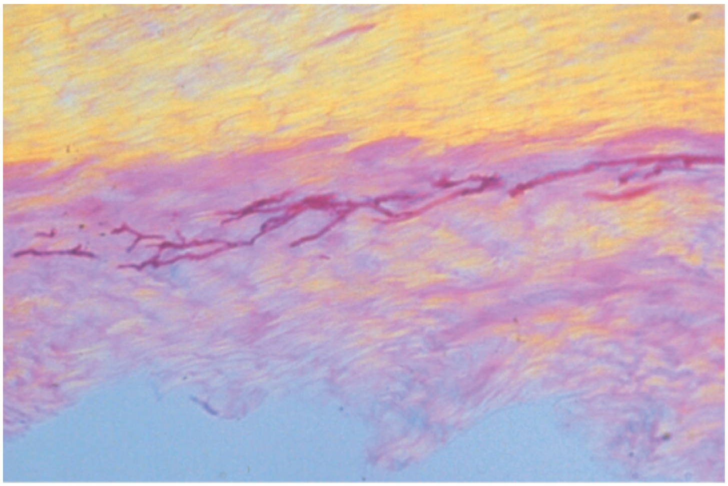


Figure 2: Yeast onychomycosis showing a twister pattern of thigmotropism of Candida pseudohyphae (PAS staining).

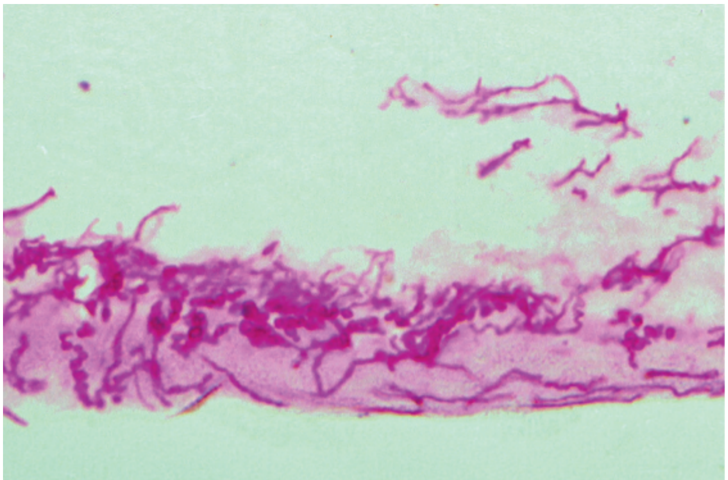


Figure 3: Tortuous thigmotropism of a non dermatophyte mold inside the mid portion of a nail plate (PAS staining, polarized light).

A transmission electron micrograph of septate or nonseptate hyphae showed numerous atypical vesicles in the actively growing hyphal tips. These vesicles are believed to originate from endoplasmic reticulum, and they contain materials and enzymes required for hyphal wall formation.

Dermatophytes are broadly classified into three groups on the basis of their natural habitats and host preferences: (a) geophilic or soil-inhabiting saprophytes, which are only occasionally pathogens for humans or animals; (b) zoophilic species, whose normal hosts are animals, but also infect humans; and (c) anthropophilic species, whose only hosts are humans. All dermatophytes considered in the present study on nails were anthropophilic species.

Dermatophytes are usually confined inside the cornified layers of the human body. The fungal tip is probably involved for promoting penetration into and incorporation inside tissues or other solid substrates. Similarly, thigmotropism probably enhances the ability of dermatophytes to explore, invade and degrade the nail apparatus. Such a process is typically explored using morphological tools. In onychomycoses, a series of observations have revealed the histological aspects of thigmotropism [1]. The thigmotropic properties of dermatophytes in nails largely maintain hyphae parallel to the overall stratification of the cornified layers. Such regular pattern is altered when the nail becomes dystrophic.

The formation of pseudohyphae by C. albicans in response to environmental stress is generally considered to be a feature of pathogenicity. Pseudohyphae likely interact with interface surfaces in a thigmotropic way by sensing the surface and rushing into pores [16, 17]. When growing on a range of surfaces in conditions favouring hyphal growth, C. albicans pseudohyphae grow in a righ-hand helical modality [20]. This is likely the reason why Candida mycelium looks wavy and twisted in histological sections.

Thigmotropic helical growth is not specific for Candida spp. A similar pattern was reported for other fungi. For instance, when growing on agar media with weak nutrient concentrations, vegetative mycelia of many fungal species exhibit spiral growth resulting from an interaction between rotating hyphal tips and the substrate. Other examples of helical growth of aerial and sporulating hyphae are identified, notably the tightly right-handed helical aerial hyphae of Trichophyton species.

During the process of NDM nail invasion the mycelium commonly shows a number of branchings. Hyphae of many fungi exhibit thigmotropism and irregular shapes which depend on tensegrity mechanisms [21–23]. Whether or not thigmotropism and tensegrity are related or independent remains unsettled.

The fungal cell wall contains polysaccharides, including glucan and chitin, as well as glycoproteins. Its precise composition varies according to the systematic position of the fungi. Likely some molecular channels take place in directing fungal growth [23–25]. When the cell is in contact with any inductive surface, stretching of the plasma membrane possibly occurs, during which channels open allowing efflux or influx of specific ions including Ca^{2+} mechanism [25]. As a result, ion concentrations are modulated inside the cell leading to the activation of cascades of events resulting in some physiological responses including hyphal shape control and thigmotropism.

Fungal thigmotropism is clearly present in cornified tissues distinct from nails including the stratum corneum [26] and hairs. Cyanoacrylate skin surface stripping represents a convenient method for exploring such mechanism [26, 27]

In conclusion, fungal thigmotropism is presently confirmed in onychomycoses. Thigmotropism appears clearly involved in the fungal pathogenicity. The aspects are in part different in dermatophytes, NDM and yeasts when these organisms are invading the nail apparatus. Such a property is likely involved in the rate of progression of the infection inside nails. Altering this fungal function might possibly represent a therapeutic target for future drugs.

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