Journal of Microbiological Methods 119 (2015) 53–58

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

# Inactivation of human pathogenic dermatophytes by non-thermal plasma

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ARTICLE INFO

*Article history:*
Received 21 August 2015
Received in revised form 25 September 2015
Accepted 25 September 2015
Available online 30 September 2015

*Keywords:*
Corona discharge;
Cometary discharge;
Decontamination of surfaces;
Low-temperature plasma;
*Microsporum*;
*Trichophyton*

ABSTRACT

Non-thermal plasma (NTP) was tested as an in vitro deactivation method on four human pathogenic dermatophytes belonging to all ecological groups including anthropophilic *Trichophyton rubrum* and *Trichophyton interdigitale*, zoophilic *Arthroderma benhamiae*, and geophilic *Microsporum gypseum*. The identification of all strains was confirmed by sequencing of ITS rDNA region (internal transcribed spacer region of ribosomal DNA). Dermatophyte spores were suspended in water or inoculated on agar plates and exposed to NTP generated by a positive or negative corona discharge, or cometary discharge. After 15 min of exposure to NTP a significant decrease in the number of surviving spores in water suspensions was observed in all species. Complete spore inactivation and thus decontamination was observed in anthropophilic species after 25 min of exposure. Similarly, a significant decrease in the number of surviving spores was observed after 10–15 min of exposure to NTP on the surface of agar plates with full inhibition after 25 min in all tested species except of *M. gypseum*. Although the sensitivity of dermatophytes to the action of NTP appears to be lower than that of bacteria and yeast, our results suggest that NTP has the potential to be used as an alternative treatment strategy for dermatophytosis and could be useful for surface decontamination in clinical practice.



## 1. Introduction

The effects of non-thermal plasma (NTP), mediated by the action of reactive or charged particles and UV light, have been previously described in numerous studies. These efforts were mainly devoted to killing of prokaryotic bacteria (Laroussi, 2005; Moreau et al., 2008; Graves, 2012) and biomolecule inactivation, e.g., protein residues on surgical instruments (Whittaker et al., 2004) or prion proteins (Von Keudell et al., 2010; Julák et al., 2011). Other potential applications in human medicine (Fridman et al., 2008; Yousfi et al., 2014) include NTP-assisted blood coagulation (Kalghatgi et al., 2007); wound healing and tissue regeneration (Stoffels, 2006; Heinlin et al., 2011), ulcus treatment (Emmert et al., 2013), angiogenesis (Haertel et al., 2014), skin disinfection (Julák and Scholtz, 2013) and cancer treatment (Kim et al., 2009; Vandamme et al., 2010). Various applications of NTP in biotechnology were recently reviewed by Scholtz et al. (2015).

Previously we have reported the influence of NTP on the survival of certain non-pathogenic microorganisms including bacteria (Scholtz et al., 2010; Scholtz et al., 2011) and micromycetes (Soušková et al., 2011; Soušková et al., 2012) in both water suspensions and on surfaces. However, the sensitivity of human pathogenic fungi to NTP is in general unknown.

The promising results with inactivation of non-pathogenic fungi, successful application of NTP in treatment of non-infectious dermatological diseases and our positive experience with treatment of dermatophytosis in human patient (Švarcová et al., 2014) encouraged us to examine the influence of NTP on the survival of several species of pathogenic dermatophytes. The results presented in this paper are important for further development of NTP use in dermatology and in decontamination of surfaces contaminated with pathogenic fungi.

## 2. Materials and methods

### 2.1. Plasma generation

Three types of electrical discharges at atmospheric pressure in air were used as the source of NTP: the positive and negative point-to-plane corona discharges were applied for decontamination of dermatophytes in water suspensions, whereas cometary discharge was applied for decontamination of spores spread on agar surface.

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http://dx.doi.org/10.1016/j.mimet.2015.09.017
0167-7012/

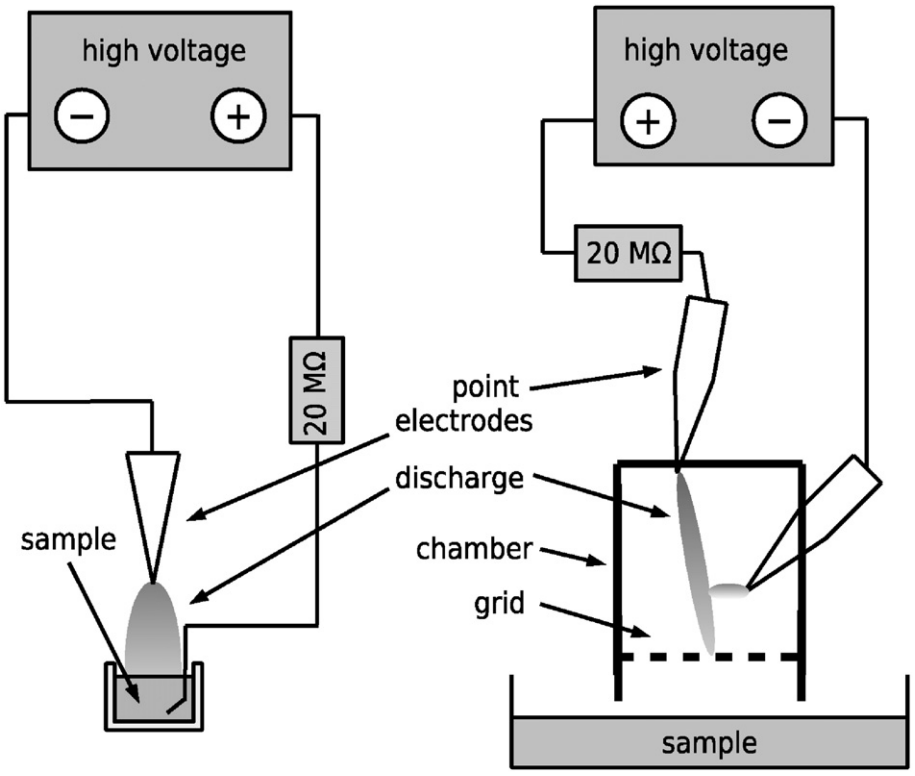


**Fig. 1.** Schematic view of the apparatus for the corona discharge exposure of water suspension (left) and for the cometary discharge exposure of agar plates (right). The polarity of electrodes in the left scheme was changed as required.

Corona discharges were generated using the previously described simple open-air apparatus (Fig. 1) (Scholtz et al., 2010). In brief, corona discharges were generated on a point electrode consisting of the tip of a syringe needle and were stabilized by the serial resistance of 20 MΩ. The grounded electrode was the surface of microorganismal water suspensions grounded with an immersed platinum wire. A micrometer screw adjusted the position of the coronizing electrode. A high voltage source, HT 2103 (Utes Brno, Czech Republic), was used to create a variable voltage up to $V = 10$ kV and current up to $I = 0.5$ mA. The corona discharges burned in a negative standard pulseless glow regime (Giao and Jordan, 1968) and in a positive regime of transient spark (Janda et al., 2011). The parameters set for the negative corona were $V = -9$ kV, $I = 300$ μA and for the positive corona were $V = 9$ kV, $I = 300$ μA. The distance between the tip of the needle and the suspension surface was adjusted to 3 mm in all cases.

Cometary discharge was generated using the previously described simple open-air apparatus (Scholtz and Julák, 2010b; Scholtz and Julák, 2010a). Briefly, the device consisted of two needles used as point electrodes in a defined geometric arrangement that were connected to the power supply via a stabilising resistor of 20 MΩ. The discharges burned between the two needle tips, creating a flaming jet that resembled the tail of a comet toward the exposed object. To enhance the efficiency of the discharges, the electrode system was enclosed in a small plastic chamber, and an electrically insulated circular metallic grid of 4 cm in diameter made of a stainless steel net with a mesh size of 1 mm was inserted between the comet and target object (Scholtz et al., 2013). This arrangement enabled treatment of various surfaces.

### 2.2. Preparation of spore suspensions

The stock cultures were prepared by taking a loopful of a 14-day-old culture, suspending it in water with Tween 85, and inoculating a drop of the resulting suspension onto the surface of Malt Extract agar (Oxoid, Hampshire, UK). The cultures were then incubated for 5 days at 25 °C. Immediately before exposure, a loopful of stock culture was suspended into a sterile buffered saline solution (pH 7.4) with Tween 85.

**Table 1**
Provenance and ITS sequence accession numbers of isolates tested in this study.

| Species | Strain no. | Provenance | ITS sequence accession number |
|---|---|---|---|
| *T. rubrum* | CCF 4879 | Isol ex sole skin, 28-year-old woman, NOV 2011, Prague, Czech Rep. | LN589973 |
| | CCF 4932 | Isol ex toenail, 47-year-old male, SEP 2013, České Budějovice, Czech Rep. | Identical |
| | CCF 4934 | Isol ex skin of the buttocks, 39-year-old male, AUG 2013, Plzeň, Czech Rep. | Identical |
| *T. interdigitale* | CCF 4878 | Isol ex chin skin, 49-year-old woman, FEB 2012, Prague, Czech Rep. | LN589974 |
| | CCF 4616 | Isol ex skin of the back, 47-year-old woman, AUG 2012, Ostrava, Czech Rep. | Identical |
| | CCF 4618 | Isol ex skin of the trunk, 50-year-old woman, AUG 2012, Kelč, Czech Rep. | Identical |
| *A. benhamiae* | CCF 4796 | Isol ex skin of the scalp, 5-year-old girl, AUG 2011, České Budějovice, Czech Rep. | LK054799 |
| | CCF 4797 | Isol ex skin of the chest, 7-year-old girl, MAR 2013, Dolní Benešov, Czech Rep. | LK054800 |
| | CCF 4848 | Isol ex skin of the face, 76-year-old woman, MAR 2012, Prague, Czech Rep. | LN589972 |
| *M. gypseum* | CCF 4625 | Isol ex skin of the arm, 51-year-old woman, OCT 2012, Plzeň, Czech Rep. | HG518406 |
| | CCF 4626 | Isol ex instep skin, 84-year-old woman, OCT 2012, Prague, Czech Rep. | LN878968 |
| | CCF 4677 | Isol ex skin of the scalp, 7-year-old boy, NOV 2011, Pardubice, Czech Rep. | HG518410 |