

RESEARCH

Plasma Sterilization of Cotton Fabrics

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ABSTRACT

The search for effective methods of sterilizing materials in hospital environments is crucial for preventing infections. Oxygen plasma has emerged as a promising alternative to autoclaving due to its potential to reduce sterilization time and improve the efficacy of the process. The present study aimed to assess the effects of exposing cotton tissues to oxygen plasma on inhibiting the growth of *Candida albicans*, aiming to contribute to a broader understanding of the applicability of this sterilization technique. Cotton tissues were exposed to oxygen plasma for varying periods of time. Morphological analysis and energy-dispersive X-ray spectroscopy (EDX) were conducted to assess potential changes in the structure and chemical composition of the fibers after plasma treatment. The results showed a significant reduction in the growth of *Candida albicans* colonies on tissues exposed to plasma, with greater efficacy observed in samples exposed for 10 minutes. EDX analysis indicated that plasma did not cause changes in the chemical composition of cotton fibers. However, morphological analysis by scanning electron microscopy revealed a direct relationship between the exposure time to plasma and the degree of destruction of the waxy cuticle of the cotton. Exposure to plasma resulted in a significant reduction in fungal growth without causing changes in the chemical composition of cotton fibers.

KEYWORDS

Oxygen; Plasma Enhanced Chemical Vapour Deposition; *Candida albicans*; cotton fabric; sterilization.

INTRODUCTION

The relentless demands of hospital environments for effective and efficient sterilization methods have driven the search for alternatives to traditional methods like autoclaving [1]. In this context, plasmas emerge as a promising solution, not only for the energy-saving benefits they provide but also for their potential to significantly reduce the time required for sterilization [2]. Concerns about energy efficiency are increasingly relevant in a world where sustainability is a growing priority [3]. Furthermore, plasmas have proven effective in eliminating a variety of microorganisms, including fungi like *C. albicans*, making them a versatile and reliable option for sterilizing a wide range of materials such as precision glassware, plastics, and

textiles [4]. This microorganism can persist in hospital environments for extended periods, especially on porous surfaces like fabrics and textiles [5]. This means that even after patient removal or superficial facility cleaning, *C. albicans* spores remain viable and pose a continuous risk of infection, especially in ICUs [6].

While autoclaves are classically recognized for their effectiveness in sterilizing materials, they have some limitations [7]. Sterilization cycles require considerable time, resulting in delays in emergency medical procedures that require sterile materials [8]. Additionally, thermosensitive materials such as plastics and precision glassware tend to deform when exposed to the high temperatures of autoclaves [9]. In contrast, plasma

processes have demonstrated the ability to sterilize in less time without compromising the integrity of materials, regardless of their nature [1].

In addition to conventional plasmas, low-pressure plasmas have also emerged as a promising alternative for material sterilization [10]. Operating under vacuum conditions, these plasmas offer greater precision in controlling ionization and molecular excitation processes, resulting in more effective and uniform sterilization [11]. Furthermore, low-pressure plasmas allow for the modulation of parameters such as electron density and plasma temperature, providing greater flexibility in adapting to different types of materials and microorganisms to be sterilized [12-13].

Different gases can be used to generate plasma; however, oxygen emerges as potentially the most effective in sterilization due to its well-known "etching" effect reported in the literature [13]. This phenomenon occurs due to the reaction of oxygen plasma with the surface of materials, resulting in the removal of small amounts of material through oxidation and volatilization processes [14-15].

Although "etching" may be undesirable in some applications, such as in the semiconductor industry where dimensional accuracy is crucial, it can be beneficial in the sterilization of textile materials by affecting the structure of the external membrane and cell wall of microorganisms [14].

In this regard, the present study advocates for investigating the effects of low-pressure oxygen plasma on the sterilization of cotton tissues contaminated with *C. albicans* [15].

MATERIALS AND METHODS

The experiment began with the preparation of 30 samples of raw cotton tissues ($1.5 \text{ cm}^2 / 1 \text{ mm}$), distributed into two groups of 15 elements each, one being the control group and the other the experimental group. The samples were cleaned with neutral detergent and water, and then dried for one hour at 50°C in a Sterilifer - SX 1.0 DTME incubator (Fig. 1).

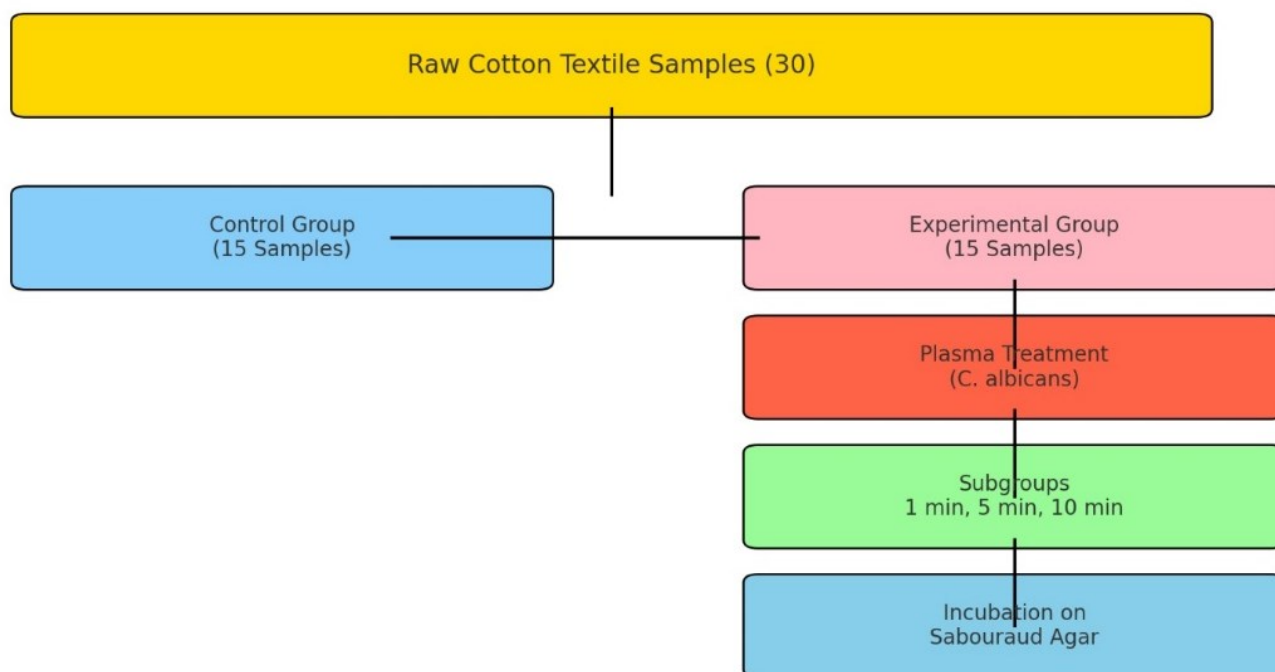


Fig 1. Flowchart of the experiment with cotton textile samples divided into control and experimental groups. The experimental group was treated with plasma after exposure to *C. albicans* and subdivided into subgroups with different exposure times.

The experimental group was immersed for 1 minute in 5 mL of Sabouraud dextrose broth (DIFCO), contaminated with 10^6 CFU/mL of *C. albicans*, and then subjected to oxygen plasma (99%) confined in a vacuum chamber, at a pressure of 2 mBarr, 420 V, and 0.03A. The samples were placed on the cathode of the reactor, immersed in the plasma sheath. For comparative purposes, both the control and experimental groups were subdivided into 3 groups of 5 elements each, according to the times they were exposed to the plasma: 1 minute, 5 minutes, and 10 minutes.

At the end of this process, the samples were immediately placed on 60 x 15 mm Petri dishes containing 5 ml of Sabouraud agar culture medium and then incubated for 48 hours at 37°C . This test provided qualitative results, being discriminated as negative when no colonies were formed (0% of the medium covered by fungi) and positive when fungi proliferated completely (100%) across the entire area of the Petri dishes. Intermediate results were calculated using IMAGE J software, considering the percentage of the medium covered by fungi.

Morphological analyses of the samples were conducted using a scanning electron microscope (SEM) (Inspect F50), which produced secondary electron emission images with an acceleration voltage of 3 kV. Changes in the chemical composition of the tissues were evaluated by energy-dispersive X-ray spectroscopy (EDX).

The collected data were reported as arithmetic means $\pm$ standard deviation. Statistical analysis was performed using Microsoft Excel 365 (Microsoft Corporation, Redmond, WA, USA) to evaluate the relationship between plasma exposure duration and the reduction in *Candida albicans* colony growth (Fig. 2).

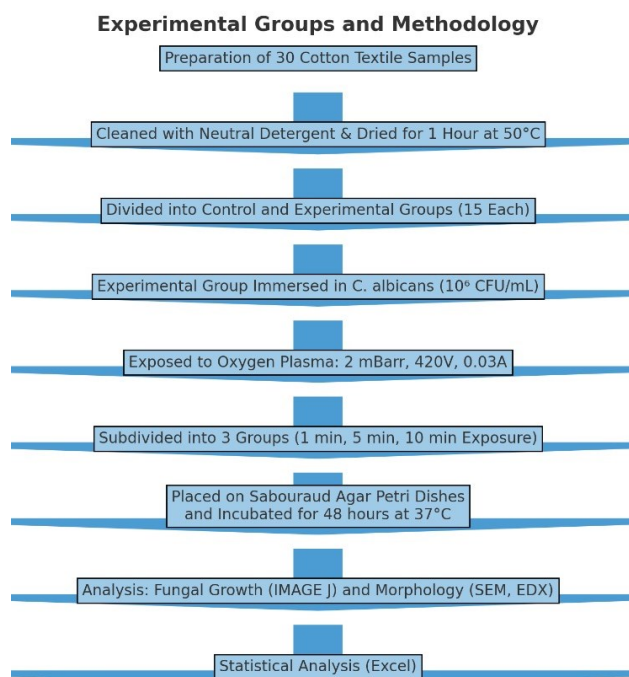


Fig 2. Schematic diagram of the experimental group and methodology applied to evaluate fungal growth on cotton textile samples after exposure to oxygen plasma. The process involves sample preparation, division into control and experimental groups, exposure to plasma at different times, and analysis of fungal growth and morphology using specific techniques.

RESULTS

The image illustrates the antimicrobial effects of plasma treatment on different groups of fabric samples, with exposure times of 1, 5, and 10 minutes. In the **1-minute group (A)**, fungal colony growth is substantial, behaving similarly to the control group and showing no signs of inhibition, indicating that the plasma exposure for this period was insufficient to produce any antimicrobial effect. In the **5-minute group (B)**, there is a noticeable reduction in colony growth compared to the 1-minute and control groups, although fungal development is still present, suggesting that 5 minutes of exposure had a moderate inhibitory effect. Finally, in the **10-minute group (C)**, fungal growth is significantly reduced, with very few colonies present, demonstrating a considerably stronger antimicrobial effect. The growth profile graph below the

images reinforces this trend, showing a substantial decrease in microbial growth as plasma exposure time increases, with the strongest antifungal effect observed in the group treated for 10 minutes (Fig. 3).

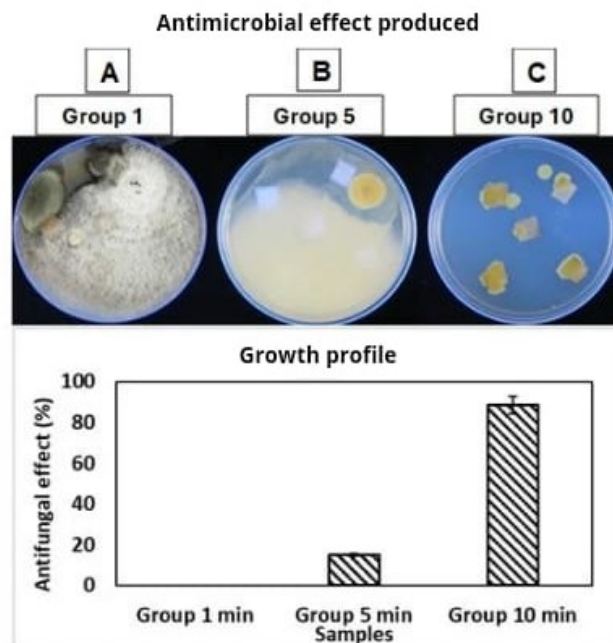


Fig. 3. Antimicrobial effect produced by argon plasma jet treatment: representative image of the control group and the 1-minute group (Group 1 Min); image of the 5-minute group (Group 5 Min); image of the 10-minute group (Group 10 Min); growth profile of the antimicrobial effect, as a function of exposure time to the plasma jet.

This reduction in the extent of fungal growth was particularly pronounced in the group exposed for 10 minutes, registering a decrease of approximately 88% compared to the control group. In turn, the group exposed for 5 minutes showed a reduction of about 15% in the extent of fungal growth. Such results suggest that the exposure period to plasma has a significant impact on inhibiting the growth of fungi in cotton tissues contaminated with *Candida albicans*.

It's worth noting that the absence of results indicating complete colony inhibition suggests that plasma treatment is not capable of completely sterilizing tissue samples. However, the reduction in fungal growth extent observed in the groups exposed for 5 and 10 minutes suggests that plasma treatment presents an inhibitory effect on fungal growth in cotton issues.

The presented image displays a scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX) analysis, representing different experimental groups. Image A corresponds to the representative spectrum of the triplicate control group, which was not treated with plasma. The fibrous structure of the samples remains intact, with no significant visual alterations, and the EDX spectrum indicates the predominance of carbon (C) and oxygen (O), with a slight trace of copper (Cu). Carbon constitutes approximately

94.6% of the sample, reflecting its organic nature. Image B refers to the group exposed to plasma for 1 minute, where the fibers maintain a structure similar to the control, still with a regular intertwining. The EDX spectrum for this group continues to show high levels of carbon (80.3%) and oxygen (68.3%), suggesting that the short treatment period did not result in significant chemical changes. Image C represents the group treated with plasma for 5 minutes. At this point, slight alterations in the surface structure of the fibers are observable, and the EDX spectrum shows the continued predominance of carbon (85%) and oxygen (76.3%), with a slight trace of copper. Finally, image D represents the group exposed to plasma for 10 minutes, where structural modifications are more pronounced, with greater opening of the fiber cuticles. The EDX spectrum reveals carbon (83.3%) and oxygen (18.4%), along with a significant increase in copper presence (12%), which may be related to contamination or the prolonged treatment duration. These results indicate that plasma exposure progressively affects the morphology of the fibers, with the most visible changes occurring in the group exposed for 10 minutes. However, the elemental composition of the samples remains largely unchanged, with carbon and oxygen consistently present across all analyzed groups (Fig. 4).

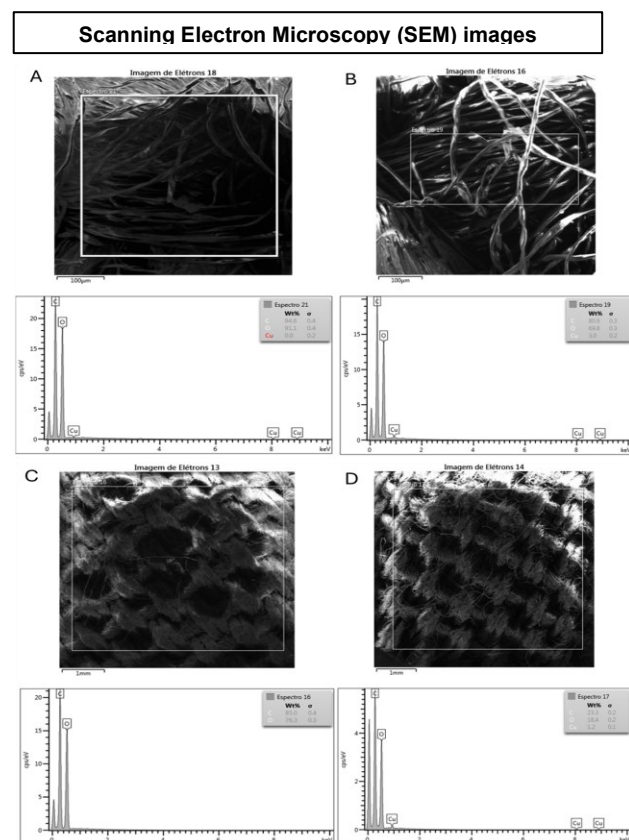


Fig. 4. Scanning electron microscopy (SEM) images of cotton samples and corresponding EDX spectra. (A) Control group, (B) sample treated with plasma for 1 minute, (C) sample treated for 5 minutes, and (D) sample treated for 10 minutes.

The detailed morphological analysis of cotton fibers, conducted at 5,000 times magnification, allowed for a meticulous observation of the structural changes occurring in response to plasma exposure. Upon examining the samples, it became evident that the duration of plasma exposure played a pivotal role in modifying the structure of the cotton fiber cuticle. Initially, when comparing fibers from the control group to those exposed for 1 minute, no significant differences in morphology were observed. Both groups exhibited a similar morphological configuration, characterized by the presence of waxy cuticles only around the fibers [16]. However, upon extending the exposure time to 5 minutes, it was noted that a portion of the cuticles was open in some fibers. This partial opening of the cuticles indicated an onset of modification in fiber structure in response to plasma treatment. Further increasing the exposure time to 10 minutes resulted in a significant intensification of cuticle opening in cotton fibers. Most fibers in the 10-minute exposure group exhibited open cuticles to a greater extent, suggesting a more pronounced alteration in fiber surface structure. These observations indicate a direct relationship between plasma exposure time and the degree of modification in cotton fiber structure (Fig. 5).

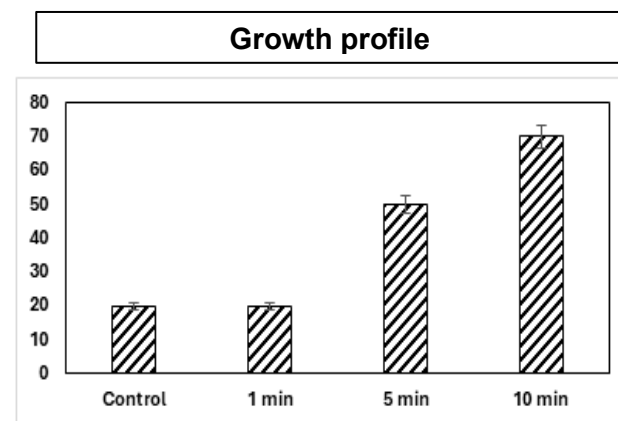


Fig. 5. Growth profile of the average roughness of the samples.

The results of contact angles measured by goniometry revealed significant variations among the different samples. It was observed that the control group exhibited an average contact angle of approximately 142.6 degrees, with a small margin of error of ± 3.1 degrees. In contrast, in samples treated for 1 minute and 5 minutes, contact angles were recorded around 143.2 and 132.2 degrees, respectively, with similar variations of ± 3.1 and ± 4.3 degrees. However, upon analyzing the sample treated for 10 minutes, a significant reduction in the contact angle was observed, reaching only 88.9 degrees, with a margin of error of ± 3.2 degrees. This substantial decrease in the contact angle suggests a change in the surface of cotton fibers after prolonged treatment, indicating possible alterations in their hydrophilic/hydrophobic properties.

These results highlight the sensitivity of cotton fibers to treatment conditions and the importance of understanding how these changes may affect their properties and future applications (**Fig. 6**).

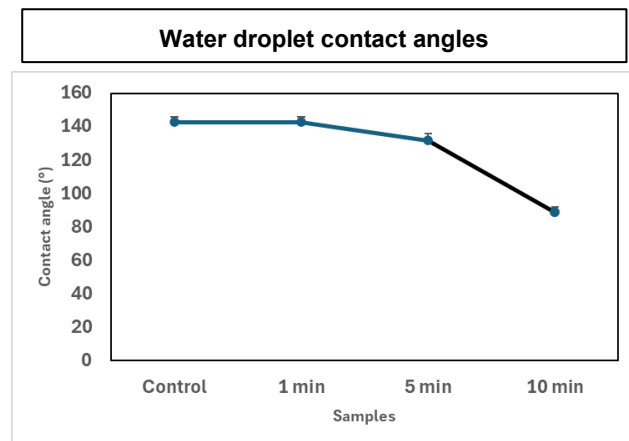


Fig. 6. Water droplet contact angles measured in all experimental groups. Standard deviations ranged from -4.3° to $+4.3^{\circ}$.

The scanning electron microscopy (SEM) images show the structural changes in cotton fibers after exposure to plasma at various magnifications. The top row presents the overall fibrous structure at lower magnifications, illustrating the arrangement of fibers in different groups. Images [A] and [B] depict representative samples, including the control, at magnifications of $500\ \mu\text{m}$ and $400\ \mu\text{m}$, respectively, where the fibers maintain their intertwined structure with no significant alterations visible. In the bottom row, higher magnifications reveal more detailed changes. In [C], at $10\ \mu\text{m}$ magnification, initial changes in the fiber cuticle begin to appear, such as roughness and slight opening in samples exposed to plasma. Images [D] and [E], representing samples treated for 10 minutes at magnifications of $10\ \mu\text{m}$ and $1\ \mu\text{m}$, respectively, show significant surface alterations, including cracks and the formation of pores in the fiber cuticle. These findings highlight the progressive impact of plasma exposure, with more pronounced structural modifications evident after prolonged exposure times (**Fig. 7**).

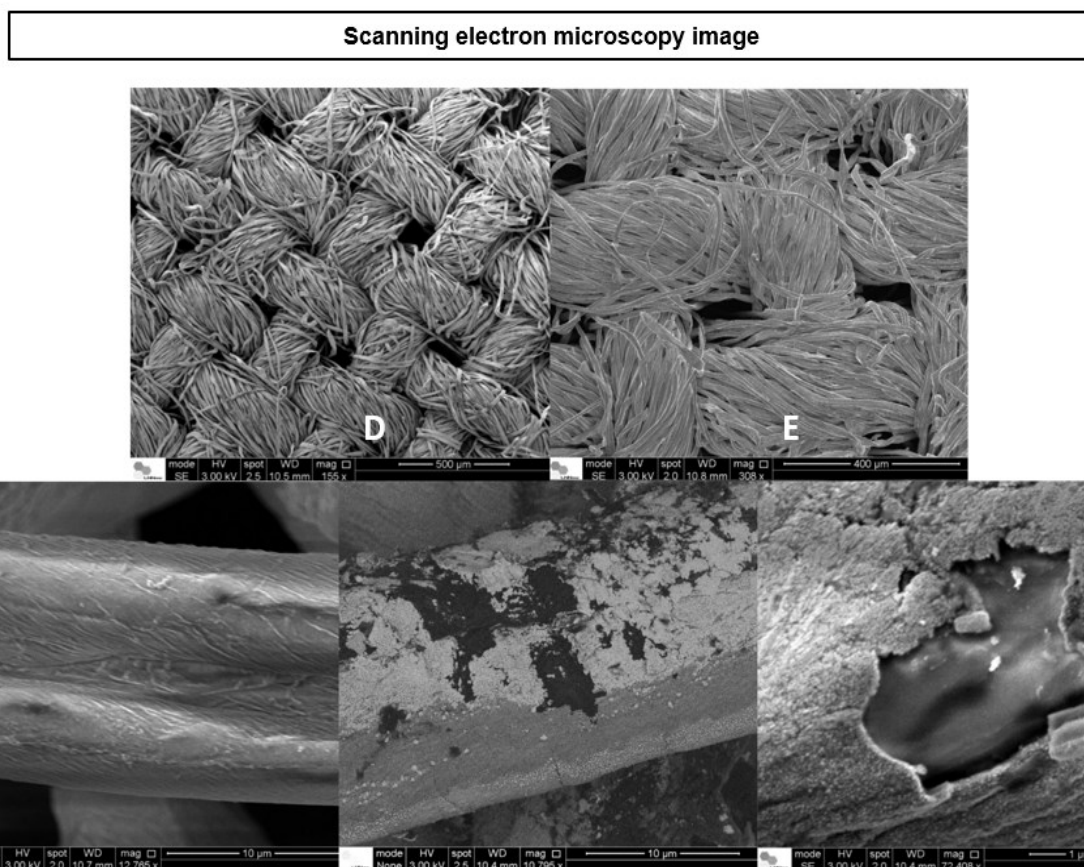


Fig. 7. Scanning electron microscopy image, with different degrees of magnification, of the control groups and elements exposed for 1 min, 5 min, and 10 min to oxygen plasma: [A] Representative sample of all groups, including the control, at $166\times$ magnification; [B] Representative sample of all groups, including the control, at $306\times$ magnification; [C] Representative sample of all groups, including the control, at $10.7\times$ magnification; [D] Representative sample of elements from the group exposed for 10 minutes to oxygen plasma; [E] Representative sample of elements from the group exposed for 10 minutes to oxygen plasma at $72\times$ magnification.

DISCUSSION

The search for effective sterilization methods for materials has been a crucial topic, especially in hospital environments where infection prevention is of paramount importance. Recently, the use of plasmas has emerged as a promising alternative to autoclaving, offering the potential to significantly reduce sterilization time and improve process effectiveness. In the present study, the effects of exposing cotton fabrics to oxygen plasma on inhibiting the growth of *Candida albicans* were evaluated, contributing to a broader understanding of this technique's applicability.

The results demonstrated that the exposure of cotton fabrics to oxygen plasma resulted in a significant reduction in the growth of *Candida albicans* colonies, with this reduction being more pronounced in samples exposed for 10 minutes, followed by those exposed for 5 minutes. These findings are consistent with previous studies suggesting the effectiveness of plasma in inhibiting fungal growth on textile materials [17].

Although autoclaving is effective in sterilization, its application is limited by material issues, making it unsuitable for delicate materials [18]. Previous studies have shown that oxygen plasma can achieve comparable levels of microbial growth inhibition without the disadvantages associated with high temperatures and is significantly faster [19]. In this study, an 88% reduction in *Candida albicans* growth was observed after 10 minutes of exposure to oxygen plasma, while conventional autoclaving methods generally require cycles of 30 to 60 minutes [20]. This suggests that oxygen plasma not only offers reduced sterilization time but also avoids potential damage to materials that may occur with autoclaving [21].

Moreover, conventional plasma methods, such as those based on gases like argon and nitrogen, have demonstrated efficacy in sterilizing surfaces and materials [22]. However, the literature highlights that oxygen plasma stands out for its ability to perform a process known as *etching*, where surfaces are "polished" by the plasma's action [23-24]. This phenomenon is particularly useful in textile disinfection, as oxygen plasma interacts with the cell wall and outer membrane of microorganisms such as *Candida albicans*, removing or damaging these protective barriers more effectively than other types of plasma [25]. Indeed, exposure to oxygen plasma for 10 minutes resulted in visible structural changes to the cotton fibers without compromising the chemical integrity of the fibers, something not observed to the same extent with other gas plasmas [26].

When comparing these results with those found in the literature, possible scenarios can be speculated. For example, some studies suggest that prolonged exposure to oxygen plasma can lead to structural damage to microorganisms, interfering with their growth and reproduction [15, 27-28], which is consistent with the findings observed in the present study, where 10-minute

exposure resulted in a more pronounced reduction in fungal growth.

However, it is important to consider that even with prolonged plasma exposure, complete inhibition of *Candida albicans* colony growth was not observed. This suggests that plasma treatment may not be fully effective in sterilizing textile samples. This observation aligns with the literature, which reports difficulty in achieving total sterilization due to the presence of biofilms or the resistance of certain microorganisms to antimicrobial agents [29].

Another aspect to consider is the possible *etching* effect of oxygen plasma on the surface of textile materials. Previous studies suggest that *etching* may increase the porosity of the material, facilitating plasma penetration and enhancing the effectiveness of the sterilization process [27]. This speculation highlights the importance of investigating not only the direct effects of plasma on microorganisms but also its impact on the structure and properties of the treated materials.

The EDX analysis revealed the predominant presence of carbon (C) and oxygen (O) in the samples, with traces of copper (Cu). Copper, known for its antimicrobial properties, has been widely reported in the literature as an effective agent in inhibiting pathogens [28-29]. However, in this study, the concentrations of copper detected did not exceed trace levels, suggesting that its contribution to the antimicrobial effect observed, especially in the group exposed for 10 minutes, was minimal. Furthermore, it is possible that the low levels of copper detected represent an artifact of the analysis, where the equipment may have identified the observed band as copper due to the absence of a more precise match. Previous studies investigating the antimicrobial effect of copper have reported significantly higher concentrations, with substantial deposits in the samples, which differs significantly from the traces detected here [30]. Thus, it is reasonable to conclude that these traces of copper were not responsible for the antimicrobial effect observed in this study, reinforcing the hypothesis that the primary mechanism of microbial inhibition was caused by the action of oxygen plasma and the *etching* process on the fibers.

When compared with previous studies, our results reinforce the idea that prolonged exposure to oxygen plasma can cause significant structural damage to microorganisms, interfering with their ability to grow and reproduce [15, 27-28]. However, even with prolonged exposure, complete inhibition of *Candida albicans* colony growth was not observed. This suggests that plasma treatment may not be entirely effective in sterilizing textile samples, which is consistent with the literature, which reports difficulty in achieving total sterilization due to the presence of biofilms or the resistance of certain microorganisms to antimicrobial agents [29].

Another aspect to consider is the impact of the *etching* process on the surface of textile materials. Previous studies suggest that *etching* can increase the material's porosity, facilitating plasma penetration and enhancing the

effectiveness of the sterilization process [27]. This effect was observed in cotton fibers, where the opening of the cuticle may have provided greater contact between the plasma and the microorganisms, increasing fungal growth inhibition [31].

In summary, the results of this study suggest that oxygen plasma treatment is an effective approach to reducing *Candida albicans* growth on cotton fabrics, with structural modifications that enhance the antimicrobial action without compromising the material's chemical integrity. The *etching* process and the generation of reactive oxygen species are the central mechanisms explaining the observed antimicrobial effect, while the copper traces detected likely did not contribute significantly to the final result. This highlights the potential of oxygen plasma as a viable alternative for sterilizing delicate fabrics in hospital settings.

CONCLUSION

The conclusions of this study indicate that the exposure of cotton fabrics to oxygen plasma under vacuum conditions significantly reduced the growth of *Candida albicans* colonies, especially in samples exposed for 10 minutes. These results confirm the efficacy of plasma in inhibiting fungal growth, suggesting its potential as an alternative sterilization method in hospital settings.

Morphological analysis revealed that plasma altered the structure of the fiber cuticles, with a progressive opening observed as exposure time increased. This structural alteration may potentially facilitate antimicrobial treatment, despite the EDX indicating that no significant chemical changes occurred in the fibers. These data suggest that plasma primarily impacts the surface structure of the fibers without modifying their chemical composition.

Although plasma considerably reduced the growth of *Candida albicans*, complete sterilization was not achieved, suggesting that biofilms or more resistant microorganisms may not be fully eliminated by this method alone. Therefore, further research is needed to optimize plasma treatment, either by adjusting variables such as pressure and voltage or by combining plasma with other antimicrobial agents or treatment cycles.

This study demonstrates the potential of oxygen plasma as an effective alternative to autoclaving, particularly by reducing treatment time and preserving fabric integrity. However, the challenge of fully eliminating resistant microorganisms highlights the importance of continuing to investigate this technology to maximize its efficacy in hospital environments.

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AUTHOR CONTRIBUTIONS

Everton dos Santos: Conceptualization, Software, Methodology, Funding acquisition, Writing- Original draft preparation.

Leticia Mello: Investigation, Data curation, Visualization, Writing- Reviewing and Editing.

Victória Colasso: Project administration, Supervision, Validation, Writing- Reviewing and Editing.

Giulia da Silva: Formal analysis, Investigation, Data Curation, Writing- Original draft preparation.

Elisa Esposito: Resources, Supervision, Validation, Data curation.

Rogério Mota: Resources, Supervision. Project administration, Validation.

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