Treatment of a Superficial Mycosis by Low-temperature Plasma: A Case Report

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Abstract: A case of dermatomycosis caused by zoophilic strain of *Trichophyton interdigitale* was treated by low-temperature plasma produced by direct current (DC) cometary discharge. The shortening of skin lesion persistence along with suppression of subjective discomfort and etiological agent was observed.

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Introduction

The rapidly developing field of low-temperature (or non-thermal) plasma use in medical applications has previously been reviewed by several authors, e.g. by Laroussi (2005, 2009), Moreau et al. (2008), Kong et al. (2009), Ehlbeck et al. (2011), Laroussi et al. (2012) or Isbary et al. (2013a). The biological effects of plasma are mediated by plenty of reactive particles, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), for a review, see Graves (2012). Till present, the plasma was mostly produced in air by dielectric barrier discharges, gliding arc, plasma jets and various corona discharges. The medical applications include mainly decontamination, disinfection and sterilization of various surfaces and liquids, studies concerning the chronic wound healing (Isbary et al., 2013b) or cancer treatment (Schlegel et al., 2013) also appeared. The microbicidal effect of plasma was studied mainly on bacteria, but suppression of fungi (yeasts and moulds) *in vitro* was also reported (Scholtz, 2005; Julák et al., 2011; Soušková et al., 2011, 2012; Sláma et al., 2013).

In previous papers (Scholtz and Julák, 2010a, b), we reported a new type of jet-like point-to-point DC electric discharge produced in atmospheric air and named the cometary discharge. Its efficiency was improved by inserting an electrically insulated metallic grid between the discharge and the exposed object, as described in Julák and Scholtz (2013) and Scholtz et al. (2013). Here, we describe an attempt to adopt this discharge for treatment of a case of dermatomycosis.

Case presentation

Patient is a 20-year-old woman student, living on a farm in a village near Prague. She was in daily contact with farm animals, including rabbits, dogs and cats. One day she observed itching and tonus in skin on the upper left quadrant of chest, the second day a circular lesion of efflorescence appeared, becoming next day red and conspicuous; this stage was denoted here as day 0. The lesion had elevated edge with numerous pinpoint pustules (*tinea corporis*, "ringworm"). Because she worked during her studies on biological applications of low-temperature plasma, she decided on her own risk not to consult a physician, but treated this efflorescence by self-medication using the cometary discharge. Smears were taken from the leading edge and central area of the affected site with a dry tampon; samples were also taken using the bacteriological loop and sterile toothbrush, but this appeared to be less effective. Samples were applied onto Sabouraud dextrose agar plate (SDA, Oxoid), where the culture of a mould appeared after 5 days of cultivation at 25 °C.

This mould was identified as zoophilic strain of *Trichophyton interdigitale* (note: such isolates were traditionally identified as *T. mentagrophytes* according to the older taxonomy) due to light brown, granular colonies with dark brown reverse on SDA, presence of numerous macroconidia and spiral hyphae in culture. The identification was verified by the DNA sequencing of the ITS rDNA. This region

was 100% identical with *T. interdigitale* subtype III (FM986758) (Heidemann et al., 2010). The isolates belonging to this subtype are considered to be of animal origin (the broad spectrum of the hosts comprise cats, dogs, guinea pigs, rabbits, etc.). The DNA isolation technique, PCR condition and sequencing were described previously (Hubka and Kolařík, 2012). The sequence of the ITS region and partial LSU rDNA was deposited into the EMBL database under the accession number HG793054. The isolate is deposited in the Culture Collection of Fungi at the Department of Botany of Charles University in Prague as CCF 4707.

The treatment started after smears intake on day 0. It consisted in application of low-temperature plasma produced by cometary discharge with inserted grid at 9 kV and 150 μA, the distance between the discharge tip and the grid was 1 cm; the same distance was maintained between the discharge and the exposed skin. This treatment was applied for 10 minutes every day on the inner half of the lesion, its other half was left untreated as a control. On the 2nd day, white tips appeared on the pimples within the whole area, the exposed part of the efflorescence started to flake off on the 5th day. On the 8th day, the itching of the exposed area becomes perceptible lower, it disappeared almost and completely on the 10th and 14th day, respectively; itching persisted on the unexposed site. Starting the 11th day, the efflorescence in the unexposed area diffused to axilla, whereas in the exposed area continued scaling and no new pimples appeared. On 19th day, the skin in the

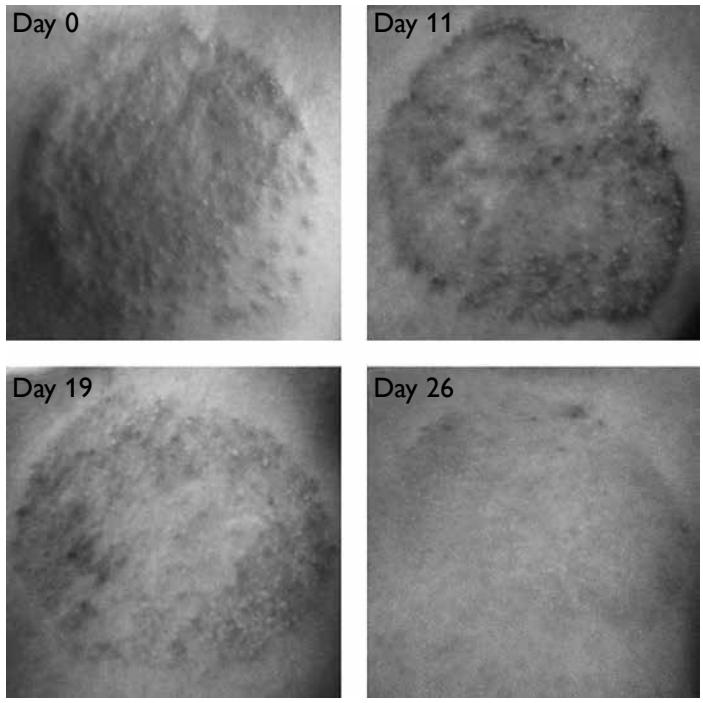

Figure 1 – The appearance of the efflorescence before treatment (upper left), during treatment (upper right and lower left) and after treatment (lower right). On all pictures, the left half of lesion was exposed to discharge.

Table 1 – Number of *Trichophyton* colonies (colony forming units) found on exposed and unexposed sites during treatment

Day	Exposed	Unexposed
0	–	48
5	26	50
9	5	250
14	3	7
19	0	5
26	0	0

exposed site was reddish and dusky but smooth-surfaced with no pimples and itching, which persisted in the unexposed site. The treatment was finished on the 24th day, when the exposed area remains unchanged, whereas in the unexposed area some isolated pustules persisted. Figure 1 documents the appearance of the lesion during treatment.

Control smears were taken from the lesion during treatment and processed as above. The results are summarized in Table 1, where the numbers of colonies grown on agar from samples taken from exposed and unexposed sites at various days of treatment are compared.

Smears were also taken from the fur and skin of domestic animals living on the farm. Numerous fungi were found, but none of them can be attributed to the genus *Trichophyton*.

Discussion

The *in vitro* resistance of fungi to low-temperature plasma is comparatively high and differs substantially among various genera. E.g. *Cladosporium sphaerospermum* in suspension is inactivated within 15 min of exposure, whereas *Penicilium crustosum* needs more than 20 min and *Aspergillus oryzae* was not completely inactivated even after 30 min. Yeast *Candida albicans* is more susceptible, being quenched within 6 minutes, whereas bacteria need only 2–4 minutes (Soušková et al., 2011). Unfortunately, the sensitivity of dermatophytes, including *Trichophyton* spp., was not yet studied *in vitro*.

In this case of benign but annoying dermatomycosis, the suppression of subjective discomfort was observed after 8th to 10th day of treatment, whereas it persisted for more than 20 days without treatment. The presence of etiological agent was lowered markedly during the initial stages and disappeared completely before 19 days of treatment. The spontaneous healing occurred, but markedly later than in treated area of efflorescence. The source of infection was not found.

The treatment of skin with low-temperature plasma represents no harm for the exposed persons, as documented by numerous studies, e.g. Julák and Scholtz (2013) or Lademann et al. (2013).

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