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Chapter 11

Observation of Fungi, Bacteria, and Parasites in Clinical Skin Samples Using Scanning Electron Microscopy

Ran Yuping, Zhuang Kaiwen, Hu Wenying, Huang Jinghong, Feng Xiaowei, Chen Shuang, Tang Jiaoqing, Xu Xiaoxi, Kang Daoxian, Lu Yao, Zhang Ruifeng, Ran Xin, Wan Huiying, Lama Jebina, Dai Yalin and Zhang Chaoliang

Additional information is available at the end of the chapter

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Abstract

This chapter highlights the description of the clinical manifestation and its pathogen and the host tissue damage observed under the Scanning Electron Microscope, which helps the clinician to understand the pathogen's superstructure, the change of host subcell structure, and the laboratory workers to understand the clinical characteristics of pathogen-induced human skin lesions, to establish a two-way learning exchange database with vivid images

Keywords: Fungi, Bacteria, Parasite, Clinical Skin Samples, SEM

1. Introduction

In dermatovereology department, skin infections by fungi, bacteria, and, parasites are very common in routine clinical practice. Differentiation and identification of these pathogens are a huge challenge and very important for the patient's diseases diagnosis and treatment. Scanning electron microscope (SEM) is a very strong tool for detection and observation of pathogens from the clinical samples that helps us obtain a direct proof of the pathogen on the surface of the skin samples of the lesion. Based on the detailed morphologic image, we can recognize the ultrastructural of the pathogen and understand the pathogenesis of the skin-infected diseases. During recent years, we collected a lot of pathogenic microorganisms' photographs taken by SEM. These pathogens include fungi (*Trichophyton violaceum*, *Microsporum canis*, *Mucor irregularis*, *Lichtheimia* (*Absidia*) *corymbifera*, *Alternaria arboresce*, *Fon-*

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secaea pedrosoi, *Aspergillus fumigatus* and *Malassezia*), bacteria (*Propionibacterium acnes*), and parasites (*Pediculosis pubis* and *Demodicid mites*) *in vivo* or *in vitro*. The diagnosis and clinical manifestation, the kinds of sample and the image of the pathogens are summarized in the Table 1.

Diagnosis	Clinical manifestation	Sample	Image of pathogen by SEM
Tinea capitis	Erythema, scales on the scalp; hair broken and hair loss	Infected hair	Fungus (*Trichophyton violaceum*)
Tinea capitis	Excessive scales and hair loss on the scalp	Infected hair	Fungus (*Microsporum canis*)
Malassezia folliculitis	Slightly pruritic, monomorphic follicular papules and pustules	Keratotic plug of pustule of hair follicle	Fungus (*Malassezia*)
Pityriasis versicolor	Erythema and scaly, hyperpigmentation or hypopigmentation of skin	Scales	Fungus (*Malassezia*)
Mucormycosis	Progressive red plaque around the inner canthus	Cultured colony	Fungus (*Mucor irregularis*)
Mucormycosis	Purulent granuloma of left forearm	Cultured colony	Fungus (*Lichtheimia corymbifera*)
Cutaneous alternariosis	An ulcer covered with crust on left anterior tibia	Cultured colony	Fungus (*Alternaria arboresce*)
Chromoblastomycosis	Red plaque in the left knee	Cultured colony	Fungus (*Fonsecaea pedrosoi*)
Primary laryngeal aspergillosis	Hoarseness, severe paroxysmal coughing and tachypnea	Biopsy tissue	Fungus (*Aspergillus fumigatus*)
Acne	Recurrent papule and pustule acne	Pustule	Bacteria (*Propionibacterium acnes*)
Pediculosis pubis	Intense itching of the scalp	Parasite	Parasite (*Pubic lice*)
Demodicid mites	Itching, multiple erythema, papules, pustules	Hair follicle plug	Parasite (*Demodex folliculorum*)

Table 1. Summary of diagnosis and clinical manifestation, kinds of sample, and the image of the pathogens observed by SEM

2. Methods

All samples for SEM were taken from clinical patients. These samples included infected hair, scales, colony of culture, and tissue of skin biopsy. The samples for SEM were fixed in 2% glutar-aldehyde for 4 h at 4 ˚C, dehydrated through four gradations of alcohol solutions (50%, 70%, 95%, 100%, progressively) for 15 min each, then soaked in isoamyl acetate for 30 min. The specimens were prepared after critical-point drying method, under which condi-

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tion they were gilded in a vacuum chamber and observed under the SEM, FEI Inspect F50, equipped with an FEG gun operated at 30 kV at high vacuum.

3. Results

3.1. Tinea capitis

Tinea capitis is a common superficial fungal infection of scalp hair follicles and surrounding skin. It often affects children rather than adults. Its pathogens are dermatophytes, usually species in the genera *Microsporum* and *Trichophyton*, such as *Microsporum canis*, *Trichophyton tonsurans*, and *Trichophyton violaceum* [1]. The clinical manifestation of tinea capitis is highly variable, depending on the causative organism, type of hair invasion and degree of host inflammatory response. Common features are patchy hair loss with varying degrees of scaling and erythema. However, the clinical signs may be subtle and diagnosis can be challenging. A number of clinical patterns exist [2]. The accurate diagnosis of tinea capitis usually depends on the laboratory investigation, mainly including direct microscopy with 10%–30% potassium hydroxide and fungal culture. It can confirm the diagnosis by detecting or isolating the causal organism by either of these two methods. Tinea capitis always requires systemic antifungal treatment. Topical treatment is only used as adjuvant therapy to systemic antifungals as topical antifungal agents do not penetrate the hair follicle. Recommended drug in systemic treatment include itraconazole, terbinafine, or griseofulvin.



Figure 1. a. A 9-year-old boy, weighing 25kg, presented to our clinic with slightly itching, multiple patchy areas of gray scaling lesions on the scalp and obvious hair loss.

We describe two cases of tinea capitis due to *T. violaceum* [3] and *M. canis* [4]. The first patient is a 9-year-old boy, weighing 25 kg, presented to our clinic because of multiple, slightly itching and patchy areas of gray scales on the scalp associated with hair loss (Fig. 1). The diagnosis of tinea capitis caused by *T. violaceum* was established by direct microscopic examination, culture, and slide-culture. The scanning electron microscope revealed that the infected hairs were destroyed by abundant fungus (Fig. 2). The boy was cured after receiving 4 weeks of systemic treatment with itraconazole 125 mg per day and topical treatment with 1% naftifine–0.25% ketaconazole cream, after wash with 2% ketaconazole shampoo once a day.

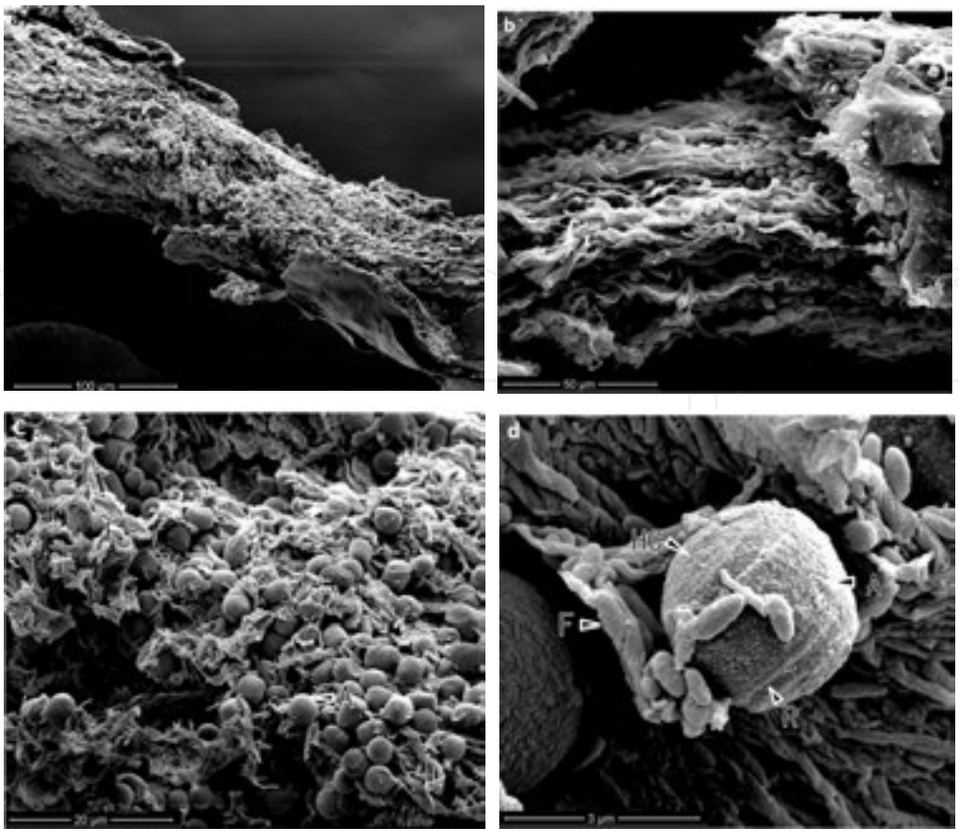


Figure 2. a-b. Cuticle layers of hair shaft were seriously destroyed and a large part had been lost, exposing the fibril cortex inside which many arthrospores were noted. c. High magnification of "a" showing the cuticle layers completely destroyed with the residual cortex fully filled with arthroconidia (A) and distorted fibril bundles (F). d. An arthrospore (A) at high magnification showing irregular convex granules on the chitinous surfaces and the poles bordered by a protruding ring structure (R), bulged by a hemispherical convex (HC). Numerous residual fibril fragments (F) noted around the spore as well as on its surface.

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The second patient is a 5-year-old boy in good health, weighing 19 kg and presented at our clinic with a 1-month history of excessive scales and hair loss on the scalp (Fig. 3a). He had been previously diagnosed with tinea capitis in a local hospital, and received oral itraconazole 100 mg per day for 14 days administered with water. However, the area of hair loss enlarged slightly. Additionally, he had a history of direct contact with a pet dog. Direct microscopic examination (with 10% KOH) of broken hair strands showed numerous spores inside as well as outside of the hair strand. Simultaneously, strands were observed under SEM, and there were many round spores in and around the hair strand (Fig. 4a, b). Fungal culture revealed yellow filamentous colonies, which were identified as *Microsporum canis* with ITS1/4-PCR, sequence-based molecular validation (Accession Number: KT003284). A diagnosis of tinea capitis caused by *Microsporum canis* was confirmed. According to his weight, the boy was treated with itraconazole using the same dose as before, but in this course each dose was administered with whole milk instead with water before. After the 14-day course, clinic assessments showed the hair loss area was smaller and without scales (Fig. 3b). With the same examinations as before, only a few spores were detectable by direct microscopic examination. The number of spores was markedly reduced in hair strands, and

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round spores were now very irregular under SEM (Fig. 4c, d). The boy continued therapy for a total of 40 days. Clinical assessment of the treatment showed that there was no apparent hair loss and all of the same examinations now appeared to be normal (Fig. 3c). Upon clinical examination during the follow-up after 3 months, there was no recurrence following the end of the treatment [4].



Figure 3. a. A 5-year-old boy presented with 1-month history of scalp scales and hair loss, who had received oral itraconazole 100 mg per day with water for 14 days; b. The patch with hair loss was smaller and without scale after oral itraconazole 100 mg per day with whole milk for 14 days; c. There was no apparent hair loss on scale after 40 days at end of treatment.

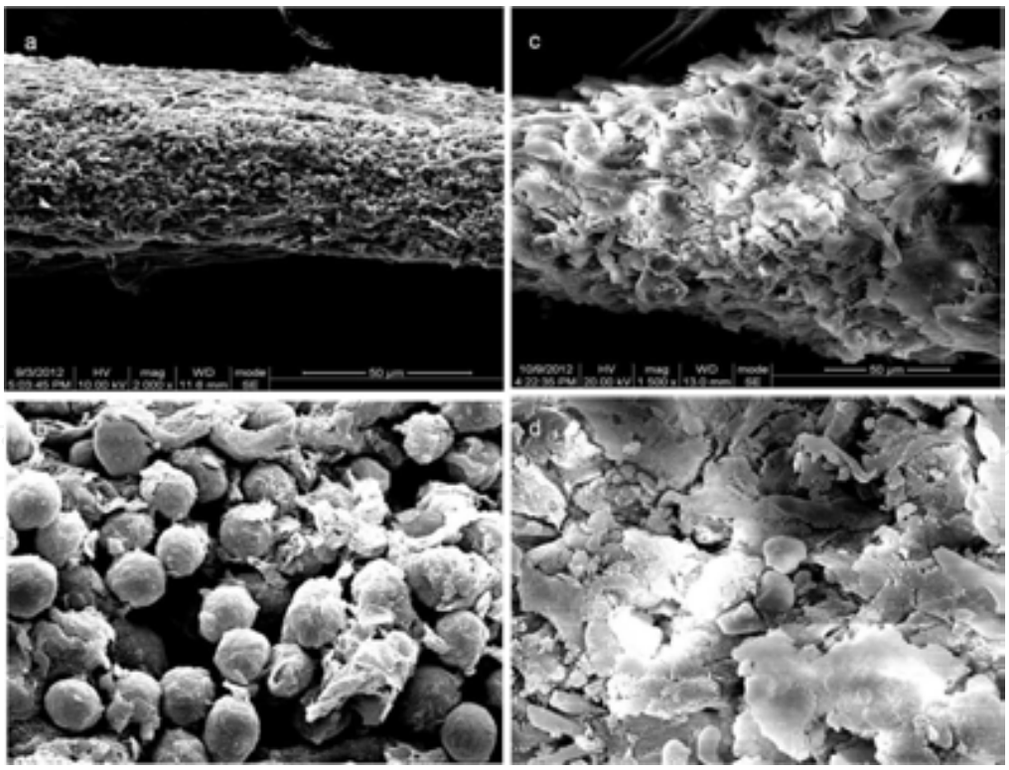


Figure 4. a-b. After oral itraconazole 100 mg per day with water for 2 weeks, broken hair strands with many round spores in and around the hair strands were evident under scanning electron microscopy (SEM); c-d. Oral itraconazole 100 mg per day with whole milk for 14 days, the number of spores were markedly reduced in broken hair strands, and spores appeared very irregular under SEM.

3.2. *Malassezia* folliculitis (*Pityrosporum* folliculitis)

Malassezia folliculitis is most commonly seen in teenagers and adults, which is characterized by pruritic, monomorphic follicular papules and pustules on the upper trunk, arms, neck and occasionally on the face. It is due to excessive growth of *Malassezia* spp. within the hair follicle, with resulting inflammation (from yeast products and free fatty acids produced by fungal lipase). Only yeast forms are observed, no hyphal forms as in pityriasis versicolor [5]. Diagnostic studies include microscopic evaluation of the presence of yeast, cultures, and biopsies. Additionally, Woods lamp can be used to illuminate the lesions, which portray a yellow-green fluorescence. Both topical and oral antifungal agents are effective agents in the treatment of *Malassezia* folliculitis and are commonly combined to hasten resolution and maintain clearance. Topical regimens include daily wash with ketoconazole shampoo 2%, then 1% naftifine-0.25% ketaconazole cream. For severe cases, it needs systemic administration of antifungal agents. Commonly used regimens include oral fluconazole 150 mg weekly for 2–4 weeks, and itraconazole 200 mg daily for 2–4 weeks [6].

The following is a case of *Malassezia* folliculitis due to *Malassezia* spp. The patient is a 25-year-old man, who was presented to our clinic because of slightly pruritic, monomorphic follicular papules and pustules on the upper trunk and neck (Fig. 5a). The diagnosis of *Malassezia* folliculitis was established by direct microscopic examination, culture, and scanning electron microscopy. The scanning electron microscope of the hair follicle from the upper trunk revealed a large number of yeast of two kinds, orbicular-ovate and globular (Fig. 5 b-c). The man was cured after receiving 4 weeks of systemic treatment with itraconazole 200 mg per day and topical treatment with 1% naftifine–0.25% ketaconazole cream after wash with 2% ketaconazole shampoo once a day.

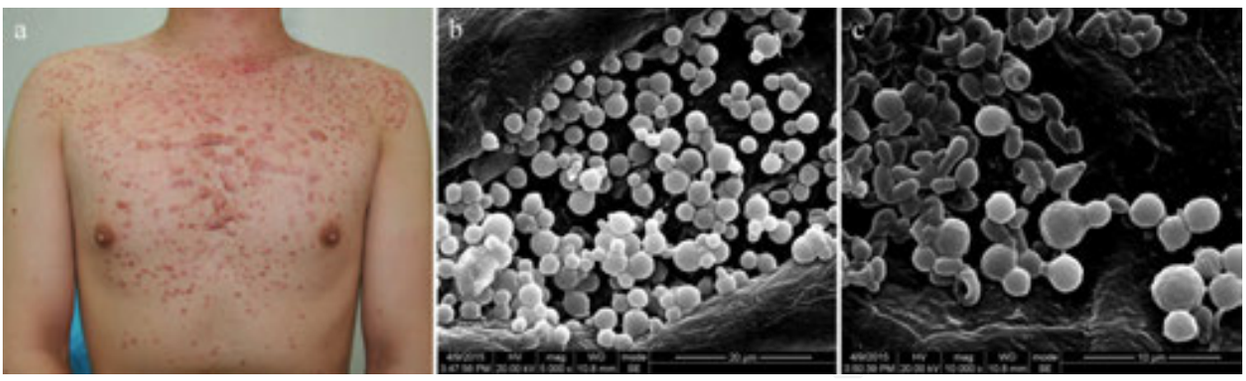


Figure 5. a. A 25-year-old man with complains of slightly pruritic, monomorphic follicular papules, pustules, and secondary keloid on the upper trunk and neck. b-c. SEM of the hair follicle from the upper trunk. These demonstrated a large number of globular or orbicular-ovate yeasts of budding daughter cell, with collar structure around the budding. b. Globular yeast. c. Orbicular-ovate and globular yeast in the same sample.

3.3. Pityriasis versicolor

Pityriasis versicolor is a superficial fungal infection of the skin and caused by *Malassezia*, a lipophilic yeast, which is part of the normal skin flora. Certain environmental, genetic, and

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a more lipid-rich environment for *Malassezia* to grow. Adolescents and young adults who are physically active are also susceptible populations [9].

immunological factors can lead to the development of disease [7]. The prevalence is as high as 50% in tropical areas [8]. Prevalence increases significantly in childhood and adolescence, probably because of increased sebum production, which allows for a more lipid-rich environment for *Malassezia* to grow. Adolescents and young adults who are physically active are also susceptible populations [9].

This is a case of pityriasis versicolor due to *Malassezia* spp. A 27-year-old man was presented to our clinic with extensive erythema and scaly for 6 months (Fig. 6a). The scaly was scraped and observed through SEM. Under SEM, numerous hyphae and spores that resemble "banana and grapes" (Fig. 6b). Treatment with oral itraconazole 200 mg bid and topical use of 1% naftifine–0.25% ketoconazole cream after wash with ketoconazole shampoo was effective.

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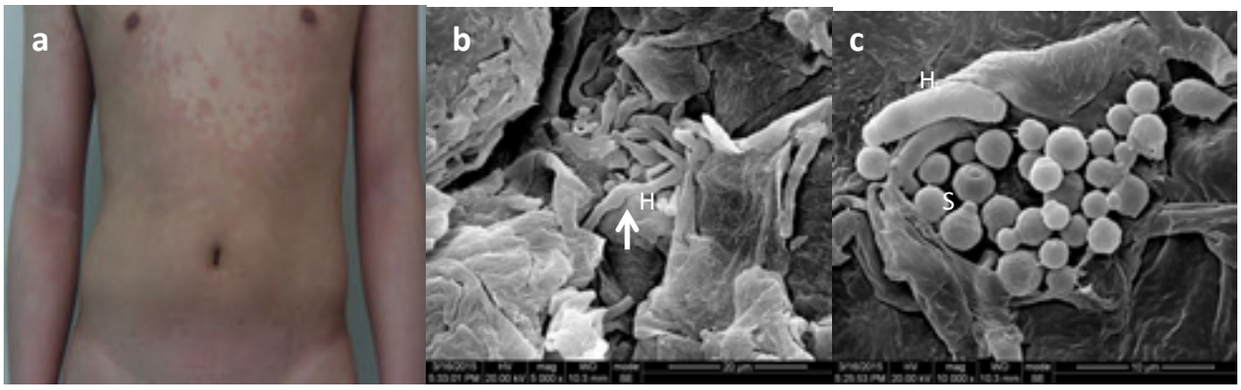


Figure 6. a. A 27-year-old man presented in our clinic with extensive erythema and scaly for 6 months. b. Under SEM, numerous hyphae (H) went through the scaly, length of which is about 10–20 μm. c. Under SEM, abundant of 3–5 μm in diameter grapes-like spherical *Malassezia* spores (S) with budding daughter cell, with collar structure around the budding, and banana-like haphae (H).

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3.4. Mucormycosis

Mucormycosis is a clinically rare and fatal opportunistic fungal infection, which invades nasal, brain, lung, gastrointestinal tract, skin, and other parts with acute, subacute, or chronic process. The routes of invasion contain respiratory tract, digestive tract, skin, and neonatal umbilical region [10]. Its pathogens are fungi in the order *Mucorales*, usually species in the genera *Mucor*, *Rhizopus*, and *Lichtheimia* (*Absidia*), such as *Mucor irregularis*, *Rhizopus stolonifer*, and *Lichtheimia corymbifera*. *M. irregularis* is a newly recognized fungal disease mainly described in China [10]. Amphotericin B and its lipid derivatives are considered as the effective drugs to treat mucormycosis.

3.4. Mucormycosis

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The following is a description of two cases of primary cutaneous mucormycosis caused by *Mucor irregularis* [10] and *Lichtheimia corymbifera* [11], respectively. One of the cases is of a 47-year-old farmer, who presented to our clinic with a history of progressive red plaque around the inner canthus (Fig. 7a), following dacryocystectomy about a year earlier. Linear, aseptate hyphae were seen by direct KOH examination and in biopsy. Fungal culture re-

presented to our clinic with a history of progressive red plaque around the inner canthus (Fig. 7a), following

vealed light-yellow filamentous colonies that were identified as *Mucor irregularis* by nucleo-

da cystectomy about a year earlier. Small, dispersed hyphae were seen by direct KOH examination and with

pronounced columellae and conspicuous collarette at the base of the columella following

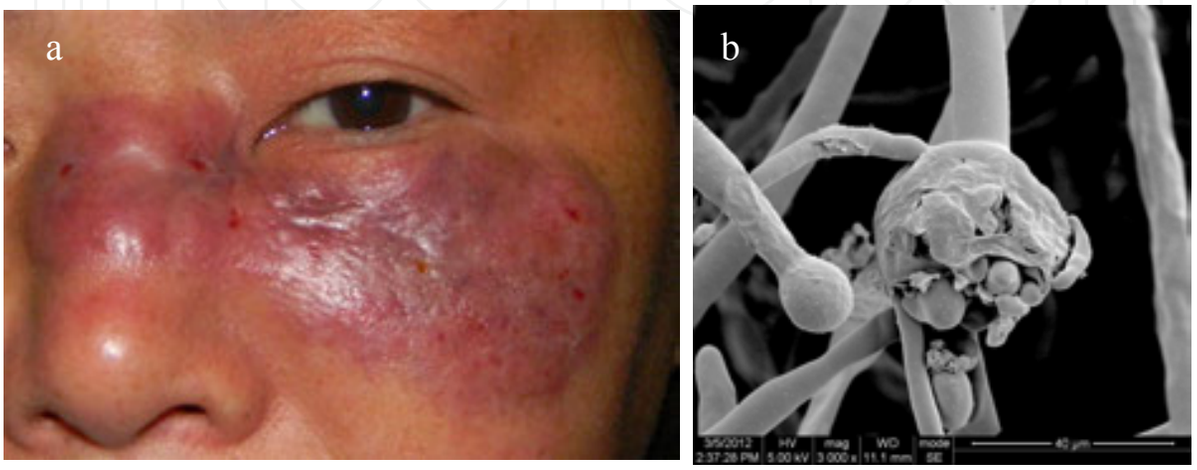
biopsy. Fungal culture revealed (Fig. 7b) filamentous colonies that were identified as *Mucor irregularis* by

ally increasing dosage. The treatment lasted 43 days, and the patient received a total 760 mg

nucleotide sequencing of rRNA gene. SEM observation revealed non-apophysate sporangia with pronounced

smooth, and fungal culture was negative. There was no recurrence for half a year through

columellae and conspicuous collarette at the base of the columella following sporangiospore dispersal (Fig.



7b). Amphotericin B and dexamethasone were used in

Figure 7. a. A 47-year-old farmer was presented to our clinic with 1-year history of progressive red plaque around the inner canthus. Faint yellow exudation was oozing from the ulceration at the center of plaque. Some scales were also observed on the plaque. b. SEM observations revealed non-apophysate sporangia with pronounced columellae and conspicuous collarette at the base of the columella following sporangiospore dispersal.

gradually increasing dosage. The treatment lasted 43 days, and the patient received a total 760 mg

amphotericin B. The patient was discharged after 2 months of treatment. The plaque became smooth, and

The other case is of a 69-year-old female farmer, who presented to our clinic with the history

of a progressive purulent granuloma of her left forearm (Fig. 8a) following a fracture of left

fungal culture was negative. There was no recurrence for half a year through telephone follow-up.

forearm about 11 months earlier. Broad, nonseparate hyphae were seen in pathologic study

with methenamine silver stain (Fig. 8b). Fungal culture revealed white filamentous colonies

that were identified as *Lichtheimia corymbifera* by nucleotide sequencing of rRNA gene. The

scanning electron microscope showed that the sporangia are slightly pear-shaped instead of

spherical. The sporangiophores of *Lichtheimia corymbifera* formed a conical apophysis and

Figure 7. a. A 47-year-old farmer was presented to our clinic with 1-year history of progressive red plaque

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Antimicrobial susceptibility test indicated that *Lichtheimia corymbifera* is most sensitive to ter-

around the inner canthus. Faint yellow exudation was oozing from the ulceration at the center of plaque. Some

binafine and intraconazole. The patient was discharged after 6 weeks of the operation and oral

intraconazole with surgery [11].

scales were also observed on the plaque. b. SEM observations revealed non-apophysate sporangia with

3.5. Cutaneous alternariosis

pronounced columellae and conspicuous collarette at the base of the columella following sporangiospore

Alternaria, an opportunistic fungus, is pigmented (also known as dematiaceous or phaeoid)

dispersal.

filamentous fungi, which are well-known soil saprophytes and plant pathogens that infre-

quently cause infection in humans. Although *Alternaria* usually infects immunocompro-

mised patients [12], in rare cases it infects healthy or immunocompetent individuals as well.

The other case is of a 69-year-old female farmer, who presented to our clinic with the history of a progressive

purulent granuloma of her left forearm (Fig. 8a) following a fracture of left forearm about 11 months earlier.

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susceptibility test indicated that *Lichtheimia corymbifera* is most sensitive to terbinafine and itraconazole. The patient was cured after 6 weeks of therapeufil alliance of oral intraconazole with surgery [11].

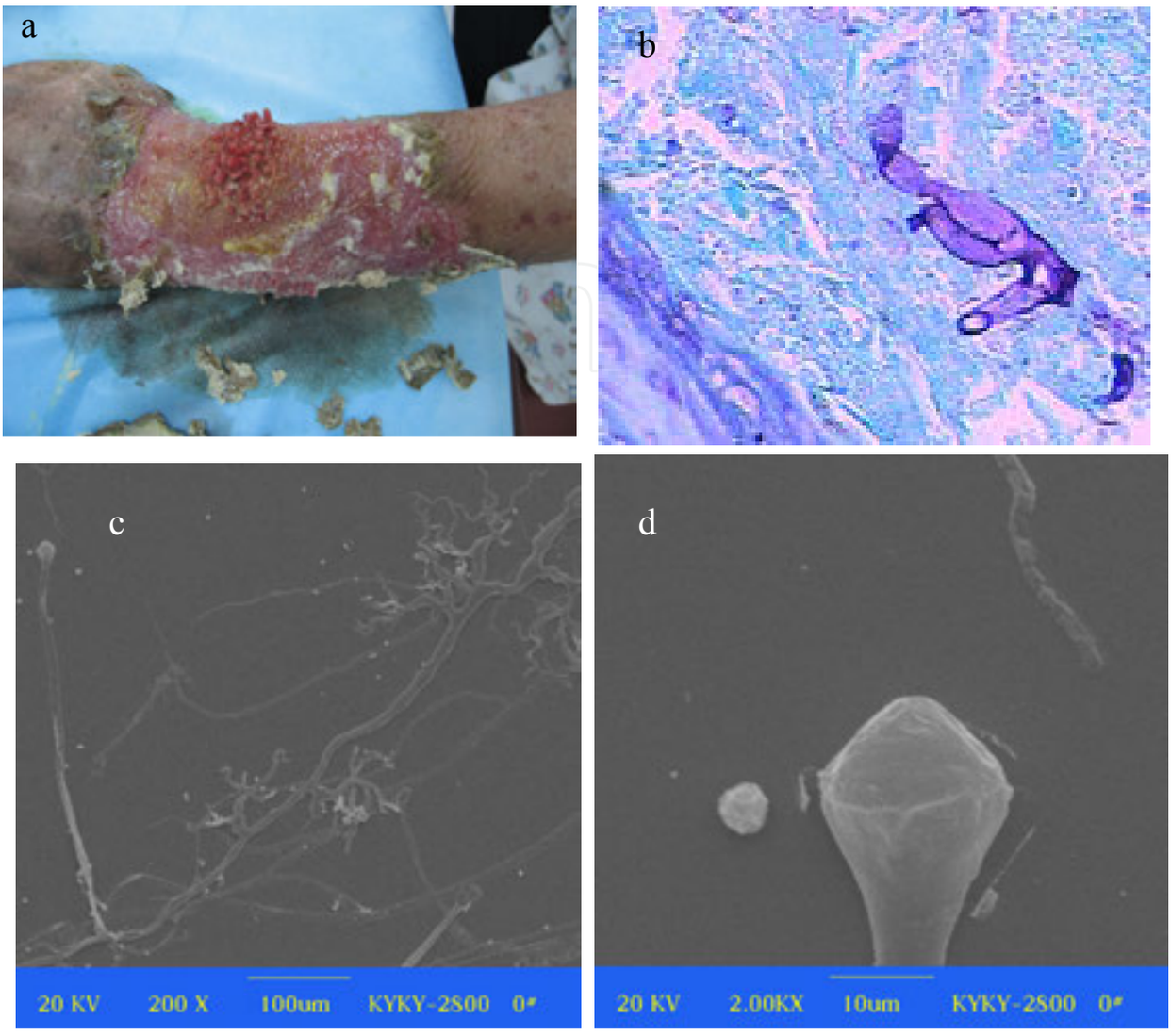


Figure 8. a. A 69-year-old female was presented to our clinic with a progressive purulent granuloma of her left forearm. b. Broad nonseparate hyphae were seen in pathologic study (methenamine silver stain, ×200). c-d. The sporangiophores of *Lichtheimia corymbifera* forming a conical apophysis and arising at points on the stolon that was between the rhizoid and not opposite them. SEM showed the sporangia were slightly pear-shaped instead of spherical (20kv, ×2000).

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There is no standard therapy for cutaneous alternariosis and the patients are usually treated with surgical resection and/or antifungal therapy.

We described the case of a healthy individual with cutaneous alternariosis due to infection with *Alternaria arborescens* [13]. A 28-year-old man presented at our clinical with a one-month history of ulcers covered with crust on his left anterior tibial (Fig. 9a). Fungal culture of the tissue revealed dark grey-white colonies with a dark-brown underside (Fig. 9b) and the SEM observation of the slide culture revealed beaked conidia (Fig. 9c). Based on the morphological features and molecular identification, the patient was diagnosed as cutaneous alternariosis. He was successfully treated with oral itraconazole and topical wet dressing of amphotericin B.

3.5. Cutaneous alternariosis

Alternaria, an opportunistic fungus, is pigmented (also known as dematiaceous or phaeoid) filamentous fungi, which are well-known soil saprophytes and plant pathogens that infrequently cause infection in humans.

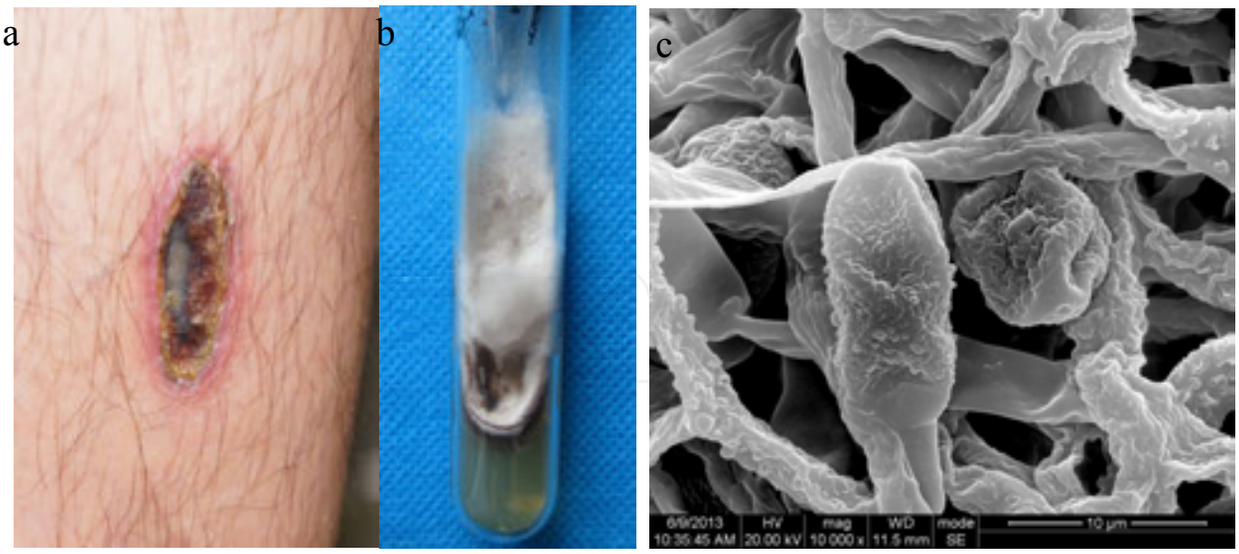


Figure 9. a. Ulcer with an overlying crust on the patient's skin of left anterior tibia. b. Fungal culture of the tissue revealed dark grey-white colonies with a dark-brown underside. c. SEM observation of slide culture revealed beaked conidia.

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3.6. Chromoblastomycosis

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Chromoblastomycosis is a chronic fungal infection of the skin and subcutaneous tissue caused by dematiaceous fungi. Common pathogenic fungi are *Fonsecaea pedrosoi*, *Phialophora verrucosa*, *Cladophialophora carrionii*, among others. These fungi exist in the natural environment in soil, water, vegetation, or wood splinters, and usually are inoculated in the skin tissue of human body by a traumatic injury. A higher incidence is reported in tropical and subtropical countries. Cutaneous lesions can be nodules, papules, and/or ulcerations and mostly affect the lower limbs. The diagnosis of chromoblastomycosis is based on direct examination, culture, and histopathology. On treatment, long courses of antifungal agents such as itraconazole, terbinafine can be used alone or in combination with surgical excision and physical treatments (cryotherapy or, mostly, thermotherapy). However, long duration of treatment is needed and cure rate of the disease is low [14].

In this part we describe a case of chromoblastomycosis due to *Fonsecaea pedrosoi*. A 34-year-old male presented at our clinic with a 12-year history of red plaque in the left knee (Fig. 10a). The patient's left knee was punctured by a fragment of a brick 12 years ago. The pathogenic fungus was isolated and identified as *Fonsecaea pedrosoi*. SEM observation: dematiaceous hyphae with many well-defined septa, conidiophores and oval brown spores arranged in a clump could be seen. The surfaces of conidiogenous cells were smooth. Oval spores were arranged around conidiophores (Fig. 10b). The patient was diagnosed as chromoblastomycosis and was treated with oral terbinafine 250 mg twice a day and thermotherapy with a small electronic heating pad (42°C, more than one hour per day) after applying topical cream containing 1% naftifine hydrochloride and 0.25% ketoconazole. The total course was 61 weeks. The crust and pruritus had disappeared and the erythema and plaque had turned smooth and soft.

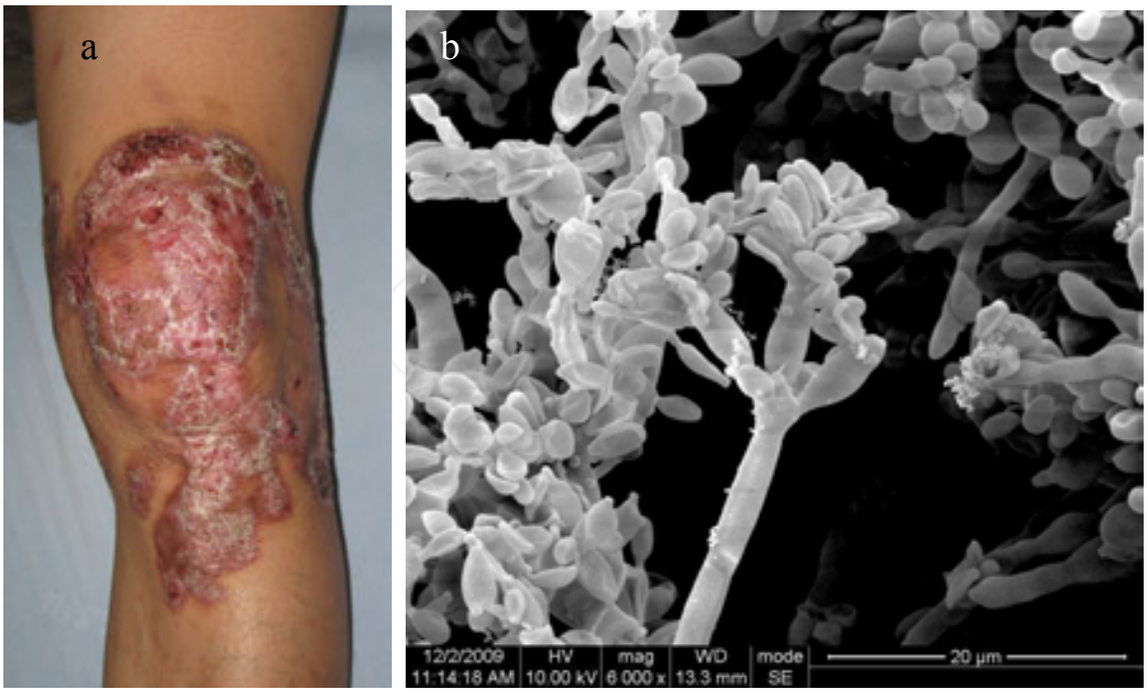


Figure 10. a) A 34-year-old male with a 12-year history of a red plaque in the left knee. b. Under SEM observation: dematiaceous hyphae with many well-defined septa, conidiophores, and oval brown spores arranged in a clump could be seen. The surfaces of conidiogenous cells were smooth. Oval spores were arranged around conidiophores.

observation: dematiaceous hyphae with many well-defined septa, conidiophores, and oval brown spores arranged in a clump could be seen. The surfaces of conidiogenous cells were smooth. Oval spores were arranged around conidiophores.

3.7. Primary laryngeal aspergillosis

Primary laryngeal aspergillosis is a rare opportunistic infection caused by *Aspergillus*. All causes leading to immunocompromisation are generally due to etiological factors [15]. For immunocompetent patients, oral sex (fellatio) may be the primary cause [16]. Airborne spore colonizes in the larynx through inhalation, then white colony grows on vocal cords or/and laryngeal ventricle. It is characterized by chronic hoarseness, with or without systemic or respiratory symptoms involving fever, cough, and tachypnea. It is usually diagnosed by laryngoscopy and biopsy. Systemic antifungal treatment is often effective.

3.7. Primary laryngeal aspergillosis

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We describe in the following a case of primary laryngeal aspergillosis due to *Aspergillus fumigatus*. The patient was a 23-year-old female undergraduate student, who presented with hoarseness, severe paroxysmal coughing, and tachypnea. Laryngoscopy revealed obvious white plaques on the swollen vocal cords and laryngeal ventricle (Fig. 11a). The diagnosis of laryngeal aspergillosis was established by the clinical manifestations and the hyphae branching at 45° angles under microscopy, SEM (Fig. 11b), and pathology. She was cured with oral itraconazole at 200 mg twice a day for 28 days.

3.8. Acne

Acne is a chronic inflammatory disease of the sebaceous–pilosebaceous system. It is estimated to affect 9.4% of the global population [17]. Acne is closely related to the combination of genetic and environmental factors, among which *Propionibacterium acnes* (*P. acnes*) plays a



Figure 11. a. A 32-year-old female undergraduate student presented with hoarseness, severe paroxysmal coughing, and tachypnea. Laryngoscopy revealed obvious white plaques on the swollen vocal cords and laryngeal ventricle. b. SEM of the biopsy tissue revealed hyphae branching at 45° angles destroyed the vocal cords tissue.

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device and surgery infections caused by *P. acnes* have also been reported. Strains of *P. acnes* have been classified into several different types [19].

prominent role [18]. Gram-positive anaerobic bacterium *P. acne* is a major commensal of the follicular plug in human skin. Other diseases such as medical device and surgery infections caused by *P. acnes* have also been reported. Strains of *P. acnes* have been classified into several different types [19].

The following is a description of a case of acne in a 24-year-old girl. She suffered recurrent papule and pustuleacne for 6 months (Fig. 12a-b). We removed the follicular plug with sterile hemostatic forceps and observed it through SEM. Under SEM, abundant rod-shaped bacteria were closely spaced in follicular plug tissue (Fig. 12c). Treatment with oral minocycline 50 mg twice a day and topical use of adapalene gel was effective.

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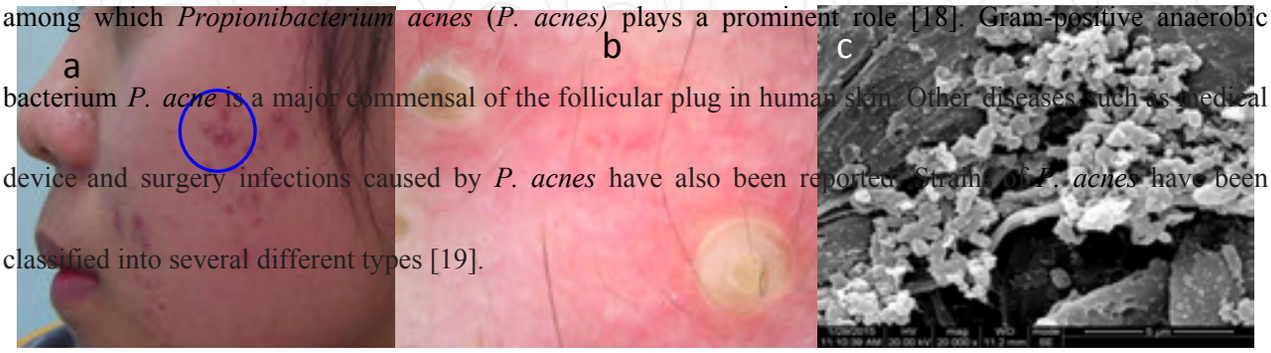


Figure 12. a. A 24-year-old girl who suffered recurrent papule and pustule acne for 6 mouths. b. Under the dermoscopy, the papule was semisphere with tawny follicular plug inside. c. Under SEM, rod-shaped bacteria were closely spaced in follicular plug tissue.

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3.9. Pediculosis

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Pediculosis is a skin disease caused by arthropods. Its pathogens are three lice species including head louse, crab or pubic louse, and body louse, which cause the *Pediculus humanus capitis, Phthirus pubis*, and *Pediculus humanus corpus,* respectively. These three insects are obligate human parasites. They are transmitted by person-to-person contact [20]. Itching in various levels of severity is the primary symptom of pediculosis. Crab louse is about 0.8–1.2 mm in length. Its legs are clawed, except for the first pair, which is shortened and vestigial. In contrast to the oval shape of head and body lice, the crab louse is almost as wide as its length, allowing it to grasp widely spaced pubic hairs [21]. Its crab appearance accounts for its name. Patients with pubic lice may be instructed to launder clothing and bedding and to avoid sexual or other intimate contact until their infestations are cured [22]. In case of infestation of head with pubic lice, the head can be shaved totally and then treated with an occlusive agent such as Vaseline petroleum jelly.

We describe a case of pediculosis on the scalp of a 6-year-old boy caused by the crab louse [23]. The boy was presented to our clinic with the complaint of intense itching of the scalp. There were some small pieces of erythema (in the circle) and a brown dot-like substance on his scalp (Fig. 13a). The dermoscopy revealed a brown parasite (0.9 mm in horizontal axes and 1.2 mm in vertical axes) with two crab-like feet adhered to the scalp (Fig. 13b). Microscopic examination and scanning electron microscope showed the detail of this insect (Fig. 13c-d). Based on these morphological findings, the diagnosis of *Pediculus humanus capitis* caused by crab louse is confirmed. Generally, pediculus humanus capitis is caused by head louse, rarely by crab louse. Where could this pathogen, crab louse, be from? After a detailed inquiry, his father was found to have pediculosis pubis that had been cured. Therefore, the boy was instructed to shave the head totally and then treated with an insecticidal tincture, which was administered to his parents as well. The boy was cured after 15 day of treatment.

3.10. Demodiciosis

Demodiciosis is a kind dermatitis caused by *Demodex*. It often presents some rosacea-1ike lesions. *Demodex* is a genus of tiny parasitic mites that live in or near hair follicles of mammals. Currently, about 65 species of *Demodex* are known [24]. Two species living on humans have been identified: *Demodex folliculorum* and *Demodex brevis*, both frequently referred to as eyelash mites [25]. The adult mites are 0.3–0.4 mm long, and 0.012–0.016 mm in diameter, with *D. brevis* slightly shorter than *D. folliculorum* [26]. Each has a semitransparent, elongated body that consists of two fused segments. Eight short, segmented legs are attached to the first body segment. The body is covered with scales for anchoring itself in the hair follicle, and the mite has pin-like mouth for eating skin cells and sebum, which accumulate in the hair follicles. The mites can leave the hair follicles and slowly walk around on the skin, at a speed of 8–16 mm per hour, especially at night, as they try to avoid light [26]. In most of the cases, the mites go unobserved, without any symptoms, but in certain cases (usually related to a disordered immune system) mite populations can dramatically increase, resulting in a condition known as *demodicosis* or *demodex mite* bite, characterized by itching, inflammation, and other skin disorders.

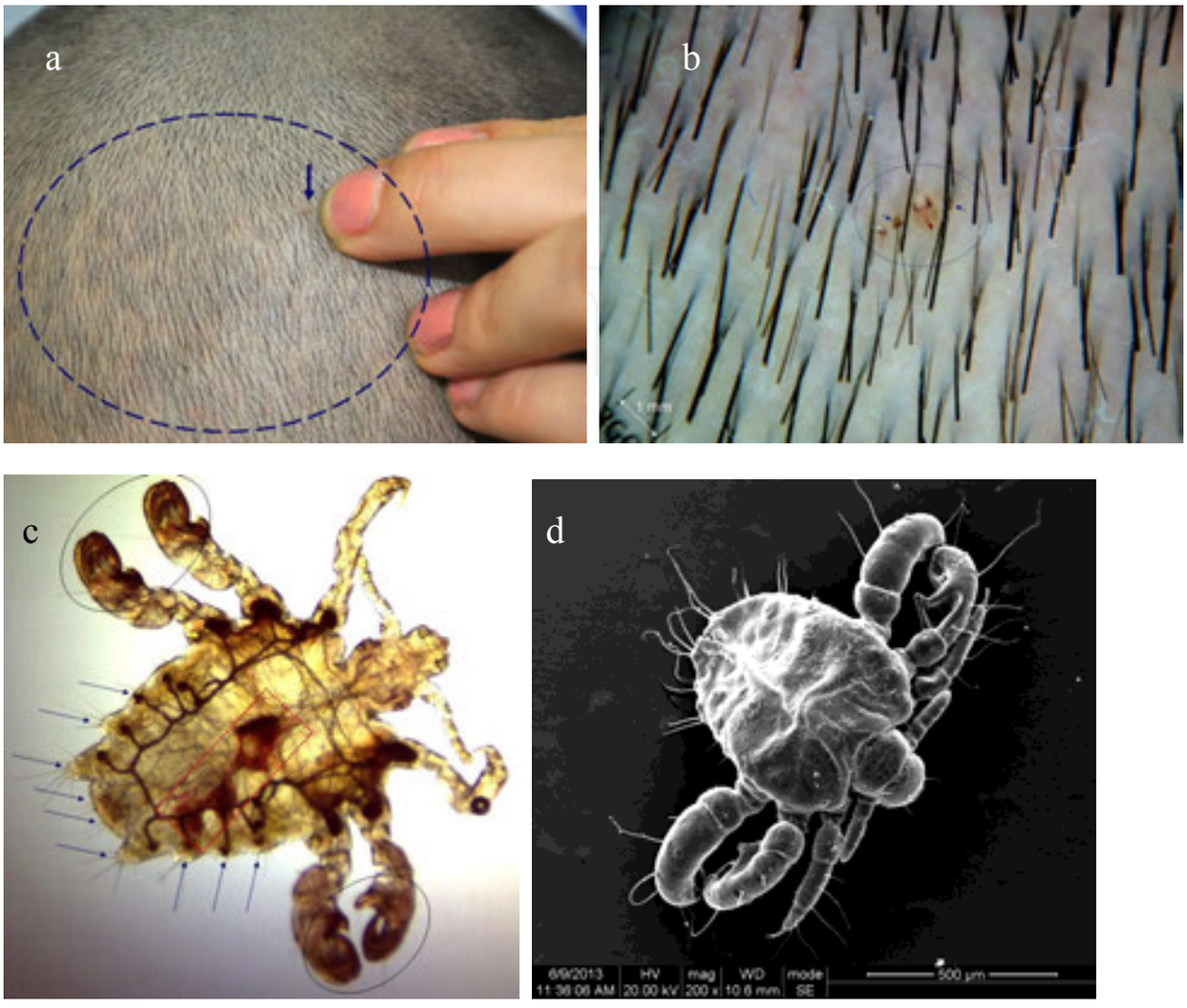


Figure 13. a. There were some small pieces of erythema (in the circle) and a brown dot-like substance on the scalp (arrow). b. The dermoscopy revealed a brown parasite (0.9 mm in horizontal axes and 1.2 mm in vertical axes) with two crab-like feet adhered to the scalp. c. Under the microscope, the parasite was characterized by a flat body like a crab and three pairs of feet in different sizes. There was an area (red box) full of blood in the middle part of the parasite. A large number of short setae (arrow) were noted at the edge of the parasite abdomen. d. The SEM showed a vivid three-dimensional ultrastructure of the parasite; the whole body was composed of three parts including spherical head, chest, and elliptical abdomen; a pair of feelers was noted on the head; the three pairs of feet were in shaped section and curved serrated claws were noted at the end of foot; short setae in different length were not only at the edge of the abdomen but also on the feet.

The following is a description of a case of demodicosis due to *Demodex mites*. The patient is a 28-year-old man, who came to our clinic because of itching, multiple erythema, papules, pustules lesions on the nose and cheek (Fig. 14a). The diagnosis of demodicosis caused by *Demodex mites* was established by direct microscopic examination. The observation of SEM revealed that the parasite consists of two segments. There were four pairs of feet on the side of the head of the parasite and its abdomen was characterized by annular striae on the surface (Fig. 14b). The man was cured after receiving 6 months of topical treatment with 7% albendazole cream once a day.

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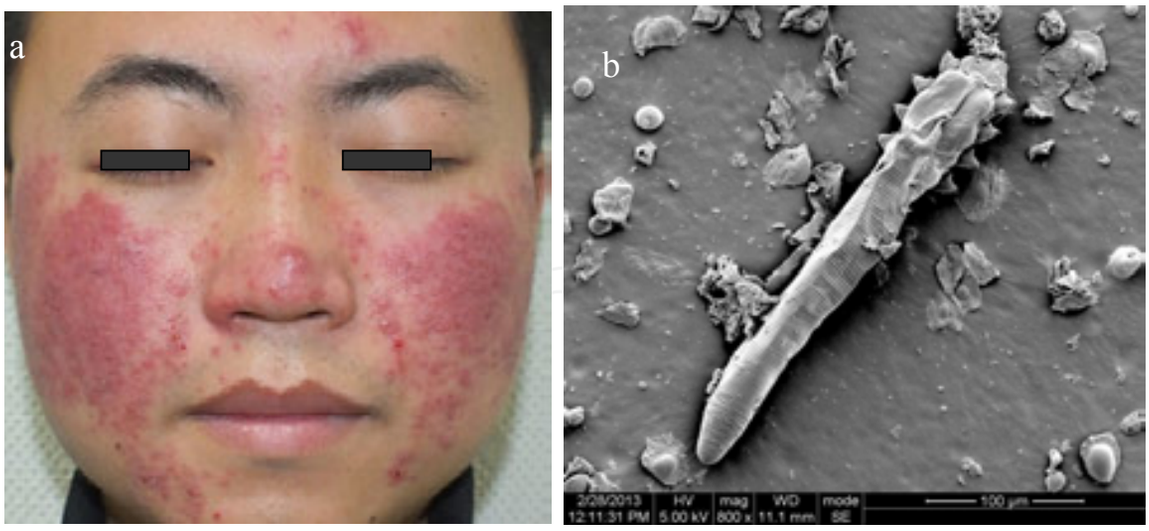


Figure 14. a. A 28-year-old man came to our clinic because of itching, multiple erythema, papules, pustules lesions on the nose and cheek. b. SEM revealed a *Demodex folliculorum*, approximately 0.33 mm in length, in the infected skin. Its elongated body consisted of two segments. There were four pairs of feet on the side of the head of the parasite, and its abdomen was characterized by annular striae on the surface.

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