



SPECIAL ISSUE RESEARCH ARTICLE

Photodynamic therapy as a potential approach for preventing fungal spread associated with the use of endotracheal tubes

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Abstract

Fungal infections related to biofilm formation on medical devices, such as endotracheal tubes (ETTs), pose significant health risks, especially during intubation procedures where fungi like *Candida* spp. can migrate into the lower respiratory tract. This study explores the use of Photodynamic Therapy (PDT) to prevent fungal cell migration from ETT surfaces to lungs, focusing on the role of curcumin as a photosensitizer. ETTs were coated with varying concentrations of curcumin, and biofilm formation was measured after applying PDT with a 50 J/cm² irradiation dose. The study found that ETTs functionalized with a one-third concentration of CUR reduced biofilm formation by 1.78 Log, significantly lowering microbial load and potentially decreasing hospital-acquired infections. Confocal fluorescence microscopy confirmed that PDT damaged the biofilm's extracellular matrix and caused detachment of dead fungal cells. Moreover, the fluorescence analysis reveals the photodegradation behavior of the photosensitizer within the tube, providing critical insights into its stability and durability, which are essential for evaluating the long-term applicability of these tubes in clinical settings. These results suggest PDT as a promising strategy to reduce fungal infections in high-risk patients, offering potential for future clinical application in preventing device-associated infections.

KEYWORDS

endotracheal tube, fungal infection, photodynamic therapy, photosensitizer

INTRODUCTION

The invasion of the bloodstream and vital organs by fungal pathogens, along with their ability to form resilient

biofilms on medical devices such as intravenous catheters and ETTs, represents a significant challenge in contemporary medicine.¹ These fungal biofilms, primarily formed by species like *Candida* and *Aspergillus*, are a

Abbreviations: CFU, colony-forming unit; CUR, curcumin; DMSO, dimethyl sulfoxide; ETT, endotracheal tube; PDT, photodynamic therapy; PVC, polyvinyl chloride; ROS, reactive oxygen species; UV-vis, ultraviolet-visible; VAP, ventilator associated pneumonia.

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major source of severe medical complications, including systemic infections that can escalate to life-threatening conditions.^{2–4}

The rapid proliferation of fungi upon entry into the bloodstream highlights the urgent need for effective interventions.⁵ The complexities associated with endotracheal intubation further increase these risks, leading to conditions such as fungemia, fungal pneumonia, and acute respiratory failure, particularly in post-surgical settings.^{6,7} Patients with prolonged intubation and compromised immunity are especially vulnerable to oral fungal colonization, which further elevates the risk of severe health complications.⁸ The migration of fungi from ETTs to the lower airways, facilitated by mechanical ventilation or the aspiration of secretions, is a critical pathway for respiratory infections.^{2,9} Moreover, the persistent presence of ETTs can compromise the integrity of the tracheal mucosa, inadvertently creating pathways for fungal infiltration beyond the upper respiratory tract, with *Candida albicans* frequently implicated in the onset of ventilator-associated pneumonia (VAP). Given this backdrop, there is a pressing need for robust preventive measures in endotracheal intubation practices, including stringent hygiene and sterilization protocols to mitigate the risks of device-associated infections.¹⁰ Early detection and vigilant monitoring for signs of fungal invasion are essential for enabling timely and appropriate therapeutic interventions.

In this context, PDT emerges as a promising therapeutic approach, utilizing the specificity of photosensitizers to absorb light at wavelengths, thereby targeting and neutralizing pathogenic cells, including fungi.¹¹ This study explores the application of PDT in conjunction with CUR-functionalized ETTs, aiming to prevent the formation of *C. albicans* biofilms and, consequently, reduce the incidence of VAP.

This investigation builds upon prior research,¹² which has shown that the incorporation of materials such as copper, zinc, essential oils, and antimicrobial polymers into medical devices (e.g., ETTs, catheters, or implant surfaces) enhances their effectiveness against microorganism proliferation through techniques such as coating, impregnation, and deposition.¹³ In the realm of material functionalization, coating¹⁴ or chemically bonding photosensitizers¹⁵ onto materials can yield devices that are highly effective against microorganisms when exposed to appropriate light sources, potentially preventing biofilm formation and mitigating device-associated infections.¹⁶

This study hypothesizes that the application of PDT to CUR-functionalized ETTs will effectively prevent the

formation of *C. albicans* biofilms, thereby reducing the risk of VAP.

This article is organized as follows: the materials and methods section detail the functionalization of ETTs and the application of PDT. The results section discusses the efficacy of this approach in preventing biofilms and reducing the incidence of VAP, followed by an analysis of the clinical implications and future research directions.

Therefore, this study aims to address a critical gap in the literature, demonstrating not only the efficacy of PDT against *C. albicans* adherence and proliferation on polyvinyl chloride (PVC) tubes but also highlighting the potential clinical application of this approach in a hospital setting, considering the durability and response of the material to light. The integration of PDT with CUR-functionalized ETTs represents an innovative strategy in the ongoing battle against fungal biofilms and their associated healthcare challenges, marking significant progress toward improving patient safety and outcomes in the context of hospital-acquired infections. The findings extend beyond the efficacy of PDT, suggesting a potential paradigm shift in preventing device-associated fungal infections and contributing to broader infection prevention strategies in clinical settings.

MATERIALS AND METHODS

Functionalization process

The functionalization process initially described and established by Zangirolami et al. (2020), involved immersing a PVC based ETT in a solution containing CUR and cesium carbonate (Cs_2CO_3) in dimethyl sulfoxide (DMSO) for 4 h at 30°C under continuous agitation. Following the immersion, the tube was thoroughly washed in DMSO for 40 min and subsequently in absolute ethanol for another 40 min, both under constant agitation. Table 1 outlines the concentrations of each sample, and the corresponding rates of reagents used throughout the functionalization process.

TABLE 1 Reagent concentrations in the functionalization process for each sample employed in 250 mL of DMSO.

Sample	Curcumin (mg/mL)	Cesium carbonate (mg/mL)
TC01	6.25	1.70
TC02	4.17	1.13
TC03	2.5	0.68

Initially, the CUR concentration within the tube was relatively high; therefore, two additional samples with lower CUR concentrations were selected to serve as benchmarks for evaluating efficiency. The amount of CUR used was also based on the previous work by Zangirolami et al.¹⁵ to maintain the proportionality and ensure comparability of the results.

Microorganisms and biofilm formation

Candida albicans strains (ATCC 90028), donated by the Oswaldo Cruz Foundation (FIOCRUZ), were used for biofilm generation. Initially, the strains were cultured on Sabouraud Dextrose Agar (SDA) (Neogem) in Petri dishes for 48 h at 37°C. Ten colonies were then collected and suspended in 10 mL of Brain Heart Infusion Broth (BHI), incubated for 18 h in an orbital shaker at 37°C and 75 rpm. The strains were subsequently washed and subjected to the same conditions for an additional 7 h. The cells were then centrifuged for 10 min at 1500 rpm and washed twice with phosphate-buffered saline (PBS), following the methodology adapted from Romano (2017).¹⁷

The initial cell concentration for biofilm growth was adjusted to an optical density of 0.38 at 530 nm, corresponding to approximately 10^9 cells/mL, with the cells suspended in BHI at a 9:1 ratio (mL). For biofilm development, a 1 cm sample of the tube (Figure 2B) was incubated with 1.5 mL of the cell and BHI suspension in a 24-well plate for 48 h.

The quantification of the biofilm was performed using the Colony Forming Unit (CFU) count method. After biofilm formation, the ETT sample was vortexed for 1 min in 10 mL of PBS. Subsequently, 100 μ L of the suspension was serially diluted and plated on SDA for 48 h to count CFUs. Each plating was performed in triplicate.

Statistical analyses were conducted employing the Mann–Whitney *U* test for non-parametric data with a 95%

confidence interval, given the small sample size (“*n*”) and the non-normal distribution of variance. Outliers were assessed using the q-Dixon test for each group individually with a 95% confidence interval.

Light source

The light source was developed by the Technological Support Laboratory (LAT) of the Institute of Physics at the University of São Paulo (USP) in São Carlos. This setup includes a laser system (Figure 1) with an irradiance of 140 mW/cm² and a peak emission wavelength of 455 nm (blue light), delivered through an optical fiber. The light was directed inside the ETT using a cylindrical diffuser positioned at the distal 2 cm of the optical fiber.

Confocal microscopy

Biofilms were observed using an inverted Zeiss® LSM 780 confocal microscope equipped with a 405 nm Diode laser and an 800 nm laser, employing single and two-photon excitation methods, respectively. The cells were stained with a live/dead dye mixture of ethidium bromide and acridine orange (50%/50%). Biofilm preparation followed the protocol detailed in section ‘Microorganisms and biofilm formation’, using smaller, flat segments of ETT with a 48-h incubation period. Three distinct sample groups were evaluated: a control group without CUR and no light exposure; a CUR dark group, which was functionalized but not exposed to light; and a PDT group, which was functionalized and irradiated with 50 J/cm².

Absorbance

Ultraviolet-visible (UV–vis) spectra were recorded in the range of 300–800 nm using a Varian Cary 50 Bio UV–Vis spectrophotometer. Samples were immersed in dimethyl sulfoxide (DMSO) for 24 h to facilitate the extraction of compounds into an aqueous solution, thereby reducing the high opacity of the solid material and enabling accurate spectroscopic analysis.

Fluorescence

Surface fluorescence spectra were recorded using a Cary Eclipse Fluorescence Spectrometer in emission scan mode, with excitation set at 435 nm and emission recorded between 300 and 600 nm. For three-dimensional

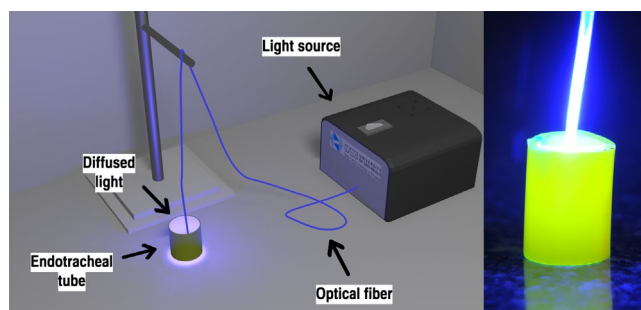


FIGURE 1 Experimental setup of light source and optical fiber illuminating a section of PVC tube alongside corresponding real-time image captured with the functionalized tube.

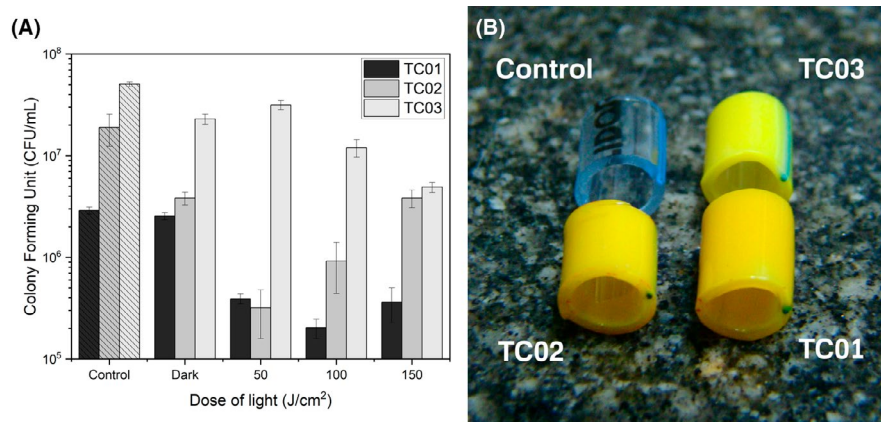


FIGURE 2 (A) Evaluation of photodynamic action of the *Candida albicans* biofilm in three concentrations of curcumin and doses of 50, 100 and 150 J/cm² ($n=6$, error bar = SD, Log₁₀ scale). (B) ETT used with varying concentrations of curcumin.

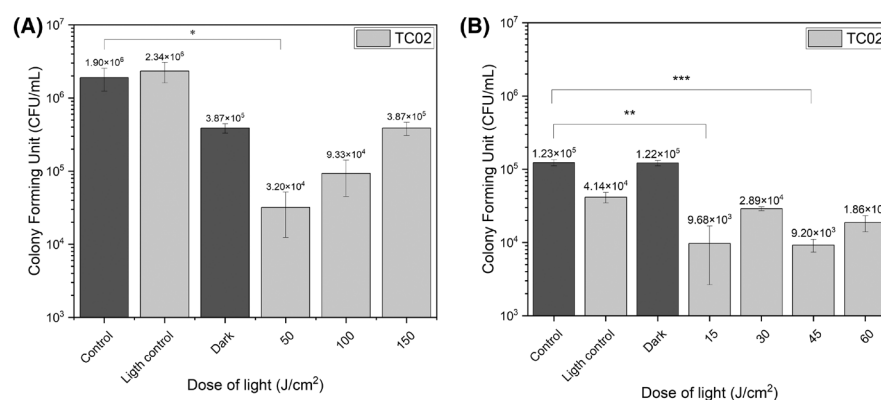


FIGURE 3 Colony Formation Unity of the TC02 sample in some ranges of light doses. * = 1.78 Logs, ** = 1.02 Logs and *** = 1.14 Logs of CFU/mL reduction, control and light control group has no curcumin in its composition, light control group was taken with a dose of 50 J/cm² of light, graph in Log scale ($n=6$, error bar = SD).

fluorescence analysis, excitation ranged from 300 to 500 nm, and emission from 300 to 600 nm. Measurements were obtained directly from the polymer surface using a fiber optic probe.

RESULTS

Microbiological results

A key limiting factor affecting the efficiency of PDT is the internalization of the photosensitizer into microorganisms.¹⁸ This challenge arises due to the relatively short lifetime of certain ROS, particularly singlet oxygen,¹⁹ which are central to the PDT process. In this study, cur is not available for internalization into fungal cells, which complicates the process further, especially in the context of biofilms, where cells are embedded in an extracellular matrix²⁰ that provides enhanced resistance, including the capacity for drug

sequestration.²¹ Consequently, a lower reduction in contamination and the need for higher radiation doses are anticipated. Therefore, the primary aim is to prevent biofilm formation rather than decontamination.

Initially, the effect of PDT was evaluated on ETTs contaminated with *Candida albicans* at three different cur concentrations used during the functionalization process, in addition to a control group without cur. Figure 2 illustrates the quantification of contamination using the colony-forming unit (CFU) count technique following the application of PDT across all groups, with light doses of 50, 100, and 150 J/cm². The results indicate that the TC02 concentration was the most effective against *C. albicans* under these conditions, demonstrated a significant CFU/mL reduction (1.78 log reduction, $p < 0.05$) with a 98.3% mortality rate.

During the functionalization process, the ETT loses its transparency due to the presence of CUR not only on the surface but also internally (Figure 2B). As a photosensitizer, CUR absorbs most of the incident light at 445 nm,

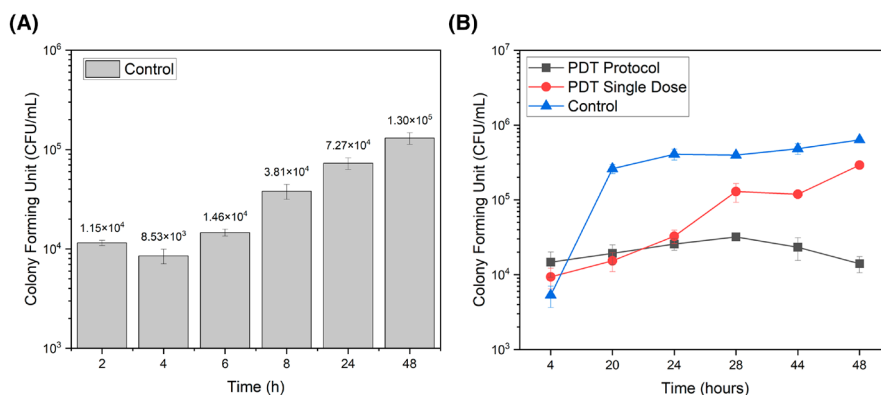


FIGURE 4 (A) Growth curve of *Candida albicans* in the TC02 sample of ETT with curcumin. (B) Comparison between control, a single dose of 50 J/cm² and a protocol involving six applications of a 50 J/cm² dose ($n=6$, error bar = SD and log scale).

resulting in a differential distribution of light between the internal and external surfaces of the ETT. VAP is primarily caused by bronchial aspiration of the internal biofilm; however, the biofilm on the external surface also contributes to the overall infection risk.

A deficiency of CUR on the ETT surface may result in fewer bioavailable molecules for light interaction on the internal surface, allowing lighter to penetrate the external surface, but potentially leading to reduced PDT efficiency. Conversely, high concentrations of CUR impede light permeation through the material, diminishing PDT efficacy on the external surface and thus affecting the overall effectiveness of the technique. The CFU/mL reduction observed at the TC02 CUR concentration represents an equilibrium between these competing factors.

Subsequently, as shown in Figure 3 for the TC02 sample, lower doses of light ranging from 15 to 60 J/cm² were tested to optimize the radiation dose. The results indicated no significant changes in CFU/mL reduction compared to the 50 J/cm² group, suggesting that lower doses do not substantially affect PDT efficacy. Notably, the dark group exhibited a non-statistically significant reduction in CFU/mL ($p>0.05$), indicating no difference between the control, light control, and dark groups in each case. Only the groups subjected to the full PDT process demonstrated a significant reduction in CFU/mL.

Reduced levels of light exposure led to lower reductions in CFU/mL, with reductions of 92.1% (1.02 logs) for 15 J/cm² and 92.5% (1.14 logs) for 45 J/cm². These findings are consistent with those reported by Zangirolami et al. (2020),¹⁵ who used a light dose of 50 J/cm² on both internal and external surfaces of ETTs across various bacterial cultures. Additionally, our results align with previous findings regarding the percentage of bacterial reduction: 72% for *E. coli*, 95% for *S. aureus*, and 73% for *P. aeruginosa*. In this study, a similar reduction in fungal cells of 98.3% (1.78 logs) was observed with 50 J/cm² using internal illumination exclusively.

Shi et al. (2016)²² reported that *C. albicans* biofilms were inhibited by 74.45% when PDT was applied using 5-aminolevulinic acid (ALA) at a dose of 300 J/cm² with a 5-h incubation time, highlighting the challenge of inhibiting biofilms. The findings by Garcia et al. (2020)²³ on the impact of biofilm growth surfaces on PDT efficacy against *C. albicans* are particularly noteworthy. Their research also suggests that applying PDT twice daily during biofilm formation may be more effective than a single application, especially when dealing with fully established biofilms. This emphasizes the need to consider biofilm development stages when designing treatment strategies for combating *C. albicans* infections.

Based on these findings, we acknowledge that fully developed biofilms pose greater challenges for elimination. Figure 4 illustrates the growth curve of *C. albicans* on the ETT surface, providing insights into the peak growth phase of the biofilm during the 48-h period. According to Chandra et al. (2001),²⁰ individual colonies begin adhering to the polymeric matrix surface within 4 h, indicating the onset of biofilm formation, although no visible extracellular matrix is present at this stage.²⁴ To proactively inhibit this process, we propose the application of PDT prior to biofilm maturation, specifically at the 4-h mark during the initial phase of biofilm formation.

The data presented in Figure 4 highlights the efficacy of the PDT protocol in preventing biofilm formation, especially when compared to the single-dose application. The PDT protocol group exhibits a marked difference in biofilm development, showing no visible formation of the biofilm matrix and a significant reduction in CFU/mL (1.65 log reduction compared to the control). This outcome suggests effective inhibition of biofilm formation when the PDT protocol is employed.

In contrast, the single-dose application demonstrates only a modest reduction in biofilm formation compared to the control group. This slight reduction may be attributed to the influence of PDT, albeit to a lesser degree than

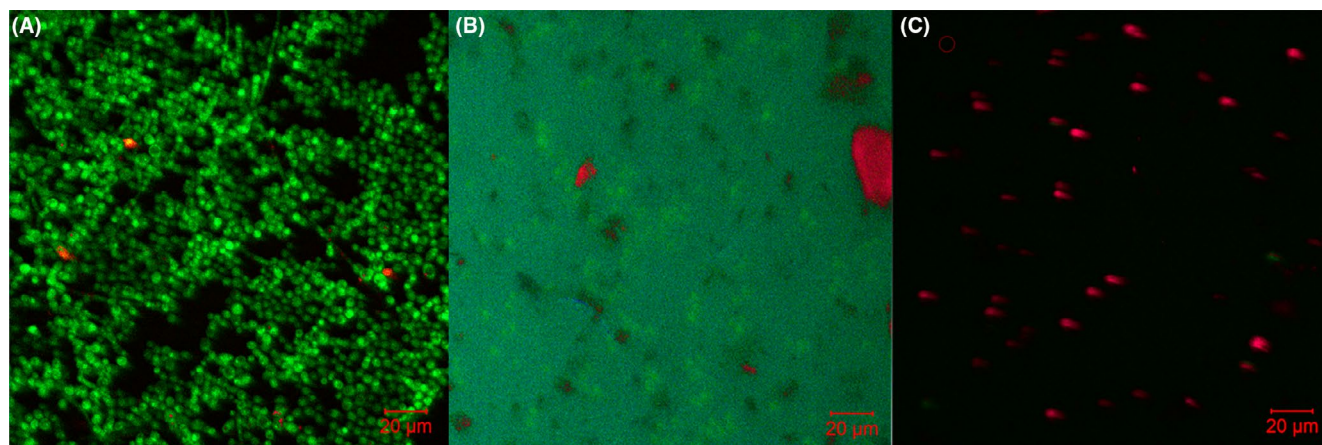


FIGURE 5 Confocal microscopy of the 48 h biofilm formation. (A) Control group PVC based without curcumin 800 nm excitation of double photon method and 40× amplification. (B) Curcumin tube control (TC02 sample) 405 nm excitation and 40× amplification. (C) Curcumin tube (TC02 sample) with 50 J/cm² of unique dose PDT application, 800 nm excitation of two-photon method, and 40× amplification.

observed with the repeated application protocol. These findings imply that a single application of PDT may not be as effective in fully preventing biofilm formation as the repeated application protocol, underscoring the importance of multiple PDT applications for optimal biofilm inhibition.

Based on the confocal microscopy analysis presented in Figure 5A, the biofilm formation observed within the CUR-deprived control group suggests the presence of predominantly healthy and viable fungal cells in substantial quantities. The image vividly illustrates the complex arrangement of biofilm along the polymeric surface plane of the tube, highlighting the extent and robustness of fungal colonization in the absence of CUR functionalization on the ETT.

Figure 5B depicts the sample treated with CUR under conditions without light exposure. This image confirms the presence of biofilm on the tube's surface but shows a reduced population of fungal cells compared to the control group. Additionally, there is a notably lower presence of red staining, indicating a reduced occurrence of dead cells, and a clustering of fungal cells. The green background of the image is due to the fluorescence emitted by CUR on the polymeric surface.

The PDT group exhibited distinct behavior, where the biofilm detached from the ETT surface following PDT application. Confocal microscopy analysis indicated the absence of both cells and biofilm remnants on the ETT surface. However, as shown in Figure 5C, cells suspended in the dye aqueous solution of the PDT group were observed, demonstrating the efficacy of PDT in inducing cell death and detachment from the tube surface. This suggests that the extracellular matrix of the biofilm was significantly affected, leading to cell death and the disintegration of the

biofilm. Importantly, the observed cells were confirmed to be dead.

Overall, the results consistently indicate that CUR prevents biofilm formation and, when activated by blue light, PDT effectively removes and kills the biofilm on the ETT surface.

Photobleaching

The usability of the ETT is closely tied to the lifespan of the functionalized photosensitizer on both the internal and external surfaces of the tube. In particular, the interaction between light and CUR on the internal wall is critical. Therefore, the behavior of bioavailable CUR is the most significant physicochemical characteristic of the functionalized ETT. Figure 6 illustrates the photobleaching of CUR in the TC02 sample when exposed to high doses (2000 J/cm²) of 455 nm laser light, equivalent to at least 12 days of light exposure under the proposed protocol (50 J/cm² per application, administered three times daily).

Figure 6A demonstrates that CUR on the internal surface degrades more rapidly compared to the external surface, primarily due to the challenges light faces in penetrating the polymeric medium. The degradation of CUR reduces by half between 700 and 900 J/cm². CUR is anticipated to remain photodynamically active up to 1800 J/cm². Once internal degradation occurs, light penetrates more effectively, delivering a higher dose of radiation to the external surface, which subsequently begins to degrade similarly to the internal surface, albeit with a delayed onset. The initial increase in the fluorescence spectrum can be attributed to the clearer background created by internal photobleaching, which enhances the reflection of the

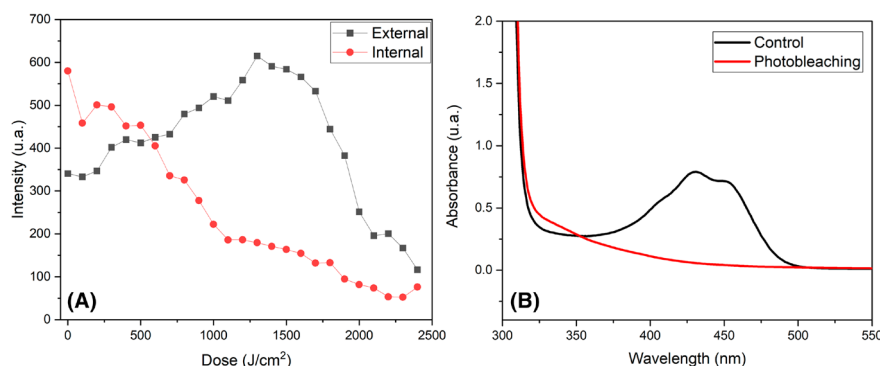


FIGURE 6 (A) Superficial fluorescence response of curcumin-functionalized ETT. The internal (red) and external (black) fluorescence behavior is shown in relation to the irradiation dose applied internally. (B) Absorbance spectra of the DMSO solution containing the byproducts from the control curcumin ETT and the sample irradiated with 2000 J/cm².

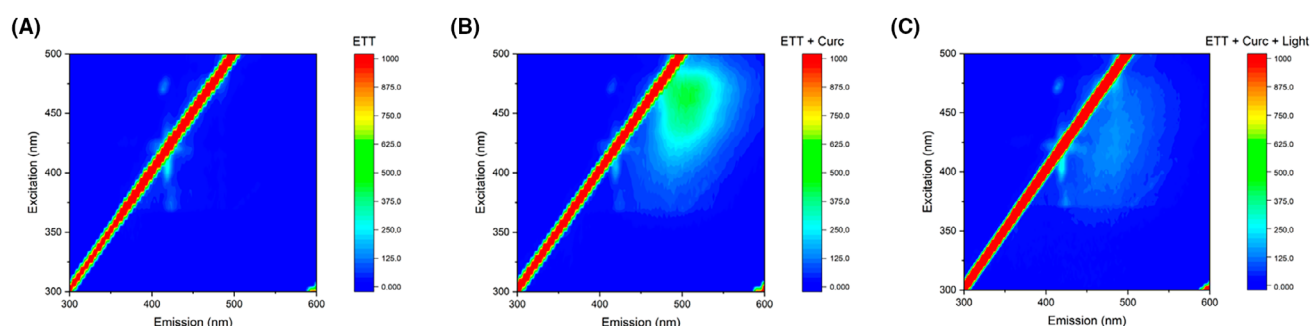


FIGURE 7 3D fluorescence profiles of (A) polyvinyl chloride (PVC)-based endotracheal tube (ETT) control, (B) curcumin-functionalized PVC ETT, and (C) irradiated curcumin-functionalized ETT.

fluorescence signal to the detector. The absorbance results presented in Figure 6B are consistent with these findings, showing the absence of CUR after irradiation, as indicated by the lack of the characteristic CUR peak at 430 nm.

In contrast, the fluorescence profile of the PVC-based ETT shown in Figure 7 remains unchanged after illumination. The same initial compounds present in the PVC ETT are detected both before and after irradiation, indicating that the PVC structure itself is unaffected by light exposure. However, the fluorescence signal from CUR decreases significantly, underscoring its susceptibility to photodegradation. This reduction in CUR fluorescence suggests that the photosensitizer undergoes degradation under prolonged light exposure, while the underlying PVC matrix remains stable. These results highlight the importance of understanding the photostability of CUR in functionalized ETTs, especially for applications requiring extended light exposure, such as antimicrobial photodynamic therapy.

DISCUSSION

The formation of *Candida albicans* biofilm on medical devices used in hospitals involves a complex dynamic

process with multiple stages, including adherence to surfaces and colonization of devices such as catheters, probes, and endotracheal tubes. This study has highlighted the importance of adopting a comprehensive strategy for combating both the formation and disruption of microbial biofilms, with a specific focus on preventing infectious diseases resulting from the formation of these complex microbial structures.

According to the findings, antimicrobial PDT must be applied during the biofilm formation process, with a particularly effective outcome achieved through a 4-h exposure to light within a 24-h interval. Confocal images indicate that the initial adhesion of *C. albicans* cells to the surface of medical devices seems blocked only after functionalization with CUR and the new structure of the endotracheal tube. Katherine Lagree (2018) suggests that surface topography may directly influence biofilm formation for various reasons.²⁵ We hypothesize that the presence of CUR, even without light application, inhibited biofilm formation by disrupting microorganism-surface interactions and avoiding attachment, as seen in the dark groups in Figures 2A and 3A. A significant variation was observed in biofilm formation in response to different CUR concentrations during the ETT functionalization

process. According to Figure 2, the TC02 sample achieved the most significant Log₁₀ reduction of 1.78 logs, which means 98.3% of biofilm cell reduction.

Namrata Raman (2014)²⁶ used multi-layer polymers loaded with antifungal agents to reduce biofilm in catheters, achieving an 83% decrease in biofilm on tubes coated with β -peptide-loaded materials. Although the approach differs, the results align with this study, which achieved a 1.78 log reduction. The importance of this reduction lies not only in the direct reduction of microbial load but also in the potential associated clinical impact. The presence of *C. albicans* biofilms in ETT can significantly increase the risk of serious lung infections, prolonged hospital stays, increased need for antimicrobial treatments, and, in some cases, increased morbidity and mortality.²⁷ Furthermore, infections associated with biofilms are more difficult to treat due to the physical barrier that biofilms provide against the penetration of antimicrobials and the presence of microorganisms in a physiological state that often makes them less susceptible to these treatments.²⁸

CUR inhibited the production of adhesins, thus reducing microorganisms' capability to adhere and form biofilms.²⁹ Typically, microcolonies would develop, and *C. albicans* biofilms would grow similarly to the cells in the control groups. However, this was not observed in the test groups, primarily due to the functionalization with the photosensitizer, as shown in the dark group in Figure 3. Additionally, CUR is known to influence the expression of genes associated with microbial adhesion, thereby altering microorganism behavior. *C. albicans* biofilms display a complex three-dimensional structure, with yeasts embedded within the extracellular matrix.³⁰ Furthermore, CUR has been shown to disrupt the extracellular matrix surrounding biofilms, weakening their structure and rendering them more susceptible to removal.

The strategy of applying the PDT protocol at 4-h intervals during the day, as a way of delaying biofilm maturation, represented an effective approach to controlling biofilms, especially on medical devices such as endotracheal tubes. Figure 4B, which illustrates the non-formation of biofilm on the surface of the ETT, demonstrates that repeated application of PDT can effectively prevent colony aggregation and subsequent biofilm maturation. PDT involves the use of a photosensitizer that, in the presence of light of a specific wavelength, produces reactive oxygen species (ROS).³¹ These ROS are capable of directly damaging microbial cells, leading to their death or inhibiting their growth. Applying this method at regular intervals before the biofilm reaches maturation may interfere with the ability of microbial cells to adhere to each other and the device surface, preventing the formation of three-dimensional structures characteristic of mature biofilms. Choosing the timing for applying PDT is crucial.

The intervention between the 6th and 8th hour of growth, as demonstrated, suggests a time when microbial cells are actively trying to establish themselves and form the initial biofilm structure. At this stage, cells have not yet developed the protective extracellular matrix that characterizes mature biofilms, making them more susceptible to the action of ROS generated by PDT. This underlines the importance of early and periodically spaced intervention to maximize the effectiveness of PDT in preventing biofilm formation. Biofilm formation on medical devices such as ETTs is a significant cause of hospital-acquired infections. These infections are particularly challenging to treat due to the resistance of biofilms to conventional antimicrobials. The demonstration that PDT can prevent biofilm formation indicates significant potential to improve patient safety by reducing the risk of device-associated infections. This also suggests a promising alternative to conventional infection control methods, which often involve the use of antimicrobials and may contribute to the development of antimicrobial resistance.

The approach used in this study to inhibit biofilm formation through PDT highlights specific challenges associated with treatment efficacy in complex biofilm environments. The presence of a dense extracellular matrix in biofilms is a significant obstacle to the penetration of photosensitizers and, consequently, to the effectiveness of PDT. This matrix, composed of polysaccharides, proteins, and extracellular DNA, not only protects microbial cells from antimicrobial agents but also limits the diffusion of photosensitizers into the biofilm, reducing accessibility to target cells. Furthermore, the strategy of linking the photosensitizer to a polymer, although innovative for increasing stability and specific localization of the treatment, may inadvertently decrease the availability of free photosensitizer in the medium. This is critical as the effectiveness of PDT depends on the direct interaction between the photosensitizer and microbial cells to generate reactive oxygen species (ROS). ROS are highly reactive and have a short lifetime, which means that their action is only effective in the immediate vicinity of their generation. Therefore, binding of the photosensitizer to the polymer may limit its ability to diffuse within the biofilm and reduce the exposure of microbial cells to ROS, resulting in lower log CFU reduction rates than expected.

The proposed 4-h protocol, applied before extracellular matrix formation, aims to prevent biofilm formation, recognizing that the effectiveness of PDT is maximized at early stages before the matrix becomes established. This preventive approach is critical considering the complexity of treating mature biofilms. Further research should concentrate on enhancing the durability of CUR or investigating the CUR byproducts generated to guarantee the sustained effectiveness of modified ETTs in medical

environments. Additionally, future studies should explore improving the photostability of CUR in modified ETTs, optimizing CUR concentrations, and evaluating alternative photosensitizers or combination therapies to maximize PDT efficacy.

This study also highlights the predominance of bacteria in the etiology of VAP, while recognizing the contribution of fungi in this context. By focusing on fungi, the research expands our understanding of the role of these microorganisms in biofilms associated with medical devices and their interactions with PDT-based treatments. The confocal microscopy images in Figure 5 show evidence of biofilm prevention. In the absence of light, biofilm exhibited normal growth on the ETT surface, whereas, in its presence, the external biofilm matrix was compromised, leading to the detachment of dead cells from the polymeric surface. Such damage is consistent with both structural and metabolic effects of ROS, preventing biofilm maintenance and inducing cell death. ROS, due to their high reactivity and short lifespan, attack nearby cellular components, including membranes, proteins, and nucleic acids. Confocal microscopy images demonstrate that, in the presence of light, the extracellular matrix of the biofilm is compromised. This structural damage is indicative of the action of ROS, which can degrade components of the extracellular matrix, such as polysaccharides and extracellular DNA, which are essential for the stability and integrity of the biofilm. Matrix degradation facilitates the detachment of dead cells from the polymeric surface, a phenomenon observed in the images. This cell detachment can be attributed to loss of adhesion mediated by matrix destruction and ROS-induced cell death. The damage caused by PDT is not limited to the physical destruction of the extracellular matrix; it also has significant effects on the metabolic function of cells within the biofilm. ROS can inhibit critical metabolic processes, damage essential cellular components, and eventually induce cell death. This dual mechanism of action—structural damage to the biofilm and impairment of cell viability—is crucial to the effectiveness of PDT in preventing the maintenance and maturation of biofilms. The visual evidence provided by confocal microscopy underscores the potential of PDT as an effective strategy to prevent biofilm formation on medical devices such as ETTs. Compromising the extracellular matrix and inducing cell death offers a dual approach to preventing the adhesion and accumulation of microorganisms, thus addressing one of the main challenges in controlling infections associated with medical devices. This study has provided valuable insights into the influence and the critical role of CUR concentration on biofilm formation and photobleaching.³² Higher concentrations not only obstructed light penetration but also compromised the efficacy of the technique. Moreover, the spatial interaction

among molecules influences ROS production and their effects on cells³³ and is instrumental in regulating cellular redox balance and cellular responses to oxidative stress.³⁴ The application of the PDT protocol prevented biofilm formation; since it interrupts cell propagation during the initial adhesion phase, hence, biofilm formation, it can find future applications in clinical settings.

CONCLUSION

The findings of this study demonstrate the effectiveness of a novel approach for preventing *Candida albicans* biofilm formation on endotracheal tubes, which is a critical issue in managing VAP. By combining CUR-functionalized ETTs with antimicrobial PDT, this research offers a dual-modality strategy that not only hinders the initial adherence of *C. albicans* to the surfaces of ETTs but also effectively eradicates the fungal cells once attached. This approach addresses a key factor in VAP development and has the potential to significantly reduce the risk of fungal infections associated with mechanical ventilation, enhancing patient safety and clinical outcomes in settings where ETTs are commonly used. While the results are promising, further studies are needed to validate the protocol's effectiveness in clinical environments and to assess its durability under prolonged use. Investigating the combined use of PDT with other antifungal agents and expanding this strategy to other medical devices prone to biofilm formation could also be beneficial. Overall, this study supports the integration of CUR-functionalized ETTs with PDT as a potent strategy against *C. albicans* biofilms. By preventing biofilm-associated infections, this approach represents a meaningful advancement in improving infection control practices and reducing the incidence of VAP in healthcare settings.

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