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## REVIEW

# In vitro models of dermatophyte infection to investigate epidermal barrier alterations

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## Abstract

Fungal infections of the skin, known as dermatophytoses, are initiated at the epidermal barrier and lead to dysfunctions of the stratum corneum and cornified skin appendages. Dermatophytosis affects a significant part of the human population and, despite the availability of effective treatments, its prevalence is still increasing. Numerous dermatophyte species are able to induce lesions in both animals and humans, with different clinical pictures and host inflammatory responses. The understanding of the infectious process and of tissue responses has been impeded by discrepancies between observations in vivo or in research models. Indeed, cells cultured as monolayers do not undergo the keratinization process required to study the adherence and invasion of dermatophytes. Animal models lack relevance to study human dermatophytosis because of species-specific differences in the development of lesions and inflammatory responses. This review focuses on the recent development of cultured human skin equivalents, which partly overcomes those limitations and allows improved understanding of the pathogenesis of dermatophytosis in human being, especially the impacts of infection on epidermal barrier integrity.

## KEYWORDS

epidermal barrier, fungal infection, model of dermatophytosis, reconstructed human epidermis, *trichophyton rubrum*

## 1 | INTRODUCTION

Dermatophytosis is a superficial fungal infection of keratinized structures caused by specific filamentous fungi named dermatophytes. In humans, the incidence of dermatophytosis is elevated and continuously increasing, rendering it a public health concern. The pathogeny of dermatophytosis remains poorly understood, partly due to the difficulties to set up a relevant model allowing the study of both the invasion of keratinized structures by fungi, and its impact on host tissue architecture and functions. Recently, the development of human

cultured skin equivalents has led to some advances. This review aims to summarize current knowledge about dermatophytosis and then focuses on in vitro models to investigate the alterations of the epidermal barrier in response to fungal infection.

## 2 | DERMATOPHYTOSIS AND DERMATOPHYTES

Dermatophytosis is an infection of superficial keratinized epidermal layers, as well as hairs and nails, which is caused by keratinolytic filamentous fungi named dermatophytes.<sup>[1]</sup> Numerous dermatophyte species are grouped according to their ecological

**Abbreviations:** AMP, antimicrobial peptide; PAS, periodic acid-Schiff; RHE reconstructed human epidermis

niches. Anthropophilic dermatophytes rely mostly on human skin for growth and dissemination in the environment and represent a threat for humans only, as they rarely infect other organisms. Zoophilic species select preferred animal hosts but can frequently infect other species including humans. Geophilic dermatophytes feed on keratinized wastes found in the soil and rarely become pathogenic. Dermatophytes were previously classified according to morphological and physiological characteristics in culture, and to clinical features of the lesions in humans or animals.<sup>[1-3]</sup> Genome sequencing, especially analysis of the polymorphisms inside the variable rDNA regions known as internal transcribed spacers (ITS), has provided phylogenetic criteria for improved species identification.<sup>[4]</sup> A revised classification of dermatophytes was proposed,<sup>[5-7]</sup> based on DNA sequences of five different loci, including ITS, on morphology and physiology in culture, and on geo-, zoo- or anthropophilic ecology (Table 1). This review is concerned with anthropophilic and zoophilic dermatophytes frequently responsible for human infections.

Dermatophytosis is responsible for 3%-4% of dermatological cases and is the most common fungal infection in humans, with a prevalence estimated around 20%-25%.<sup>[8,9]</sup> In addition, its prevalence is continuously raising due to increased risk factors such as sport activities, type 2 diabetes, vascular diseases or ageing. Modern mobility further increases the dissemination of anthropophilic

dermatophytes that extend in previously poorly affected geographical areas.<sup>[10,11]</sup> Among the species capable of infecting human skin, *Trichophyton rubrum* is the most frequently involved, being responsible for 50%-90% of dermatophytoses in humans.<sup>[9,12]</sup> The annual health expense cost of dermatophytosis is estimated to more than 500 million of US dollars.<sup>[13]</sup>

## 2.1 | Dermatophyte infections induce various clinical pictures

The clinical signs of dermatophytosis result from both the degradation of keratinized tissues caused by fungal processes, as well as from the specific immune response of the infected host. Zoophilic species, probably less adapted to human hosts, generate more severe inflammatory responses than anthropophilic species.<sup>[6,14]</sup> Usual signs include dryness, desquamation, cracks and erythema of the skin of the feet, scalp or other body locations. Infections in hairless areas and nails, principally due to *Trichophyton rubrum* and *Trichophyton interdigitale*, are the most frequent in industrialized countries. Scalp infections, mainly observed in developing countries, are preferentially due to *Microsporum canis*, *Trichophyton tonsurans* and *Trichophyton violaceum*.<sup>[9]</sup> For instance, failure to adequately disinfect the hair cutting tools favours the dissemination of dermatophytes.<sup>[15]</sup>

**TABLE 1** Main species of dermatophytes<sup>[5,7-9,14,16,23]</sup>

Species (former taxonomy)	Ecological niche (preferred host)	Clinical picture in humans	Epidemiology
<i>Epidermophyton floccosum</i>	Anthropophilic (human)	Tinea pedis Tinea unguium Tinea cruris	
<i>Microsporum audouinii</i>	Anthropophilic (human)	Tinea capitis Tinea corporis	Mainly found in sub-Saharan Africa
<i>Microsporum canis</i>	Zoophilic (cat, dog)	Tinea capitis Tinea corporis	
<i>Nannizia fulva</i> ( <i>Microsporum gypseum</i> ) <i>Nannizia gypsea</i> ( <i>Microsporum gypseum</i> ) <i>Nannizia incurvata</i> ( <i>Microsporum gypseum</i> )	Geophilic	Tinea capitis (rarely)	Most common geophilic species
<i>Trichophyton benhamiae</i> ( <i>Arthroderma benhamiae</i> )	Zoophilic (guinea pig)	Tinea capitis Tinea corporis	
<i>Trichophyton interdigitale</i>	Anthropophilic (human)	Tinea pedis Tinea unguium	Second most common species worldwide
<i>Trichophyton mentagrophytes</i>	Zoophilic (dog, cat, rabbit)	Tinea corporis Tinea capitis	
<i>Trichophyton rubrum</i>	Anthropophilic (human)	Tinea pedis Tinea unguium Tinea corporis	Most common species worldwide
<i>Trichophyton tonsurans</i>	Anthropophilic (human)	Tinea capitis Tinea corporis	
<i>Trichophyton violaceum</i>	Anthropophilic (human)	Tinea capitis Tinea corporis	Most important species in Africa

In most human cases, dermatophytosis lesions remain superficial, confined to the epidermis. Histologically, dermatophytic lesions exhibit fungal components (arthroconidia, filaments) restricted to the cornified layer of immunocompetent patients. Intercellular oedema and acanthosis, a thickening of the epidermis that results from increased keratinocyte proliferation, are sometimes observed.<sup>[16]</sup>

Unfrequently, dermatophytes invade the dermal tissue, particularly after local trauma in patients with chronic infection.<sup>[17,18]</sup> In immune-deprived patients, dermatophytosis may involve subcutaneous tissues and even deep organs, possibly becoming a life-threatening disease in the absence of appropriate treatment.<sup>[19,20]</sup>

Although dermatophytosis is usually not a severe condition, its impacts on the quality of life are significant. In addition to local pain and unpleasant feelings around lesions, patients suffer psychologically due to aesthetic features of lesions and their social consequences.<sup>[21,22]</sup>

## 2.2 | Contamination by dermatophytes is favoured by risk factors and genetic predispositions

The dissemination of dermatophytes in humans occurs by direct contact with an infected patient or animal. It can also result from contact of the skin with contaminated items, as fungal arthroconidia remain infectious for more than 1 year in the environment.<sup>[23]</sup>

Numerous risk factors favour dermatophytosis, illustrating the importance of maintaining effective epidermal and immunological barriers to protect cutaneous tissues. Decreased epidermal barrier efficiency, induced by scratches, nail alterations or low level of sebum secretion before puberty, as well as elevated environmental humidity, may contribute to promote dermatophytosis.<sup>[24]</sup> Impaired peripheral blood circulation due to age or other causes, with consecutive diminished nutrient availability, reduced oxygenation, and delay in migration of immunocompetent cells or in production of antimicrobial peptides (AMP) at the site of infection may favour the infectious process. As a result of alterations of peripheral blood circulation and nerve endings, diabetes leads to an almost threefold increased risk of dermatophytosis, especially foot and nail tinea.<sup>[25]</sup> Moreover, frequent close contacts with domestic, livestock or wild animals are linked to susceptibility to dermatophytosis.<sup>[24]</sup> Sport practice, especially in indoor facilities and swimming pools, increases the barefoot exposure to potentially contaminated areas.<sup>[26]</sup> Excessive foot perspiration in closed shoes is also detrimental.<sup>[10]</sup> These factors are probably responsible for an elevated prevalence of dermatophytosis from 15% in the general population to 70% in subjects that engage in regular sport practice.<sup>[27-29]</sup>

Since the 1990s, links between the familial history and the susceptibility to dermatophytosis have been reported,<sup>[30,31]</sup> and several genetic predisposition factors have been identified.<sup>[32]</sup> Some HLA haplotypes protect against, whilst others increase the risk of dermatophytosis.<sup>[33-35]</sup> Mutations in gene CLEC7A, which encodes protein dectin-1 that binds to fungal  $\beta$ -glucans,<sup>[36]</sup> as well as mutations in the signalling pathways involved in the immune antifungal response,

such as CARD9 and STAT3,<sup>[37,38]</sup> are linked to increased occurrence of dermatophytosis. More recently, low copy numbers of gene DEFB4, encoding the antimicrobial peptide (AMP)  $\beta$ -defensin-2, were shown to increase susceptibility to dermatophytosis.<sup>[39]</sup> AMPs are involved in the skin defence against pathogens, including bacteria, virus or fungi, through direct antimicrobial activities, as well as through immunomodulatory effects and reinforcement of the epidermal barrier.<sup>[40,41]</sup> The AMP  $\beta$ -defensin-2,  $\beta$ -defensin-3, RNase 7, S100A7 and cathelicidin LL-37 inhibit in vitro the growth of various dermatophytes species, including *T. rubrum*, and are overexpressed in response to the presence of dermatophytes in monolayer cultures of keratinocytes, and in skin biopsies from patient with dermatophytosis.<sup>[42-44]</sup>

## 3 | DERMATOPHYTE BIOLOGY AND MECHANISMS OF CUTANEOUS INFECTION

Like other eukaryotic cells, cells of dermatophytes are characterized by the presence of a nucleus and organelles, including mitochondria and vacuolar membrane-limited compartments involved in the storage, distribution and recycling of metabolites.<sup>[45]</sup> In the fungal cell membrane, ergosterol replaces cholesterol, which is specific to animal cells, to modulate fluidity of the phospholipid bilayer and to serve as a precursor of other cell components. The ergosterol synthesis pathway is thus regarded as one particularly interesting target for antifungal treatments.<sup>[46-48]</sup> Fungal cells are further surrounded by a cell wall responsible for mechanical resistance, cell shape and rigidity.<sup>[49,50]</sup> The cell wall plays a role in adhesion between fungi themselves, and between fungi and other living cells. It is composed of polysaccharides such as  $\beta$ -glucans and chitin, and glycoproteins.<sup>[51]</sup> Chitin chains are interconnected by hydrogen bonds to form several layers and are covalently bound to networked  $\beta$ -glucans and glycoproteins on their external side.<sup>[45,52-54]</sup> Components and thickness of fungal cell walls vary between species. Still poorly characterized in dermatophytes, it is composed of chitin,  $\beta$ -glucans, mannans and galactomannans.<sup>[51,55]</sup>

### 3.1 | Dermatophytes are filamentous fungi and produce two types of spores

Dermatophytes are filamentous fungi, which mean that they develop into tubular structures named hyphae, made of interconnected aligned fungal cells bordered by an uninterrupted wall. The hyphae are regularly divided into cell compartments by septa made of peripheral rings with a composition similar to that of the cell wall. Pores through these septa allow communication between cytoplasm of multiple cell compartments along the entire hypha.<sup>[45]</sup>

Contrary to active hyphae, the spores are quiescent unicellular elements with low metabolic activity.<sup>[45]</sup> Their cell wall is thicker than that in hyphae, providing an extreme mechanical resistance, and their high lipid and glycogen content, serves as energy storage for eventual reactivation. Spores are physiologically designed to

disperse in the environment and to survive in adverse conditions. Upon improvement of environmental conditions, spores reactivate and develop into new hyphae.

The anthropophilic dermatophytes undergo asexual reproduction using mitosis.<sup>[56]</sup> Hyphae can produce two types of spores through formation of conidia or arthroconidia.<sup>[45]</sup> Conidia result from lateral or terminal budding of a hypha; microconidia are single individuals, whilst macroconidia comprise several conidia attached together that result from successive budding events. In contrast, arthroconidia are produced through the fragmentation of hyphae at site of septa. Conidia are solely produced in vitro on Sabouraud medium, whereas arthroconidia are produced by dermatophytes in vivo, indicating that the environment is critical to the type of sporulation and growth.<sup>[57]</sup>

Fungal colonies are formed from activated spores, when they encounter favourable conditions.<sup>[45,58]</sup> Regarding pathogenic dermatophytes, those conditions are fulfilled when arthroconidia adhere to adequate tissues of a tolerant species. First, spores swell by rehydration and form new cell wall components in a nonpolarized way. Simultaneously, synthesis of nucleic acids and proteins concurs to create new emerging tubular structures, creating new cell content as well as an elongating and thickening cell wall. When the cell volume becomes large enough, nuclear division occurs and a septum appears to separate the cells into two interconnected compartments, creating a hypha. Each compartment may continue this process, building new compartments and elongating the hyphae. In this way, hyphae are able to grow, progressively elaborating diverging ramifications and ultimately large colonies. Interconnected hyphae created from one spore are named the mycelium.

### 3.2 | Dermatophytes infect the skin through different steps

When human skin is infected by dermatophytes, three steps can be recognized.<sup>[59,60]</sup> First arthroconidia adhere to the host epidermis through specialized proteins present on the fungal surface<sup>[61,62]</sup> and proteases released by fungi, such as subtilisin 3.<sup>[63-66]</sup> In a second step, called germination, arthroconidia detect favourable conditions and initiate the reactivation of metabolic activity and their growth into hyphae.<sup>[58]</sup> During the third step, referred to as invasion, hyphae produced from the germinating tubes invade the epidermal cornified layer, whilst digesting keratin into small peptides and amino acids.<sup>[67]</sup> Finally, the hyphae start producing new arthroconidia that are released locally to infect other individuals or body sites. The steps of skin infection by dermatophytes, as well as the impacts on the epidermal tissue, are illustrated in Figure 1.

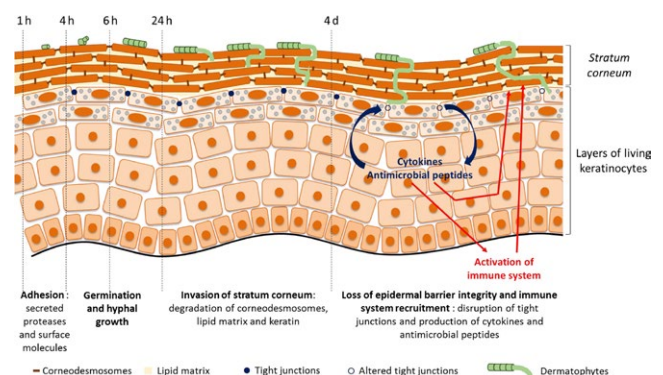
## 4 | IN VITRO MODELS OF DERMATOPHYTOSIS

Several experimental models have been developed to characterize the mechanisms whereby dermatophytes to invade host tissues, to understand the immune response and to evaluate the efficacy

of antifungal treatments. For instance, suspension of isolated cornified keratinocytes,<sup>[68]</sup> fragments of cornified layer isolated by tape-stripping methods<sup>[69]</sup> and pieces of nails or hairs<sup>[67]</sup> have been used. These protocols are convenient to grow dermatophytes, but are devoid of living keratinocytes and therefore cannot be used to model the epidermal response.

To address those limitations, keratinocytes cultured in monolayers<sup>[70-73]</sup> and neutrophils<sup>[74]</sup> have been used to observe the cell response to the presence of dermatophytes, through the detection of released cytokines and AMPs. However, as keratinocytes cultured in submerged conditions do not proceed to full keratinization, these models are not adequate to study the adhesion and invasion by dermatophytes.

In parallel, in vivo animal models, such as guinea pig<sup>[64]</sup> or mouse,<sup>[75]</sup> have been explored. However, the severity of lesions and the extent of inflammatory responses vary considerably according to the host and the dermatophyte species.<sup>[6,14]</sup> A consistent in vivo model of infection should thus be developed using the natural host. As *T. rubrum* does not naturally infect non-human species, the development of an animal model is not fully relevant.<sup>[76]</sup> Despite those limitations, in vivo models of *T. rubrum* dermatophytosis using guinea pig<sup>[77]</sup> or mouse<sup>[78,79]</sup> have been reported, but the protocols required multiple application of spores or abrasive treatments to initiate some cutaneous infection. Those in vivo models yielded useful information about pathogenic mechanisms, such as



**FIGURE 1** Schematic summary of epidermal infection by the anthropophilic dermatophyte *Trichophyton rubrum*. One hour after infection by contact, arthroconidia adhere to the host epidermis by a complex mechanism involving fungal secreted proteases and surface molecules. After 4 h, germination has started and arthroconidia produce germ tubes which grow to form segmented hyphae detected on the epidermal surface from the sixth hour of infection. As soon as 24 h after application of arthroconidia on the epidermis, and for the 3 d following infection, hyphae invade the cornified layer by progressing through intercellular spaces and inside corneocytes, most likely as a result of degradation of corneodesmosomes, extracellular lipid matrix and intracellular keratin. On the fourth day of infection, hyphae reach the granular layer. At this stage, integrity of the epidermal barrier is lost in response to alteration of tight junctions. Simultaneously, keratinocytes exhibit enhanced expression and release of proinflammatory cytokines and antimicrobial peptides, which will then be responsible for control and eventual resolution of the infection



the role of fungal keratinases<sup>[64,80,81]</sup>, about immune responses of the host,<sup>[75,79]</sup> and allowed testing of antifungal compounds.<sup>[77,82,83]</sup> Cutaneous explants freshly isolated from various species including humans,<sup>[64-66,84-86]</sup> represent an interesting alternative. However, their limited availability, together with the large variability between the different donors, severely restrict their use. To palliate those limitations, cultured reconstructed human epidermis was used to study the development and analysis of *T. rubrum* dermatophytosis.

Reconstructed epidermis or epidermis on collagen lattice including fibroblasts mimics closely superficial cutaneous tissues, as they exhibit a cornified layer at their air-liquid interface, like in vivo. They allow studies of the interactions between pathogens and epidermal keratinocytes, and of subsequent barrier alterations. Studies on *Candida albicans*<sup>[87]</sup> or on percutaneous migration of helminths<sup>[88]</sup> have demonstrated the potential of reconstructed tissues to investigate safely and ethically some infectious processes that occur in human skin.

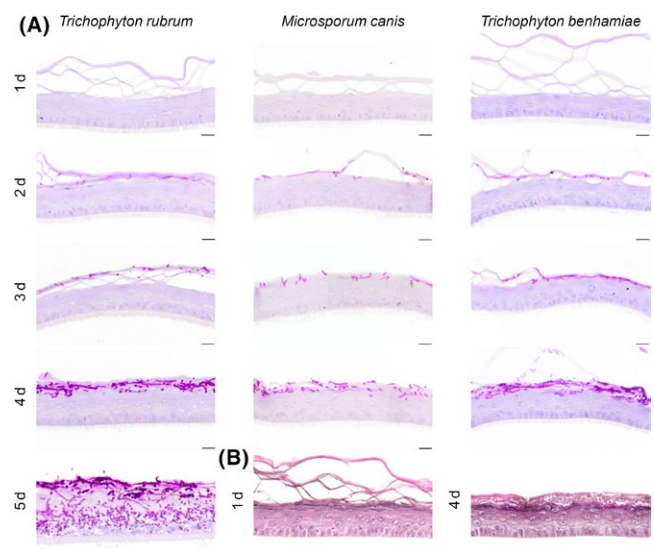
Several models of dermatophytosis on reconstructed human epidermis (RHE) have been reported. In 1995, a first report confirmed the efficacy of terbinafine as an antifungal compound in a reconstructed tissue.<sup>[89]</sup> More recently, two models of dermatophytosis based on commercially available reconstructed skin models, EpiDerm (MatTek)<sup>[73]</sup> and EpiSkin<sup>®</sup>,<sup>[90]</sup> have been published. Both utilized conidia as infectious material. However, conidia have never been observed in dermatophytosis lesions in patients, where only arthroconidia are identified.<sup>[57]</sup> Therefore, the use of arthroconidia seems more adapted, and the production of arthroconidia in vitro was carried out, using a culture medium poor in nutrients, combined with an elevated CO<sub>2</sub> partial pressure.<sup>[91]</sup> An in vitro model of dermatophytosis using arthroconidia and reconstructed feline epidermis was set up to evaluate the efficacy of antifungal molecules<sup>[92]</sup> and allowed demonstration of a crucial role of the fungal protease subtilisin 3.<sup>[63]</sup> We recently illustrated the use of arthroconidia in an in vitro model of RHE infection with different strains of *T. rubrum*, as well as with zoophilic *Microsporium canis* and *Trichophyton benhamiae*.<sup>[93]</sup> Interestingly, RHE infection by dermatophytes can be monitored in tissue sections using histochemical periodic acid-Schiff (PAS) staining of chitin, a component of the fungal cell wall (Figure 2). Prior to PAS, digestion of glycogen by  $\alpha$ -amylase was carried out to suppress glycogen staining in suprabasal keratinocytes.<sup>[93]</sup> This morphological approach, together with the quantitation of fungal elements using PCR amplification of dermatophyte 18S rDNA, proved sensitive enough to localize dermatophytes in RHE and to monitor the infection over time. In this system, the adhesion of arthroconidia to RHE happened within 1 hour and a topical application of miconazole was able to prevent or abolish fungal infection.<sup>[93]</sup>

## 5 | RECONSTRUCTED HUMAN EPIDERMIS ALLOWS TO CHARACTERIZE BARRIER ALTERATIONS UPON INFECTION

Upon dermatophyte infection of RHE, signs of leakage of the barrier were observed 4 days or later after infection. Achterman et al<sup>[73]</sup>

described release of LDH activity from RHE (EpiDerm from MatTek) after culture with conidia from various dermatophyte species. Of interest, this release did not occur after the exposure of RHE to heat-killed fungi. LDH release becomes obvious when dermatophytes remain in close contact with the epidermal tissue for at least 4 days. A similar timeframe allowing tissue invasion followed by destruction of the cultured epidermis after 10 days was confirmed by Liang et al<sup>[90]</sup> who used the EpiSkin<sup>®</sup> model, which includes dermal components, to probe *T. rubrum* infection. Their study also showed that conidia applied at low density were less prone to extend into deep layers after 4 days of infection. Using our "Open Source" RHE model recommended for nonanimal testing due to its openness, transparency and collaborative development,<sup>[94-97]</sup> it appeared that several dermatophyte species and strains remained restricted to the cornified layer after 4 days of infection at densities selected for infection.<sup>[93]</sup>

To understand the response of epidermal tissue to dermatophyte infection, cell signalling and cytokine release have been assayed upon infection of RHE. Even if immune components are absent from the RHE models investigated so far, the robust responses of keratinocytes suggest that they sense the presence of hyphal fungi upon contact. This led us to monitor the decrease in the barrier efficiency during *T. rubrum* infection, to characterize the invasion of the cornified layer by ultrastructural analysis and to study inflammatory or antimicrobial responses.<sup>[98]</sup> The epidermal barrier was found



**FIGURE 2** Progressive invasion of reconstructed human epidermis (RHE) by different dermatophyte species. A, RHE was infected by topical application directly on the cornified layer of *Trichophyton rubrum* IHEM 13894, *Microsporium canis* IHEM 21239 or *Trichophyton benhamiae* IHEM 20163 arthroconidia, respectively, at densities of 1700 per cm<sup>2</sup>, 17 000 per cm<sup>2</sup> and 53 per cm<sup>2</sup>. RHE was then processed 1, 2, 3, 4 d after infection for histology, using periodic acid-Schiff (PAS)-staining after  $\alpha$ -amylase treatment and hemalun counterstaining of tissue sections. B, RHE infected by topical application of *Trichophyton rubrum* IHEM 13894 arthroconidia was processed 1 and 4 d after infection for histology, using HE-staining of tissue sections. Scale bars: 20  $\mu$ m

deficient after 4 days of infection, by (a) monitoring the transepithelial electrical resistance through RHE; (b) estimating the Lucifer yellow outside-in permeation using fluorimetry or histology; and (c) assessing inside-out diffusion of biotin upwards through the RHE. Accordingly, tight junctions were disorganized when the barrier efficiency was impaired. In addition, signs of activation of defense mechanisms confirmed potential roles of keratinocytes. Of interest, p38 MAPK inhibitor PD169316 hampered the development of *T. rubrum* infection on RHE, indicating that this signalling pathway is somehow involved during dermatophytosis, at least in vitro.<sup>[98]</sup> In this context, it is worth noting that activation of p38 MAPK in epithelial cells has recently been associated with alterations of tight junctions.<sup>[99]</sup> Altogether, data obtained so far result in identification of the MAPK p38 signalling pathway as a major component of epidermal response.<sup>[73]</sup> Thus, we hypothesized that the p38 pathway could induce or modulate the immune response leading to expression and release of proinflammatory genes and proteins.<sup>[73]</sup>

## 6 | LIMITATIONS AND PERSPECTIVES OF IN VITRO INVESTIGATION OF DERMATOPHYTOSIS

In vitro modelling of dermatophyte infection allows investigation of the mechanisms involved, by providing conditions to grow these highly selective fungi in an environment similar to what they encounter in vivo. Human skin equivalents cultured at an air-liquid interface produce a functional cornified layer that allows the study of adhesion and invasion by dermatophytes, and contains living keratinocytes able to react to infection. These models have the advantage to mimic infection of the human skin without ethical limitations. They seem more appropriate than in vivo animal models due to host-related differences in the severity of lesions and in inflammatory responses. The fact that skin equivalents are composed solely of keratinocytes allows to highlight their specific roles.

On the other hand, in vitro three-dimensional RHE models suffer some limitations, notably the lack of immune cells, hampering investigations of immune responses. The absence of microbiome and absence of sebum, as well as the culture conditions characterized by the absence of friction and by an elevated relative humidity, probably act together to limit the normal epidermal barrier efficiency, rendering the human skin equivalents more susceptible to dermatophyte infection than their in vivo counterparts.

Altogether, data obtained using in vitro and in vivo models of dermatophytosis allow to outline key steps of dermatophyte infection of human epidermis (Figure 1). Upon seeding with a defined inoculum, adhesion of arthroconidia to the cornified layer happens 1 hour after contact<sup>[93]</sup> by a complex mechanism involving fungal secreted proteases, such as subtilisin 3,<sup>[63-66]</sup> and surface molecules.<sup>[61,62]</sup> Four hours after infection, germination starts. Arthroconidia produce germ tubes which grow to form segmented hyphae detected on the epidermal surface from the sixth hour of infection.<sup>[69,90,93]</sup> After 24 hours, and for the 3 days following infection, hyphae invade the

cornified layer by progressing through intercellular spaces and inside corneocytes, most likely as a result of degradation of corneodesmosomes, extracellular lipid matrix and intracellular keratin.<sup>[69,84,90,93]</sup> On the fourth day of infection, hyphae reach the granular layer. At this stage, integrity of the epidermal barrier is lost in response to alteration of tight junctions.<sup>[93]</sup> Simultaneously, keratinocytes exhibit enhanced expression and release of proinflammatory cytokines and AMP,<sup>[73,93]</sup> responsible for control and eventual resolution of the infection through activation of the immune system. Because studies in vitro<sup>[70,71,73,74,100,101]</sup> and in vivo<sup>[75,79]</sup> revealed a release of pro-Th1 (IL-12, IFN $\gamma$ ) and pro-Th17 (IL-1 $\beta$ , TGF- $\beta$ , IL-6) cytokines upon dermatophyte infection, a cooperation between these two pathways is likely to provide protective response against dermatophytosis. However, in vitro studies which relied on the stimulation of immune cells by spores or components of dermatophytes are not suited to study interactions between keratinocytes and immune cells. On the other hand, studies of animal models cannot assess the human immune response to dermatophytosis. To study the immune system activation, human models including epidermal tissue and cells of the immune system are required. The development of a human skin equivalent comprising both keratinocytes and immune cells will require major technical improvements, for example, design a matrix that allows immune cells migration and/or define a culture medium for optimal growth of both cell types. Progress has recently been made in that direction. A reconstructed skin equivalent was cocultured with macrophages below the culture insert to measure cytokines production by macrophages in responses to topical application of LPS.<sup>[102]</sup> Van den Bogaard et al.<sup>[103]</sup> designed a coculture of human skin equivalents populated with T cells. In this model, pre-activated T cells, added underneath the reconstructed tissue, were able to migrate into the dermis and to induce production of cytokines and AMP by keratinocytes. Adjustment of this model would be very helpful to assess the behaviour of naïve T cells and the crosstalk with keratinocytes upon infection with dermatophytes. Another coculture model was developed to evaluate the transepithelial migration of neutrophils through lung epithelial cells cultured in monolayer, in responses to topical infection by *Escherichia coli*.<sup>[104]</sup> That model could potentially be adapted to skin equivalent infected with dermatophytes. A sophisticated method designed to assess the migration of dendritic cells in responses to sensitizing substances<sup>[105]</sup> could also be used to monitor the release by keratinocytes of factors influencing dendritic cells.

In conclusion, human skin equivalents currently represent a reliable model to study the pathogenic mechanisms of human dermatophytosis, such as adhesion, invasion and disruption of the epidermal barrier. Further improvements, especially development of coculture with immune cells, would broaden their relevance to the human in vivo context.

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## CONFLICT OF INTEREST

The authors state no conflict of interest.

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