Influence of a plasma jet on the viability of *Candida albicans*

**Abstract:** A rapid growth of interest in low temperature plasma in medicine in recent years has given rise to a number of different applications. We report a low cost kHz driven argon plasma jet with flow rate less than 0.15 L min\(^{-1}\) suitable for treatment of small areas infected with the wide-spread yeast pathogen *Candida albicans*. The plasma jet has been applied for inactivation of *C. albicans* seeded on an agar surface as well as yeast suspensions. The effects of different treatment parameters (gas composition, distance and treatment time) have been studied on the efficiency of yeast inactivation. *C. albicans* viability was evaluated by XTT assay and by measuring diameters of circular transparent zones.

**Keywords:** plasma jet, *C. albicans*, XTT assay

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1 Introduction

In recent years, large attention has been paid to the application of non-equilibrium atmospheric pressure plasmas in different fields of science, medicine and technology [1-3]. Atmospheric pressure plasmas can provide many biochemically active agents like UV photons, electrons, radicals, electronically and vibrationally excited molecules, which seem to be an effective alternative to many existing sterilization methods [4-6].

One of the promising applications is the inactivation of various microorganisms and microbial biofilms. Candidiasis, caused by *Candida* species, is the most common fungal infection in humans and animals. *Candida albicans* is considered the most prevalent fungal biofilm-forming pathogen that causes life-threatening infections by colonizing polymers used in medical devices [7-11].

Recently, some research groups have dealt with the inactivation of yeasts using different atmospheric pressure plasma sources [12-18]. A He-O\(_2\) (2 L min\(^{-1}\), 3% O\(_2\)) plasma source was used for inactivation of *C. albicans* cells inoculated in Sabouraud's medium [15]. A pure He plasma source (0.15 L min\(^{-1}\)) has been used for samples on agar surfaces. These samples were evaluated by measuring diameters of circular inhibitory zones. The largest diameter achieved was 6 mm after 100 s of treatment [17].

In the present work, we have studied the microbiology efficacy of a miniature atmospheric pressure plasma jet with low consumption of Argon gas and dissipation power on *C. albicans* yeast cells. We have investigated the dependence of the antifungal response on the following parameters of the plasma jet: a) Plasma jet distance (between the sample and the end of the plasma jet capillary); b) Gas mixtures (Ar + 0-6% O\(_2\)).

The viability of *C. albicans* biological samples was studied for two different media: i) agar surface and ii) suspension. In the former case, viability was ascertained by measuring diameters of circular transparent, while in the latter case, XTT assays were carried out.

2 Experimental procedure

A schematic view of the experimental apparatus is shown in Fig. 1. The source of non-equilibrium plasma is a hollow needle-to-cylinder electrode configuration fed by argon-oxygen gas mixtures. The main features of this apparatus are as follows: gas: Ar + 0-6% O\(_2\), flow rate: 100 cm\(^3\) min\(^{-1}\), voltage: 7 kV\(_{pk-pk}\) at 12 kHz operating frequency. The discharge was created in a glass capillary with inner and outer diameters of 0.5 mm and 1 mm respectively, and then released to air in the form of a torch-like post-discharge.

Electrical discharge parameters were measured using an oscilloscope (Agilent technologies DSO 1012A)
connected to current (Persson current 2877) and high-voltage (Tektronix P6015A) probes. The total power used on the discharge system was calculated from the area of a Lissajous figure obtained from the applied voltage and integrated charge (Fig. 2). The optical emission spectra (OES) of the plasma jet were measured using a Jobin-Yvon H 25 monochromator coupled with a CCD multichannel detector (resolution: 0.1 nm). OES of the plasma jet revealed the dominancy of 2nd+ N2 emission bands (C3Πu → B3Πg, 300–440 nm), OH band (306–310 nm) and the Ar emission (4p → 4s) in the 680–850 nm region (Fig. 3). NO band emissions (230-320nm) were also observed, however the emission intensity was very low. No significant emission line corresponding to oxygen at 777 nm was detected in pure Argon or in any admixture of oxygen (1-6% O2 in Ar).

2.1 Materials and methods

The yeast strain C. albicans CCY 29-3-32 (serotype A) was obtained from the Culture Collection of Yeasts (CCY, Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia).

2.2 Preparation of C. albicans suspension

Yeast cells cultivated on slant agar were inoculated to YNB medium enriched with 0.9% glucose and cultivated for 30 h at 28°C. After cultivation, the growth medium was removed by centrifugation and yeast cells were washed with phosphate buffered saline. The suspension of C. albicans was used for the preparation of stock solution (~ 10^6 cells mL^-1).

2.3 Evaluation of C. albicans viability

The influence of the plasma beam on C. albicans was tested by two methods - growth of C. albicans on the agar surface and viability of C. albicans in suspension.

2.4 Plasma jet treatment of C. albicans on agar surface

Five drops of stock suspension (each of 100 μL) were placed on the agar surface in the Petri dishes. The influence of miniature plasma argon source efficiency was tested for different exposure times (30-120 s), distances (4-12 mm)
and for different admixture of oxygen in the range of 1-6% with Argon. The plasma jet was oriented perpendicular to the samples. After plasma treatment, all plates were incubated for 48 h at 37°C. A ruler was used to measure the diameters of the inhibitory zones, where no colony forming units (CFU) were present on the agar plates. The average value was calculated from 10 samples for every measurement.

2.5 Plasma jet treatment of \textit{C. albicans} suspension

The stock suspension was pipetted to a round-bottomed polystyrene 96-well microtiter plate (30 μL in all wells). The plasma jet was directed perpendicular to the bottom of the wells. The distance between the jet and surface of the suspension was constant (9 mm). The number of wells analysed was 196 for both gas compositions (pure argon and 1% O\textsubscript{2} in argon). The exposure of the yeast varied from 10 to 150 s for each sample at room temperature (300 K) and atmospheric pressure. Two hours after the plasma irradiation, the viability of the samples was measured by an XTT test [19]. This test utilizes XTT \textit{[2,3-bis (2-methoxy-4-nitro-5-sulfophenyl-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide]}, which is reduced by mitochondrial dehydrogenases of metabolically active yeast cells to a deep-red formazan product. The results have been calculated using a calibration curve, which was obtained by measuring the absorbance at a wavelength of 490 nm across the suspension for different concentrations of yeast cells. As a positive control, the untreated \textit{C. albicans} suspension was used, whereas the negative control consisted of an autoclaved suspension.

3 Results and discussion

3.1 Irradiation of samples

In all samples the gas alone was tested to control the drying effect of the gas flow on \textit{C. albicans} cells. These tests showed no influence on microbiological efficacy (data not shown).

3.2 Agar plates

After plasma irradiation of \textit{C. albicans} for different exposure times and following incubation of agar plates, the inhibitory zones were detected (Fig. 4). The diameter of inhibitory zones increases with prolongation of treatment time. Areas were calculated assuming a circular shape.

In Fig. 5 the average values of the growth inhibition zone area versus exposure time and distance between sample and plasma source are shown. In all measurements of \textit{C. albicans} growth, a mixture of 1% O\textsubscript{2} to Ar was used (Figs. 4, 5). The admixture of a larger amount of oxygen into the plasma source did not result in significant improvement in inhibitory zones; the effect was rather the opposite (negative). This is most likely due to quenching of the discharge by the higher concentration of oxygen in the gas mixture. The increasing treatment time together with the decreasing distance enhanced the antifungal effect on agar surfaces, as hypothesized. It is interesting to note that in spite of the relatively small cross section of the plasma jet (inner diameter of the glass capillary d=0.5mm, plasma cross section in capillary ~ 0.8 mm\textsuperscript{2}), and thus narrow plasma jet, the inhibited area is substantially larger (between 3 - 35 mm\textsuperscript{2}) depending on the treatment time and distance of the plasma jet from the sample. The larger inhibited area on the sample is due to diffusion of 

![Figure 4: Growth of \textit{C. albicans} exposed for a) 30, b) 60, c) 90 and d) 120 s to DBD argon-oxygen (99:1) plasma jet of 4 mm length (48 h after treatment).](image-url)
active species, primarily in the sample rather than in the surrounding air. This is because the active area does not increase with increasing sample distance.

Fig. 6 represents the dependence of decontaminated surface area on distance between the end of the capillary and the treated surface for different Ar-O\textsubscript{2} gas mixtures at the same treatment time of 120 s. The inhibition areas in this case were within 4-32 mm\textsuperscript{2}. Considerably decrease of the inhibition zone was observed for oxygen admixture above 1%.

The dependence of the decontaminated surface area versus different Ar-O\textsubscript{2} gas mixtures and treatment time has also been studied and was calculated for five different gas compositions (Fig. 7).

In all cases the growth of inactive zones was detected, however, the slopes for different oxygen admixtures reflect a decrease of antifungal response with increase of oxygen concentration. We explain this behaviour with the discharge quenching by oxygen within the capillary, and thus with decreased production of the active species responsible for inactivation. With increasing distance between the plasma jet and the sample, the flight-time of species in air increases from 0.3 to 0.9 ms, which allows reaction between the reactive species and particles in the post-plasma responsible for their transformation and decay before reaching the sample. According to Schidt-Bleker \textit{et al.} [20], the major reactive species that hit the samples are O\textsubscript{3}, NO\textsubscript{2}, NO and H\textsubscript{2}O\textsubscript{2}. According to Kutasi \textit{et al.} [21], the oxygen admixture to argon influences the electron energy distribution function (EEDF) by decreasing the average energy of electrons and reducing the high energy tail of the EEDF, as well as reducing metastable Ar states in the plasma. In our opinion, this results in a decrease in the dissociation of molecules and a decrease in the production of reactive species. The time dependence seems to be linear for all gas compositions, but the slopes of the lines decrease with increasing O\textsubscript{2} admixture. The highest antifungal effect was observed for pure argon for a treatment time of 120 seconds. The absence of emission from O lines in the plasma is mainly due to the radiative lifetime of the transition of O(\textsuperscript{P}) to O(\textsuperscript{S}), which is only 27 ns and therefore not observed in the postplasma region. Detailed reaction schemes for the processes in Ar-O\textsubscript{2} mixtures are presented in [22].

3.3 Plasma jet treatment of \textit{C. albicans} suspensions

The survival of \textit{C. albicans} cells in suspension was monitored by XTT assay, which is related to the mitochondrial dehydrogenase activity of metabolically active cells. The living cells reduce the XTT reagent to formazan and consequently there is a change of colour (Fig. 8). The red wells (columns with plasma-untreated cells) represent living yeast cells. With increasing duration of plasma treatment (10 s to 150 s), the number of surviving cells decreases, the reduction of XTT stops and the colour of the reaction mixture changes to yellow. From a qualitative point of view, the yeast cells were killed after 60 s of plasma treatment.

The viability of \textit{C. albicans} cells was monitored after treatment with Ar and (Ar + 1% O\textsubscript{2}) (Fig. 9). The surviving cells were quantified by XTT assay using a calibration
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The XTT assay enables the quantification of living cells in the range of $10^6 – 10^3$ cells mL$^{-1}$. The absorbance values thus remained unchanged below the lower concentration limit of *C. albicans* cells. The decrease of living *C. albicans* cells was 25.8% after 10 s of Ar treatment, and 13.3% after (Ar + 1% O$_2$) treatment. The considerable decrease of living cells was monitored after 30 s of plasma treatment: a 65.2% decrease was noted for Ar) and a 45.1% decrease for Ar + 1% O$_2$. As can be seen in Fig. 9, the viability of *C. albicans* cells after 60 s of plasma treatment decreased to 0.4% (Ar) and 2% (Ar + 1% O$_2$). Thus, 60 s of plasma treating was sufficient to kill *C. albicans* cells under the given reaction conditions. The temperature of the plasma plume did not play a role in the plasma treatment, it was approximately the same as ambient air.

**4 Conclusions**

The miniature kHz driven low gas consumption argon plasma jet process was evaluated for antimicrobial efficacy against *C. albicans*. Despite the narrow plasma plume generated by the plasma jet, multiple larger inhibition zones on agar surfaces were observed compared to the diameter of the plasma plume. In the case of yeast suspensions, reduction of viability to less than 0.4% from the starting microbiological concentration of $10^6$ cells mL$^{-1}$ was detected. In both tests, analyses indicated that higher admixtures of oxygen do not lead to higher killing efficiency for *C. albicans*. This is most likely due to quenching of discharge by the oxygen present in the gas mixture.

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**References**

[1] Daeschlein G., Woedtke T. von, Kindel E., Brandenburg R., Weltmann K.-D., Junger M., Plasma Process. Polym., 2010, 7, 224–230

[2] Brandenburg R., Ehlbeck J., Stieber M., Woedtke T. von, Zeymer J., Schlüter O., Weltmann K.D., Contrib. Plasma Phys., 2007, 47, 72

[3] Weltmann K.-D., Brandenburg R., Woedtke T. von, Ehlbeck J., Foest R., Stieber M., Kindel E., J. Phys. D: Appl. Phys., 2008, 41, 194008
[4] Boudam M.K., Moisam M., Saoudi B., Popovici C., Gherardi N., J. Phys. D: Appl. Phys., 2006, 39, 3494
[5] Dobrynin D., Fridman G., Friedman G., Fridman A., New J. Phys., 2009, 11, 26
[6] Akishev Y. et al., IUPAC, Pure and Applied Chemistry 2008, 80, 1953
[7] Harriott M.M., Noverr M.C., Antimicrob. Agents Chemother., 2009, 53, 3914
[8] Concia E., Azzini A.M., Conti M., Drugs, 2000, 69, 5
[9] Arendrup M.C., Epidemiology of invasive candidiasis, Curr. Opin. Crit. Care, 2010, 16, 445
[10] Niermann B., Boke M., Sadeghi N., Winter J., Eur. Phys. J. D 2010, 60(3), 489
[11] Wang C., Srivastava N., Eur. Phys. J. D, 2010, 60(3), 465
[12] Song Y. et al., Plasma Process. Polym., 2012, 9, 17
[13] Daeschlein G. et al., Plasma Process. Polym., 2012, 9, 380
[14] Koban I. et al., New J. Phys., 2010, 12, 073039
[15] Xiong et al., Phys. Plasmas, 2010, 17, 123502
[16] Yamazaki H. et al., Dental Materials Journal, 2011, 30(3), 384
[17] Pociota A. et al., Journal of Electrostatics, 2010, 68, 128
[18] Rupf S. et al., Journal of Medical Microbiology, 2010, 59, 206
[19] Kuhn D.M., Balkis M., Chandra J., Mukherjee P.K., Ghannoum M.A., J. Clin. Microbiol., 2003, 41, 506
[20] Schmidt-Bleker A., Winter J., Iseni S., Dünnbier M., Weltmann K.-D., Reuter S., J. Phys. D: Appl. Phys., 2014, 47
[21] Kutasi K., Guerra V., Sá P., J. Phys. D: Appl. Phys., 2010, 43, 175201
[22] Xiong Q., Nikiforov A.Y., Lu X.P., Leys C., J. Phys. D: Appl. Phys., 2010, 43, 415201