

## Application of post-discharge region of atmospheric pressure argon and air plasma jet in the contamination control of *Candida albicans* biofilms

Anelise Cristina Osório Cesar Doria\*, Camila Di Paula Costa Sorge, Thaisa Baesso Santos, Jhonatan Brandão, Polyana Alves Radi Gonçalves, Homero Santiago Maciel, Sônia Khouri, Rodrigo Sávio Pessoa

**Abstract Introduction:** *Candida* species are responsible for about 80% of hospital fungal infections. Non-thermal plasmas operated at atmospheric pressure are increasingly used as an alternative to existing antimicrobial strategy. This work investigates the action of post-discharge region of a non-thermal atmospheric plasma jet, generated by a gliding arc reactor, on biofilms of standard strain of *Candida albicans* grown on polyurethane substrate. **Methods:** Samples were divided into three groups: (i) non-treated; (ii) treated with argon plasma, and (iii) treated with argon plus air plasma. Subsequently to plasma treatment, counting of colony-forming units (CFU/ml) and cell viability tests were performed. In addition, the surface morphology of the samples was evaluated by scanning electron microscopy (SEM) and optical profilometry (OP). **Results:** Reduction in CFU/ml of 85% and 88.1% were observed in groups ii and iii, respectively. Cell viability after treatment also showed reduction of 33% in group ii and 8% in group iii, in comparison with group i (100%). The SEM images allow observation of the effect of plasma chemistry on biofilm structure, and OP images showed a reduction of its surface roughness, which suggests a possible loss of biofilm mass. **Conclusion:** The treatment in post-discharge region and the chemistries of plasma jet tested in this work were effective in controlling *Candida albicans* biofilm contamination. Finally, it was evidenced that argon plus air plasma was the most efficient to reduce cell viability.

**Keywords:** Gliding arc plasma, Biofilm, *Candida albicans*, Cell viability.

### Introduction

*Candida* species are found in the gastrointestinal tract in 20 to 80% of the healthy adult population. These commensal microorganisms become pathogenic if there are changes in host's defense mechanisms or compromised anatomical barriers secondary to burn or invasive medical procedures. The incidence of nosocomial yeast infection has increased substantially in recent decades leading to mortality rates up to 60% (Ruiz et al., 2013). Because of the medical importance of these microorganisms, the use of new technologies is necessary for their control.

Non-thermal electrical plasmas operated at atmospheric pressure (approx. 101 kPa) are increasingly gaining attention as a potential approach to the eradication and control of infection and/or bacterial or fungal contamination. As an antimicrobial strategy, the advantages of non-thermal plasmas operated at atmospheric pressure are the simple design, low cost of construction/operation, usage of nontoxic gases, with operating conditions of gas at or near room temperature, and no harmful residues (Gaunt et al., 2006; Kong et al., 2009; Laroussi, 2002). In

last years, much effort and progress have been done in order to elucidate the exact mechanisms leading to bacterial or fungal inactivation by the action of electric plasma (Alkawareek et al., 2012; Chen et al., 2014; Laroussi, 2002; Mai-Prochnow et al., 2014; Taghizadeh et al., 2015; Traba and Liang, 2015). It is considered that several plasma products play an important role in this process, namely reactive oxygen species (ROS), such as ozone, atomic oxygen, superoxide, hydrogen peroxide and hydroxyl radicals (Kong et al., 2009), and reactive nitrogen species (RNS), UV radiation, and charged particles (Gaunt et al., 2006; Laroussi and Leipold, 2004; Ma et al., 2008).

In this work, it was investigated the effect of the post-discharge region of a non-thermal atmospheric plasma jet, generated by a gliding arc reactor, with mixtures of argon and air, on standard strain biofilms of *Candida albicans* (ATCC 10231) grown on polyurethane substrate, the main constituent of central venous catheter.

\*e-mail: [ane.doria@gmail.com](mailto:ane.doria@gmail.com)

## Methods

The fungal suspensions of standard strain ATCC® (American Type Culture Collection) of *C. albicans* (10231) in the concentration of  $10^6$  colony forming units per milliliters (CFU/ml) in Dextrose Sabouraud broth (DIFCO) were prepared, in which polyurethane plates, 2 mm thick and 2 cm<sup>2</sup> area, were inserted under sterile conditions. After this, the samples were incubated at 37 °C for 48 hours, under constant agitation (110 rpm) in an incubator shaker, and washed with phosphate buffer (PBS, pH  $7.2 \pm 0.1$ ) to remove non-adherent cells (Vasconcelos et al., 2014). The samples were divided into three groups: (i) control group that was not treated; (ii) the group treated with the argon plasma, and (iii) the group treated with argon plus air plasma. The experiments were performed in triplicate and the treatment with each of the two types of plasma was performed on different days. Figure 1 shows the experimental setup, and Table 1 presents the process parameters used in this work.

A gliding arc reactor was used to generate a reactive plasma environment composed of plasma jet and post-discharge regions. The gases used were argon (99.5% purity) and compressed-air generated from medical/orthodontic compressor. To generate the plasma, a 60 Hz 7500 V voltage transformer was used. Using a variac, the discharge current was maintained at around 19 mA for all investigated processes. A sample holder was placed 30 mm below the reactor, so as the sample was treated in the post-discharge region,

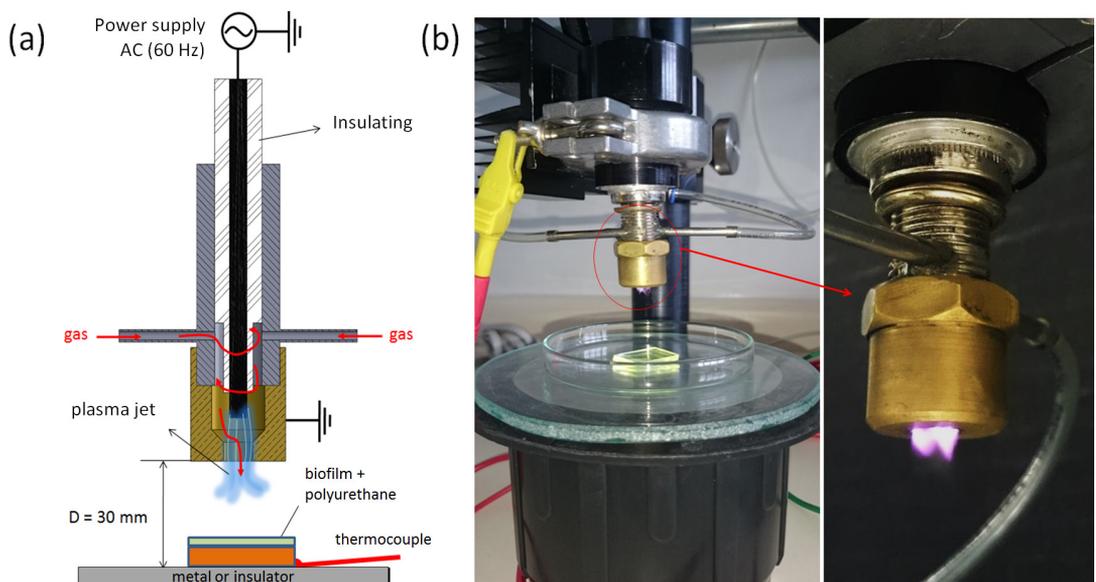
thus preventing a high heating of the substrate surface (maximum temperature of 43 °C) by the plasma jet.

After treatment, the biofilm plaques on the samples were detached in PBS using a vortex shaker. A volume of 100  $\mu$ l of the inoculum of the control group and of the treated groups were plated on Sabouraud agar and incubated at 37 °C for 48 h, after which the CFU/ml count was performed. The results are expressed as percent reduction of the number of colonies after the treatment with respect to the initial number before the treatment. Finally, 20  $\mu$ l of the inoculum were used to perform the test of cell viability by the trypan blue technique (adapted from Saad-Hossne et al., 2004).

The magnified images of the samples were obtained by scanning electron microscopy (SEM from Zeiss,

**Table 1.** Parameters used during plasma treatment.

Plasma	Argon	Argon + Compressed air
Gas flow (L/min)	10	6 + 4
Root mean square current - $I_{RMS}$ (mA)	19.8	19.3
Root mean square voltage - $V_{RMS}$ (V)	700	1850
Discharge power (W)	13.9	35.7
Frequency (Hz)	60	60
Nozzle-to-sample distance (mm)	30	30
Treatment time (min)	10	10



**Figure 1.** (a) Scheme of the experimental setup used in the treatments. (b) Photo of the plasma plume. The distance between biased-electrode and grounded electrode was around 1 mm, and the hole diameter in grounded electrode is 5 mm.

model EVO MA 10) and the surface roughness was evaluated by optical profilometry (OP, from Veeco®, model NT9100).

## Results

The results of the quantitative analyses, presented in Table 2, show a reduction in the number of CFU/ml of about 85.0% for group ii (argon plasma only) and of 88.1% for group iii (argon plus air plasma). The cell viability was 33% for group ii and 8% for group iii in comparison with group i (non-treated), the viability of which was set to 100%.

SEM and OP images of each analyzed sample (substrate and groups i-iii) are shown in Figures 2 and 3,

**Table 2.** Reduction percentage for counting of colony forming units (CFU) and cell viability.

Group	CFU/mL	% of reduction	% of viable cells
i	$3.00 \times 10^5$	-	100
ii	$4.50 \times 10^4$	85.0	33
iii	$3.56 \times 10^4$	88.1	8

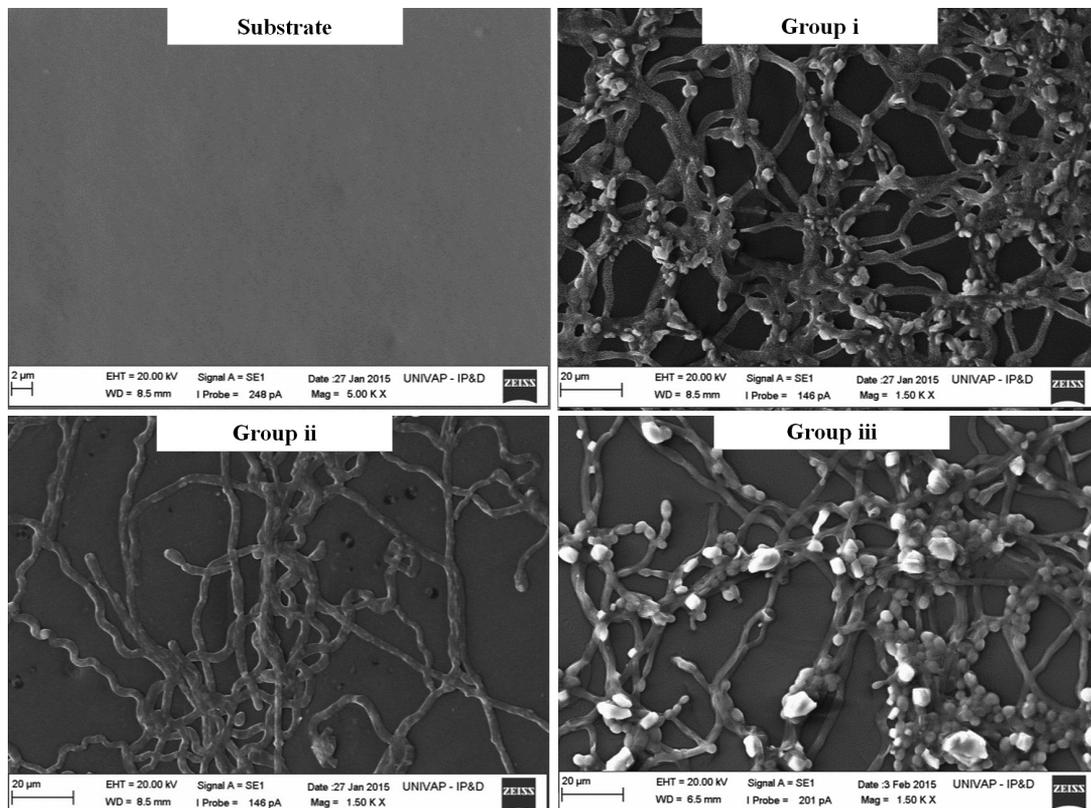
(i) not treated; (ii) treated with argon plasma; (iii) treated with argon plus air plasma.

respectively. The OP results revealed that the substrate has a smooth surface with a measured quadratic roughness ( $R_q$ ) of approximately  $0.09 \mu\text{m}$ . By other hand, it was observed an increasing in up to two orders of magnitude ( $R_q = 4.75 \mu\text{m}$ ), when the biofilm was cultivated on it. Additionally, it was possible to observe a difference between the surface roughness of the untreated and treated samples ( $2.27 \mu\text{m}$  and  $1.90 \mu\text{m}$  for groups iii and iv, respectively), that can be related to the plasma treatment.

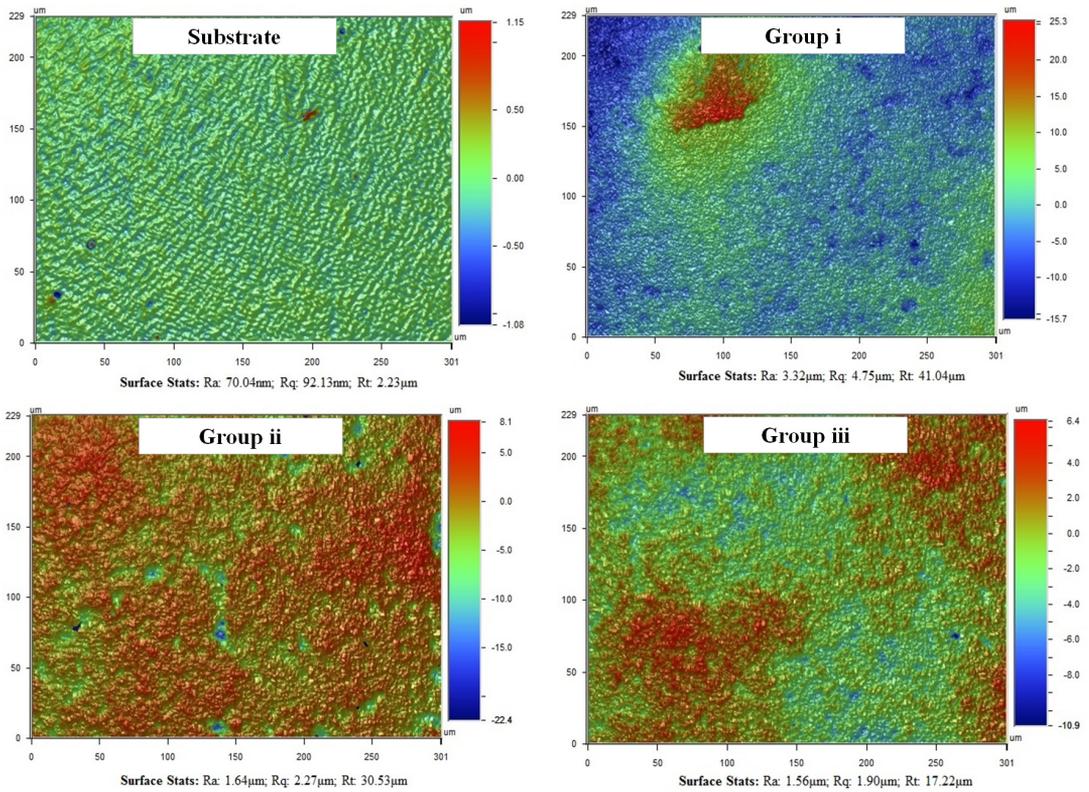
## Discussion

From the CFU/ml counting, in both plasma jet compositions up to 80% efficacy was obtained in the control of the yeast colonies grown on polyurethane substrate. On the other hand, cell viability tests indicated that only about 8% of the yeast cells treated with argon plus air plasma could survive, proliferate, and/or generate other cells, demonstrating that the argon/compressed-air plasma was the most effective in *Candida albicans* biofilm inactivation.

The SEM images provided qualitative information on the samples, such as confirmation of the presence of biofilm, and distribution and morphology of the



**Figure 2.** Images of scanning electron microscopy of the substrate surface. Groups: i) untreated; ii) treated with argon plasma; and iii) treated with argon plus air plasma.



**Figure 3.** Images of optical profilometry of the substrate. Groups: i) untreated; ii) treated with argon plasma; and iii) treated with argon plus air plasma.

microorganisms. On the other hand, with the OP images it was possible to obtain quantitative data on the surface roughness of the samples, which was reduced by ~50% after treatment with the post-discharge plasma. This effect was possibly caused by increase of ROS and RNS due to insertion of air in argon plasma, which might have provoked catalytic inactivation of the microorganisms, despite the persistence of the biofilm organization. The quantitative analysis suggests a possible loss of biofilm mass with plasma treatment, which is not fully apparent in the SEM images probably because the latter were taken from a much smaller area.

As final remarks, it is highlighted that the treatment of *Candida* biofilms in post-discharge region of an atmospheric gliding arc plasma jet is an interesting alternative to treat surfaces contaminated by microorganisms, without the direct exposure to plasma environment that otherwise would cause a fast heating of the biological sample and the polymeric substrate. This issue is relevant when the treated surface is temperature sensitive, being worth to note that the temperature measured at the surface of the polyurethane during the post-discharge treatment was 43 °C at most.

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## References

- Alkawareek MY, Algwari QT, Laverty G, Gorman SP, Graham WG, O'Connell D, Gilmore BF. Eradication of *Pseudomonas aeruginosa* biofilms by atmospheric pressure non-thermal plasma. *PLoS One*. 2012; 7(8):e44289. PMID:22952948.
- Chen C, Liu DX, Liu ZC, Yang AJ, Chen HL, Shama G, Kong MG. A model of plasma-biofilm and plasma-tissue interactions at ambient pressure. *Plasma Chemistry and Plasma Processing*. 2014; 34(3):403-41. <http://dx.doi.org/10.1007/s11090-014-9545-1>.
- Gaunt LF, Beggs CB, Georghiou GE. Bactericidal action of the reactive species produced by gas-discharge nonthermal plasma at atmospheric pressure: a review. *IEEE Transactions on Plasma Science*. 2006; 34(4):1257-69. <http://dx.doi.org/10.1109/TPS.2006.878381>.

Kong MG, Kroesen G, Morfill G, Nosenko T, Shimizu T, van Dijk J, Zimmermann JL. Plasma medicine: an introductory review. *New Journal of Physics*. 2009; 11(11):1-35. <http://dx.doi.org/10.1088/1367-2630/11/11/115012>.

Laroussi M. Nonthermal decontamination of biological media by atmospheric-pressure plasmas: review, analysis, and prospects. *IEEE Transactions on Plasma Science*. 2002; 30(4):1409-15. <http://dx.doi.org/10.1109/TPS.2002.804220>.

Laroussi M, Leipold F. Evaluation of the roles of reactive species, heat, and uv radiation in the inactivation of bacterial cells by air plasmas at atmospheric pressure. *International Journal of Mass Spectrometry*. 2004; 233(1-3):81-6. <http://dx.doi.org/10.1016/j.ijms.2003.11.016>.

Ma Y, Zhang G-J, Shi X-M, Xu G-M, Yang Y. Chemical mechanisms of bacterial inactivation using dielectric barrier discharge plasma in atmospheric air. *IEEE Transactions on Plasma Science*. 2008; 36(4):1615-20. <http://dx.doi.org/10.1109/TPS.2008.917165>.

Mai-Prochnow A, Murphy AB, McLean KM, Kong MG, Ostrikov K. Atmospheric pressure plasmas: infection control and bacterial responses. *International Journal of Antimicrobial Agents*. 2014; 43(6):508-17. <http://dx.doi.org/10.1016/j.ijantimicag.2014.01.025>. PMID:24637224.

Ruiz LS, Khouri S, Hahn RC, Silva EG, Oliveira VK, Gandra RF, Paula CR. Candidemia by species of the *Candida parapsilosis* complex in children's hospital: prevalence, biofilm production and antifungal susceptibility. *Mycopathologia*. 2013; 175(3-4):231-9. <http://dx.doi.org/10.1007/s11046-013-9616-5>. PMID:23404576.

Saad-Hossne R, Saad-Hossne W, Prado RG. Efeito da solução aquosa de fenol, ácido acético e glicerina sobre o tumor ascítico de Ehrlich. Estudo experimental in vitro. *Acta Cirurgica Brasileira*. 2004; 19(1):54-8. <http://dx.doi.org/10.1590/S0102-86502004000100009>.

Taghizadeh L, Brackman G, Nikiforov A, van der Mullen J, Leys C, Coenye T. Inactivation of biofilms using a low power atmospheric pressure argon plasma jet; the role of entrained nitrogen. *Plasma Processes and Polymers*. 2015; 12(1):75-81. <http://dx.doi.org/10.1002/ppap.201400074>.

Traba C, Liang JF. The inactivation of *Staphylococcus aureus* biofilms using low-power argon plasma in a layer-by-layer approach. *Biofouling*. 2015; 31(1):39-48. <http://dx.doi.org/10.1080/08927014.2014.995643>. PMID:25569189.

Vasconcelos LC, Sampaio FC, Albuquerque AJR, Vasconcelos LCS. Cell viability of *Candida albicans* against the antifungal activity of thymol. *Brazilian Dental Journal*. 2014; 25(4):277-81. <http://dx.doi.org/10.1590/0103-6440201300052>. PMID:25250489.

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## Authors

Anelise Cristina Osório Cesar Doria<sup>1\*</sup>, Camila Di Paula Costa Sorge<sup>1</sup>, Thaisa Baesso Santos<sup>1</sup>, Jhonatan Brandão<sup>2</sup>, Polyana Alves Radi Gonçalves<sup>2,3</sup>, Homero Santiago Maciel<sup>1,2,3</sup>, Sônia Khouri<sup>1,4</sup>, Rodrigo Sávio Pessoa<sup>1,2,3</sup>

<sup>1</sup> Laboratório de Biotecnologia e Plasmas Elétricos, Universidade do Vale do Paraíba – UNIVAP, Av. Shishima Hifumi, 2911, Urbanova, CEP 12244-000, São José dos Campos, SP, Brazil.

<sup>2</sup> Laboratório de Nanotecnologia e Processos a Plasma, Universidade do Vale do Paraíba – UNIVAP, São José dos Campos, SP, Brasil.

<sup>3</sup> Departamento de Física, Instituto Tecnológico de Aeronáutica – ITA, São José dos Campos, SP, Brazil.

<sup>4</sup> Núcleo de Estudos Farmacêuticos e Biomédicos, Faculdade de Ciências da Saúde, Universidade do Vale do Paraíba – UNIVAP, São José dos Campos, SP, Brazil.