Action of an Argon/Water Vapor Plasma Jet in the Sterilization of Silicone Contaminated with *Candida albicans*

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ABSTRACT: This work evaluates the influence of argon/water vapor plasma jet exposure time on surface sterilization of a silicone substrate contaminated with *Candida albicans*. For this purpose, gliding arc-type geometry was used to generate a nonthermal plasma jet at an applied power on the order of 20 W under water vapor carried by an argon flow of 5 L/min. *C. albicans* biofilms were grown on silicone plates with a 2cm² area. Samples were treated with a plasma jet for different exposure times, resulting in the following sample groups: nontreated (A) or treated with plasma for 2 min (B), 5 min (C), 10 min (D), and 20 min (E). Experiments were performed in triplicate. To evaluate the action of the plasma jet on the contaminated samples, colony-forming units (CFUs) were counted before and after plasma exposure. Scanning electron microscopy (SEM) was used to analyze the morphological modification of the treated substrate, whereas Fourier transform infrared spectroscopy (FT-IR) was used to analyze the bare substrate after exposure to plasma. Optical emission spectroscopy was used to investigate the plasma species in the ultraviolet range. Results indicated reductions in CFUs of 42.4%, 74.2%, 83.7%, and 97.5% for groups B, C, D, and E, respectively. SEM images showed that groups D and E suffered more external cellular changes with shriveled, swollen, and/or deformed aspects. These effects can be attributed to reactive species in the plasma such as hydroxyl radicals (OH), which were increased considerably with the presence of water vapor in the mixture with argon gas. A quality change in the surface of the silicone material after 20 min of plasma treatment was observed with the results of the SEM and FT-IR analyses. Finally, the argon/water plasma jet promoted only a slight increase in substrate temperature (up to 310 K) for the investigated conditions, which is very convenient for thermal-sensitive material sterilization.

KEY WORDS: atmospheric plasma jet, gliding arc, water vapor, argon, *Candida albicans*, plasma sterilization

I. INTRODUCTION

Hospital infections can result from a patient's unbalanced microbiota and the introduc-

tion of microorganisms into the susceptible body. Other factors that are no less important include passive entry from external sources such as pathogens, contaminated hands of health professionals, or failures in the cleaning process or disinfection cycle on the material's surface. Preventive and assertive strategies are needed to break the chain of hospital infections.¹ Panoramicallly, 60% of hospital infections are related to fungemia, and 80% of these infections are related to the main pathogen *Candida albicans*.² This fungus is considered opportunistic, has pronounced clinical relevance due to its greater virulence capacity, and may be involved in both colonization and/or infection processes.²⁻⁷

At present, the removal of *C. albicans* and other microorganisms on material surfaces is carried out by various sterilization processes, including the use of heat, heat mixed with humidity and high pressure, chemical solutions, or electromagnetic radiation.³ The effectiveness of these traditional sterilization methods is well established. However, in recent years, efforts have increased to determine alternative methods of sterilization that are less toxic to the environment, are easy to operate, and have minimal operator exposure to occupational hazards. In this context, studies have pointed to the use of electric atmospheric plasma as a promising and revolutionary sterilization technique to combat fungi and other microorganisms.^{3,8} The action of plasma on the organic material is effective in reducing the load of microorganisms by the synergistic action of active agents such as charged particles, chemically reactive species, photons, ultraviolet (UV) radiation, and electric fields that act on multiple cellular metabolic processes and microorganism components.^{3,9}

Recently, Doria et al.³ reviewed studies on the sterilization of fungi of the *Candida* genus by atmospheric plasmas. They confirmed that understanding of the mechanisms of action of the plasma in this type of microorganism have been little studied. Okawa et al.¹⁰ recently investigated the use of a glow discharge plasma in a mixture gas of helium and oxygen at atmospheric pressure for inactivation of *C. albicans*. They demonstrated the efficiency of plasma in destroying the peripheral cellular structure of *C. albicans*, and attributed that the antifungal effect is due to excited atomic oxygen in helium plasma. Therefore, due to the clinical importance in the control of *C. albicans* contaminations, a growing attention has been given in studies related to the antifungal action of atmospheric plasmas in biomedical tools. In this context, it is interesting and relevant to investigate an alternative method for the disinfection of a common and frequently used material in the hospital environment, such as the medical elastomer polydimethylsiloxane (PDMS) or silicone. The bulk properties of silicone make it particularly attractive for medical applications: it is chemically and physiologically inert, is resistant to sterilization and disinfection, and has properties that are less favorable to microorganism proliferation.¹¹ Even so, contamination by microorganisms is common in most polymer materials used in environments with high humidity, such as the human body, in which the interaction with the surface of the polymer can sometimes cause the polymer to decompose and consequently alter functionalities such as hydrophobicity.¹¹ This work aims to evaluate the influence of the exposure time of an argon/water vapor plasma jet in the sterilization

of silicone substrate surface contaminated with *C. albicans*.

II. MATERIALS AND METHODS

In this study, samples of contaminated silicone were used. Preparation of the fungal suspension of a standard strain of *C. albicans* (10231; American Type Culture Collection, Manassas, VA) *in vitro* was performed on Sabouraud dextrose broth in a concentration compatible to 0.5 on the McFarland turbidity scale (106 CFU/mL). Then 40 mL of this suspension was added to Erlenmeyer flasks containing silicone substrates (3R Plásticos, São Paulo, SP, Brazil) with an area of 1 cm² and thickness of 0.3 cm. In sequence, the samples were maintained on an incubation shaker with constant stirring at 110 rpm and temperature at 310 K for a period of 48 hours. The samples were then washed with phosphate-buffered saline (PBS) solution to remove nonadhered cells and preserve their integrity on the surface of the substrate.¹²

After the samples were prepared, they were randomly divided into five groups. Group A contained untreated (control) samples, whereas groups B–E contained samples exposed to electric plasma for different durations (group B, 2 min; group C, 5 min; group D, 10 min; and group E, 20 min). Each treatment was performed in triplicate.

The samples were treated in the postdischarge region of an atmospheric pressure plasma jet generated in a vortex gliding arc reactor, as illustrated in Fig. 1. To generate the plasma, a high-voltage transformer (7.5 kV, 60 Hz) was used to bias the center electrode as illustrated in Fig. 1a. A high voltage resistance (1 k Ω) protects the transformer in the event of an electric arc. We used a digital oscilloscope (Tektronix 2024b; Tektronix, Beaverton, OR) together with voltage and current probes (Tektronix P6015A and P6021A, respectively) to measure the electrical voltage, current, and discharge power. A Variac transformer (VARIAC, Cleveland, OH, USA) was used to maintain the discharge current at around 20 mA for all investigated conditions. Here, the water vapor was carried out to the reactor by passing a flow of 5 L/min of argon gas (purity of 99.95%) through a flask containing deionized water at room temperature. The distance between the substrate nozzle was fixed at 10 mm for all treatments. To measure the temperature of the substrate during the sterilization process, a k-type thermocouple was placed below the substrate. For more details on the plasma jet system, see Doria et al.¹²

After exposure to plasma, the treated samples were immersed in 5 mL of PBS solution and cell detachment was performed with assistance from a vortex stirrer. A 0.1 mL volume of the inoculum from the control group and treated groups was plated on Sabouraud dextrose agar and incubated at 310 K for 48 h. The number of CFUs/mL was counted using an electronic colony counter (Phoenix Luferco, Araraquara, SP, Brazil). The results were compiled for calculation of the colony reduction rate. We used a statistical inferential method to calculate the one-way analysis of variance (ANOVA), followed by application of a *post hoc* test (Tukey's multiple comparisons test), to compare the treatment groups against the negative control. A level of 5% was considered significant ($P < 0.05$).⁵

Morphological modification of the treated substrate surface was analyzed by scan-

FIG. 1: (a) Schematic of a vortex gliding arc reactor with gas insertion apparatus. (b) Photograph of the reactor and plasma jet. DI, deionized; HV, high-voltage; OES, optical emission spectroscopy.

ning electron microscopy (SEM) (model EVO MA 10; Zeiss, Oberkochen, Germany). To investigate the chemical bonds, infrared measurements were performed with attenuated total reflectance (ATR) Fourier transform infrared spectroscopy (FT-IR) on a 400 IR spectrometer (PerkinElmer, Waltham, MA, USA) at a resolution of 2 cm^{-1} . Each ATR spectrum was recorded with the blank ATR cell as the background.

Optical emission spectroscopy (OES) was used to investigate the plasma species in a UV-visible range of 200–500 nm. We used an Acton Spectra Pro SP-2500i monochromator with a Pixis 256 charge-coupled device camera (Princeton Instruments, Trenton, NJ), allowing a resolution of 0.05 nm. The spectrum was obtained via the Lightfield program, which allowed determination of the integration time for composing the spectrum, which was then displayed on a computer screen.

III. RESULTS

Using ANOVA, we verified that the mean values between the treatment groups were statistically significant ($P = 4.2\text{E-}07$) after treatment with plasma. With this variation

test, the possibility that the results were merely causal was rejected. After this step, the Tukey test was performed to identify the paired groups that responded significantly to the treatment. As shown in Table 1, all groups (B, C, D, and E) responded significantly to plasma treatment compared with the control group (A).

In addition, we evaluated the dispersion and distribution of CFUs of the control group and groups treated with atmospheric plasma (Fig. 2). There was greater heterogeneity in the values of the groups treated at times ≤ 5 min (B and C). However, the groups treated for > 5 min (D and E) showed lower dispersion of the results (i.e., the treatment was more adequate for the uniform reduction of CFUs along the substrate). Note that the position of the median line for groups B, D, and E resulted in a symmetrical distribution.

TABLE 1: Tukey test: comparison of paired groups after application of atmospheric plasma

Comparison Between Groups	Standard Error	Alpha Value	P Value
B-A	15.97	0.05	0.03
C-A	15.97	0.05	4.84E-5
D-A	15.97	0.05	4.18E-5
E-A	19.91	0.05	2.74E-5

FIG. 2: Dispersion and distribution of the CFU count of the control group and groups treated with atmospheric plasma. CFU, colony-forming unit.

However, the data for group C were positively asymmetric by the deviation from the midline to the lower quartile.

Figure 3 presents photographs of the seeded plates before (control) and after plasma application. Group E presented the best response to treatment, in both the rate of reduction in CFUs and in the distribution of the resulting values.

Figure 4 shows the colony reduction rate of *C. albicans* as a function of plasma jet exposure time. Samples exposed to plasma for 2 min (group B) had a 42.4% reduction. Groups C (5 min) and D (10 min) had reductions of 74.2% and 83.7%, respectively. Finally, group E (20 min) had the highest reduction of 97.5%, which is equivalent to a reduction of 2 log.

Figure 5 shows the temporal temperature variation of the substrates for all of the investigated groups during exposure of the contaminated surface to the plasma stream. The starting point for all groups was the local temperature (~ 297 K). From this value, the treated groups showed a trend of increase and subsequent saturation of temperature.

FIG. 3: Photography of the seeded plates before (control, group A) and after application of the plasma (groups B, C, D, and E)

FIG. 4: *C. albicans* colony reduction rate as a function of the plasma exposure time

FIG. 5: Temperature behavior in the substrate during and at the end of the application of plasma samples of the contaminated groups in the silicone surface.

Figure 6 presents the optical spectra of the argon and argon/water vapor plasma jet in the wavelength region of 200–400 nm. In Fig. 6a, for pure argon plasma, besides Ar I spectral lines at the visible region (not shown here) and bands that belong to the second positive system of nitrogen [$N_2(C^3\Pi_u \rightarrow B^3\Pi_g)$], the presence of the OH band [$OH(A^2\Sigma^+ \rightarrow X^2\Pi)$] in the emission spectra was also observed. With the insertion of water vapor, a drastic increase of the OH band was observed (see Fig. 6b).

Figure 7 illustrates the SEM images of the surface of the contaminated silicone after

FIG. 6: Optical spectrum in the UV range for the following conditions: (a) pure argon at 5 L/min and (b) water vapor with argon at 5 L/min. The main species of nitrogen and water vapor were identified. UV, ultraviolet.

plasma treatment.

Figure 8 shows the FT-IR spectra of the surface of the noncontaminated silicone substrate after application of the argon/water vapor plasma jet. A broad absorption peak appeared at around 3400 cm^{-1} , indicating hydroxyl groups.¹³ Absorption bands at a wavenumber range of $2750\text{--}3000\text{ cm}^{-1}$ are attributed to the vibration mode of the C–Hn stretching.¹⁴ At 1411 cm^{-1} , there is an absorption band characteristic of the deformation of the methyl groups. The strong absorption band at 1256 cm^{-1} is attributed to the vibration mode of the –Si–CH₃ stretching.¹⁵ The strong absorption bands at 1072 and

FIG. 7: Micrographs of the contaminated silicone substrate surface after application of the water vapor plasma jet. (a) Group B (exposed to 2 min). (b) Group C (exposed to 5 min). (c) Group D (exposed to 10 min). (d) Group E (exposed to 20 min).

1013 cm^{-1} are attributed to the vibration mode of the Si–O–Si stretching. The absorption band at 782 cm^{-1} is assigned to the vibration mode of the asymmetric Si–O–Si stretching, while the absorption band at 689 cm^{-1} is related to the deformation of the Si–O–Si bonding.¹⁶

IV. DISCUSSION

The results presented in Fig. 4 corroborate the work of Maisch et al.,¹⁷ who studied the application of atmospheric plasma at room temperature for inactivating *C. albicans* biofilm without thermally damaging heat-sensitive materials. They verified that the reduction of fungal counts on polyurethane substrate is correlated with higher plasma exposure time. The explanation is that the longer the samples remain in contact with the plasma, the greater the concentration and action of reactive species on the organic material.¹⁷ Another study compared the effects of plasma action on fungi and Gram-negative and Gram-positive bacteria and found that effectiveness was related to biocidal effects. Nishime¹⁸ observed that for efficient plasma sterilization of a polymeric sample contaminated with fungus (*C. albicans*), almost twice the exposure time is required in comparison to a sample contaminated with bacteria (*Pseudomonas aeruginosa*). The justification was based on the structural external morphological presentation of fungi. The thickness of the external cell wall is thick, establishing a higher stiffness in relation to the bacteria.¹⁸

Figure 4 also shows that the first 2 min of the plasma exposure reduced the *C. albicans* colony in 42.4%. If this value accompanies the same ratio per minute, there would

FIG. 8: FT-IR spectra of the surface of the noncontaminated silicone substrate after application of the water vapor plasma jet. FT-IR, Fourier transform infrared spectroscopy.

be a total expected time to inactivate *C. albicans* in < 5 min, a fact not observed experimentally. An explanation would be the process of chemical saturation of the plasma in a given time. To maintain an increasing action of reactive species from the plasma on the contaminated substrate, it would be necessary to change the parameters of the system during the process (as the discharge voltage and/or current). However, this promotes an increase in the temperature on the substrate, probably hindering its use in heat-sensitive substrates.

Figure 5 presents results of temperature monitoring of the substrate during the sterilization process. As can be seen, the maximum temperature obtained after 20 min of treatment was around 310 K. In general, we observed that a saturation of the substrate

temperature begins to occur only after 10 min of treatment. From the point of view of CFU reduction rate, group E responded better to the treatment due to higher plasma exposure time.

Chemical analysis of the plasma jet was performed with the OES technique. Figures 6a and 6b show the OES spectra in UV range for the plasma jet operated only with argon gas and argon plus water vapor, respectively. The insertion of water vapor in the argon plasma jet causes a drastic increase in the intensity of the hydroxyl (OH) group (up to 2 orders of magnitude), thereby allowing us to infer the active participation of these species in the process of reducing the CFU rate of *C. albicans* sedimented in silicone. Indeed, despite a very short lifetime, OH is one of the most important species in the plasma sterilization process.¹⁹ Moreover, Chen et al.²⁰ also showed that the increase in OH concentration is directly related to the formation of high oxidant H_2O_2 species.

Figure 7 presents SEM micrographs of *C. albicans* cells on a silicone surface for control and plasma-treated samples. The components of *C. albicans* present on the silicone surface, treated for 2 and 5 min, respectively, suffered minimal external changes while maintaining their cell structures with a natural shape (i.e., oval). On the other hand, groups D and E underwent large changes, with a “wilted, swollen, and/or deformed” appearance, possibly due to the oxidation, degradation, or rupture of the cell wall. Cell nonconformities can be related to the action of the hydroxyl radical on protein linkages in the cell outer membrane.²¹ Consequently, extravasation of the cytoplasmic content to the extracellular medium occurs.

In the search for details about the mechanisms of action of OH radicals, Khosravian et al.²¹ conducted an experiment that simulated the molecular dynamics on the microorganism *Staphylococcus epidermidis*. Strong evidence was found on the action of the OH on the cell membrane, breaking the protein bonds of the polysaccharide rings. Maia and Bicudo²² and Yost and Joshi²³ also studied the action of the OH radical as being the main element of the reactive oxygen species (ROS) group responsible for cell inactivation. This radical causes oxidative stress by breaking down binding of membrane compounds, leading to irreversible changes in the microorganism. In addition, oxidized molecules can also combine with the genetic material of the microorganism and induce additional damage.²³ Other issues related to ROS, such as their biocidal mechanisms of action, still need to be elucidated.²³

Finally, to determine whether the argon/water vapor plasma sterilization technique is effective for treating the silicone substrate, it is important to ensure that their morphologic and chemical characteristics remain intact. Experiments were performed on the uncontaminated substrate for different treatment times studied in this work. SEM micrographs indicate that the surface morphology changed after 20 min of treatment, demonstrating that the chemical species generated by the plasma caused some corrosion of the silicone material surface (Fig. 7). Chemical analysis of the substrate surface by the FT-IR technique (Fig. 8a) corroborated the SEM results, showing that a slight change in the intensity of the chemical bonds Si–O–Si and –Si–CH₃ occurs for the 20-min condition. Moreover, the formation of C–Hn bonds was observed (Fig. 8b). This observation

demonstrates the high reactivity of the argon/water plasma jet on silicon surface during long exposure time, since, although it did not promote a significant surface warming (approximately 36–38°C, Fig. 5), it caused an effect of chemical corrosion on the surface of the material.

V. CONCLUSION

This work investigated the influence of the exposure time of an argon/water vapor plasma jet in the sterilization of a silicone substrate surface contaminated with *C. albicans*. The treatment was carried out in the postdischarge region and proved to be an interesting alternative to sterilization techniques, due to the fast inactivation process and the low temperature increase of the substrate material. This last issue is relevant when the treated surface is temperature sensitive, and it is worth noting that the temperature increase of the silicone during treatment was ≤ 310 K. A point to be improved is the corrosion effect of material that occurs when higher time periods are required.

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