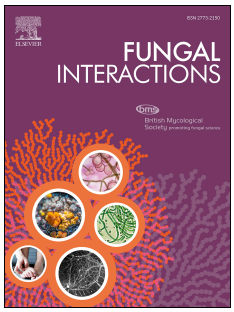

Modulating *Aspergillus fumigatus* biofilm formation: Antifungal-induced alterations in conidium-abiotic surface interactions

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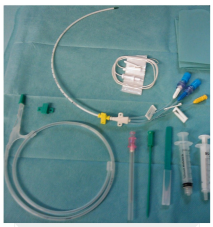
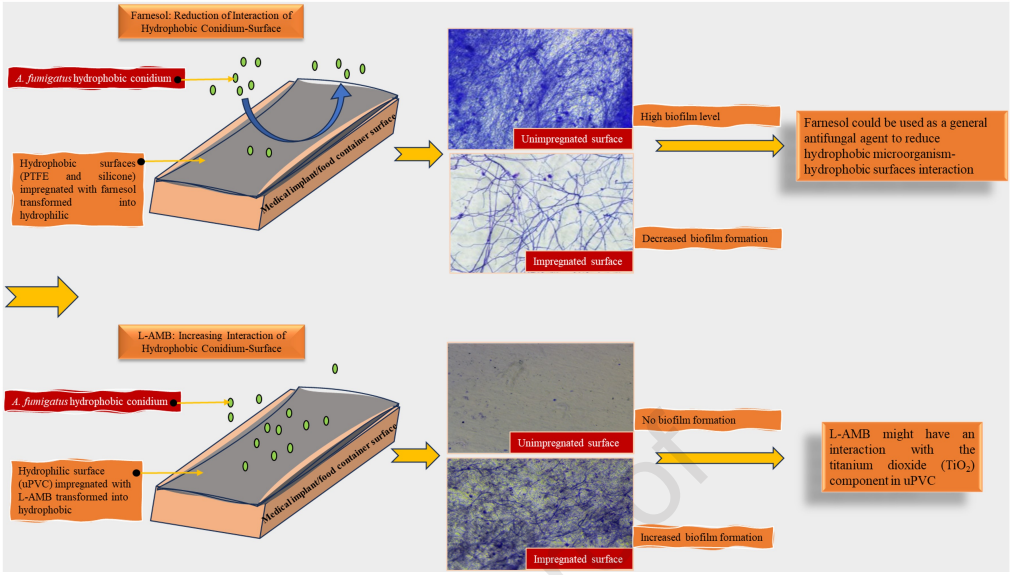
Modulating *Aspergillus fumigatus* Biofilm Formation: Antifungal-Induced Alterations in Spore-Abiotic Surface Interactions



Aspergillus fumigatus conidium can grow on medical implants, as well as on the surfaces of materials used in the food industry



Modulating *Aspergillus fumigatus* Biofilm Formation: Antifungal-Induced Alterations in Conidium-Abiotic Surface Interactions

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Abstract

Biofilm prevention on surfaces supporting microbial growth is an alternative strategy to manipulating microbial cells. This study focuses on *Aspergillus fumigatus*, a prominent airborne fungal pathogen. We exposed glass, acrylic, high-density polyethylene (HDPE), Nylon 6, polytetrafluoroethylene (PTFE), silicone, and unplasticized polyvinyl chloride (uPVC) surfaces to antifungal agents (triclosan, liposomal amphotericin-B (L-AMB), tyrosol, and farnesol) to study *A. fumigatus* conidium-abiotic surfaces interactions.

The total protein concentrations of *A. fumigatus* mycelia were quantified after growth in both a broth medium and on agar, subsequent to treatment with the agents. The hydrophobicity of chosen surfaces and the fungus was assessed using the contact angle and the microbial adhesion to hydrocarbons (MATH) assays, respectively, when subjected to antifungal agents. Moreover, *A. fumigatus* biofilms on uPVC and PTFE were evaluated through transmission flow-cell culture and optical microscopy.

Hydrophobic surfaces (PTFE and silicone) impregnated with farnesol transformed into hydrophilic. Conversely, L-AMB altered the surface properties of uPVC from hydrophilic to hydrophobic, potentially as a result of L-AMB's interaction with the TiO_2 component in uPVC. Considering the effect of antifungals on conidia, *A. fumigatus* conidia surfaces exhibited a shift from hydrophobic to hydrophilic characteristics under the influence of these agents.

Keywords

Antifungal agent, *Aspergillus fumigatus*, Biofilm formation, Fungal-surface interaction, L-AMB, Surface hydrophobicity

Introduction

Exposure to *Aspergillus fumigatus* in healthcare settings has been associated with opportunistic invasive fungal infections, a significant cause of mortality and morbidity in patients with severe neutropenia or immunosuppression [1]. To prevent fungal-solid surface interactions and, consequently, the formation of biofilms in healthcare facilities and food processing facilities, it is essential to find a way to reduce the initial attachment of conidia to surfaces.

Recent intervention strategies are intended to avoid initial medical device colonisation, minimise microbial cell attachment to the device, penetrate the biofilm extracellular polymeric substances (EPS) and destroy the associated cells, as well as treatment based on gene inhibition of cell attachment and biofilm formation [2]. The development of fungicidal coatings is also an effective method for eliminating/reducing biofilm formation and hence overcoming the pathogens' drug resistance [3]. Similar to medical implants, the surfaces of materials used in the food industry can be altered [4,5,6,7]

Microbial cell attachment to surfaces is influenced by several factors including Brownian movement, van der Waals attraction, gravitational forces, surface hydrophobicity (or surface electrostatic charges), and cell hydrophobicity [8]. The effect of cell surface hydrophobicity on microbe attachment to biotic and abiotic surfaces has advantages and disadvantages. Hydrophobic cells can be useful in removing aromatic and xenobiotic organic chemicals from the environment. Meanwhile, the hydrophobic characteristics of microbial surfaces encourage adherence to abiotic and biotic surfaces as well as penetration of host tissues. Hydrophobic microorganisms are more invasive, trigger diseases that are difficult to treat, and damage surfaces by forming biofilms [9]; on the other hand, they can readily accumulate on organic

pollutants and decompose them. Hydrophilic microorganisms also play a considerable role in removing organic wastes from the environment because of their high resistance to hydrophobic chemicals [10].

Under various environmental and growth conditions, *Candida albicans* cells can exist in either a hydrophilic (water- attracting) or hydrophobic (water- repelling) state [11]. Cell surface hydrophobicity (CSH) serves as a biophysical indicator of a cell's inclination towards a hydrophobic or hydrophilic environment. Cells exhibiting higher CSH display a preference for a hydrophobic setting, whereas those with lower CSH tend to remain in aqueous environments. This characteristic has significant implications for fungal virulence and the formation of biofilms [12].

Proteins seem to play a crucial role in the cell surface hydrophobicity (CSH) of *A. fumigatus* [13]. In *Aspergillus* spp., these proteins include hydrophobins, small proteins characterized by hydrophobic domains enabling interactions with surfaces exhibiting hydrophobic properties [12].

Surface hydrophobicity and hydrophobic interactions play a crucial role in the non-specific adhesion of *C. albicans* to host tissues or implanted medical devices [14]. Hydrophobic cells, due to the interaction of water molecules within themselves being stronger than with hydrophobic or non-polar particles, tend to be excluded from the water or aqueous environment [11]. Consequently, hydrophobic microorganisms remain in close proximity to the liquid-solid interface. This characteristic allows hydrophobic cells to readily interact with and adhere to solid surfaces, while hydrophilic cells typically disperse in the aqueous environment [11].

Unplasticized polyvinyl chloride (uPVC), a hydrophilic surface [10,15], and polytetrafluoroethylene (PTFE), a superior hydrophobic surface, were among the surfaces used in this study [16].

uPVC is used for a variety of applications, including drainage systems, food-hygiene (food-contact and packaging for food), cosmetics, and medications, among others [17]. PVC resin, calcium carbonate ($CaCO_3$), and titanium dioxide (TiO_2) typically constitute the chemical composition of uPVC [18]. In one study, a thin layer of TiO_2 was put to the acrylic resin that serves as the substrate for dentures [19]. This boosted the resin's hydrophilic characteristics, reduced food accumulation, and had an inhibitory effect on the adhesion of microbes. A TiO_2 coating can also be found on a wide range of medical devices; the TiO_2 coating on catheters, for example, has antimicrobial properties [20]. PTFE is frequently used in the design of heart valves and vascular grafts for cardiovascular engineering [21].

To undertake chemical modification on the surfaces, one method is to apply fungicides to them. The macrolide polyene antibiotic liposomal amphotericin-B (L-AMB), which is used to treat a variety of fungal infections, is one of the widely used substances of this kind [22]. However, depending on the fungal susceptibility, drug concentration, and pH, L-AMB may act either as fungistatic or fungicidal [23]. When L-AMB interacts with ergosterol-containing fungal membranes on biotic surfaces, the membranes depolarize and become more permeable. This interaction leads to the release of monovalent ions and eventual cell death [24]. L-AMB has both hydrophobic and hydrophilic portions, making it an amphoteric molecule [25]. It was concluded that AMB was effective in inhibiting the growth of fungal therapy but not in preventing fungal infection. However, in a study by Talas et al. (2019), it was revealed that AMB can prevent fungal infections by inhibiting germination and hyphal growth. They explained that this phenomenon suggests that reduced adhesion and biofilm formation could serve as a defence mechanism for fungi [26].

As another approach, microbiostatic antimicrobials are applied to the surfaces [27]. Notably, among these therapeutics, those specifically targeting the quorum sensing (QS) signalling pathways show promise [28], providing a targeted and effective strategy to disrupt microbial communication and inhibit microbial growth on treated surfaces.

Triclosan, a diphenyl ether derivative used as an antiseptic, has shown to have antifungal effects on *A. fumigatus* via interrupting the QS signalling system [29].

The sesquiterpene alcohol farnesol, a hydrophobic compound, was found to play a quorum quenching (QQ) role in *A. fumigatus* by interfering with the structure of the fungal cell wall and hyphal polarity [30,31]. The production of farnesol by *C. albicans* represents the first identified quorum-sensing system in a eukaryote [32]. In *C. albicans*, the presence of accumulated farnesol has notable effects on both dimorphisms, by inhibiting the transition from yeast to mycelium, and biofilm formation [33]. Chen et al. (2004) discovered tyrosol as a quorum-sensing molecule (QSM) generated by the *C. albicans* SC5314 strain. Their research indicated that, unlike farnesol, tyrosol acts as a stimulator of the yeast-to-hypha conversion process [34]. Notably, when farnesol and tyrosol compete directly, tyrosol does not alter the quorum-sensing activity of farnesol [33]. The impact of tyrosol, a phenolic compound, on *A. fumigatus* has not yet been reported.

Considering the above, this paper investigates the antibiofilm role of the selected applied agents on the interaction of *A. fumigatus* conidia and abiotic surfaces used in medical implants and food processing facilities. The findings contribute to the mitigation of biofilm formation in the specified applications.

Materials and methods

The fungus and its maintenance

A. fumigatus ATCC46645, was obtained from the Culture Collection of the University of Westminster, London, UK. Stock cultures of *A. fumigatus* maintained on potato dextrose agar (PDA) (Merck, Dorset, UK,) were propagated in potato dextrose broth (PDB) (Fisher Scientific, Loughborough, UK).

Abiotic surfaces

The surfaces (Silicone; uPVC; PTFE, also known as Teflon and manufactured by Dupont Co.; HDPE; Glass; Nylon 6; and Acrylic) used in this study were all provided by Goodfellow Cambridge Ltd, Huntingdon, UK.

Antifungal agents

One-centimetre surface segments were impregnated by immersion in a solution containing antibiofilm agents [triclosan (Sigma-Aldrich, Dorset, UK), L-AMB (Thermo Fisher Scientific, Leicestershire, UK), tyrosol (Merck, Dorset, UK), and farnesol (E,E isomer; Merck, Dorset, UK)] at their minimum inhibitory concentration at 50% (MIC_{50}). The surfaces were gas sterilized using ethylene oxide and subsequently immersed overnight in the solutions of the agents at 48°C to coat both internal and external surfaces, followed by an 8-h drying period at room temperature. This dipping procedure was repeated twice. The surface segments were then allowed to dry for an additional 24 h.

Extraction of proteins from *A. fumigatus* mycelia for the protein assay

Two sets of PDA plates were prepared; (i) Agar plates supplemented with the agents (triclosan, L-AMB, tyrosol, and farnesol): Untreated inoculum (100 μL) was added to the plates, and (ii) Agar plates prepared without the agents. Agents-treated inoculum (100 μL) were added to the plates. The supplemented agar plates carrying *A. fumigatus* conidia were incubated for one week.

Freeze-dried mycelium was prepared from colonies grown on PDA as described by Al-Samarrai et al. (2000). Briefly, a single colony was excised from the agar, cleansed, and suspended in 1 mL of distilled water. After pipetting for fragmentation, CsCl (1 g) was added, followed by centrifugation. The mycelium was separated, leaving agar at the bottom. After two washes with distilled water, the mycelia were freeze-dried for a minimum of 12 hours and stored at −20°C [35].

To analyse the protein concentration in a broth medium, inoculum was added to 1.8 mL of RPMI-1640 in a 2 mL collection tube and treated with triclosan, AMB, tyrosol, and farnesol at their MIC_{50}s. Following a 40-h static incubation in RPMI-1640 at 37°C, proteins from the fungal cultures were extracted, as detailed below.

To extract proteins from the mycelia, trichloroacetic acid (TCA) precipitation method was used [36]. Finally, the mycelia protein concentration was calculated using Bradford protein assay [37].

Contact angle and wetting properties

Water contact angle (WCA) measurement offers a method to evaluate the hydrophobicity of a mycelial mat in filamentous fungi [12]. WCA measurements were performed (First Ten

Angstroms FTA125 general purpose goniometer, Portsmouth, UK) with a 6 L drop of MQ water (Millipore) placed on the surface of choice (Silicone; uPVC; PTFE; HDPE; Glass; Nylon 6; and Acrylic). The surfaces were impregnated with triclosan, L-AMB, tyrosol, and farnesol. Untreated surfaces were used as the controls. Three WCA were measured per sample at room temperature via the sessile-drop method.

The contact angle of a liquid drop on a surface is determined by using Young's equation [38] (Equation 1).

$$\mathrm{COS}\,\theta\mathrm{y} = (\mathrm{Y}sv - \mathrm{Y}sl)/\mathrm{Y}lv \qquad \text{Equation 1}$$

where γ_{lv}, γ_{sv}, and γ_{sl} represent the liquid-vapor, solid-vapor, and solid-liquid interfacial tensions, respectively, and θ_y is the contact angle.

Small contact angles (<90°) correspond to high wettability, while large contact angles (>90°) correspond to low wettability. Super hydrophilic surfaces are defined as having contact angles of less than 10°, and superhydrophobic surfaces are defined as having contact angles of more than 150° [39].

Transmission flow-cell preparation

Flow-cell device (FC 281-PC, BioSurface Technologies Corporation, United States) was used to mimic the *in vivo* environment. Coupons of each PTFE and uPVC surfaces were prepared as the un impregnated control and impregnated with the agents at their MIC_{50}s, defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation [40]. The surfaces were incubated for 48 h at 37 °C in the Flow-cell device while inoculated PDB medium passed over them. The surfaces were

188 then stained with crystal violet 0.5% (w/v) dye, and optical microscopy was used to take
189 images of the biofilms.

190 Determination of hydrophobicity by using microbial adhesion to
191 hydrocarbons assay

192 The microbial adhesion to hydrocarbons (MATH) assay [41] is a common method for
193 determining CSH in fungi [12]. Conidia of a 14-day cultures were taken from PDA plates
194 (three samples were collected for each treatment with triclosan, L-AMB, tyrosol, and farnesol
195 MIC_{50}s). Phosphate buffered saline (PBS) was used as a negative control, and *A. fumigatus*
196 strain was used as a positive control. Treated and untreated control isolates were washed with
197 a saline solution (0.9% w/v). Subsequently, a saline solution containing conidia was covered
198 with 300 µL hexadecane in glass tubes. The tubes were vortexed for at least three periods of
199 30 seconds. After standing for 15 minutes at room temperature, the hexadecane phases were
200 carefully removed and discarded. The tubes were then cooled to 5°C. The absorbance of the
201 resulting conidial suspension was measured at 470 nm, and the Hydrophobic Index (HI) was
202 calculated based on three independent samples using equation 2. In this assay, entities with
203 HI>0.7 are considered hydrophobic.

204 $$[(A470 \text{ of control}) - (A470 \text{ of hexadecane treated sample})]/A470 \text{ of control}$$

205 Equation 2

206 Statistical analyses

207 Values presented in the results are the means of triplicate experiments and the standard error
208 of the mean (SEM) is shown as error bars. The SPSS software was used for paired sample T-
209 Test calculation showing data sets that were deemed not significantly different (N.S. > 0.05)

and data sets that were significant at different levels: *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 and ****P ≤ 0.0001.

Results

A. fumigatus mycelia proteins extraction for the protein quantity assay

The comparison of protein levels in *A. fumigatus* mycelium grown in RPMI-1640 broth medium with those grown on agar revealed reductions in the latter environment (Fig. 1). Notably, inoculum treatment with triclosan and farnesol (****P ≤ 0.0001) and subsequent grown on agar plates exhibited stronger inhibitory effects compared to the other agents. Additionally, agar treatment with tyrosol and farnesol (****P ≤ 0.0001) showed a stronger inhibitory effect compared to the other agents when the inoculum was grown on them. PDA is a combination of abiotic and biotic components, with the agar being abiotic and the potatoes and dextrose being biotic. The stronger impact of certain treatments in a broth medium, and on agar suggests that the substrate plays a crucial role in fungal growth dynamics.

Investigating the effect of the antifungal agents on dynamic nature of the variety of abiotic surfaces

The WCA measurements reveal variations in the hydrophobicity of different solid surfaces (glass, acrylic, HDPE, nylon 6, PTFE, silicone, and uPVC) before and after impregnation with triclosan, L-AMB, tyrosol, and farnesol (supplementary file, Fig. S1, and Fig. 2).

It was observed that the hydrophilic surface of glass (initial contact angle θy=9°) exhibited an increased hydrophilicity upon treatment with L-AMB (θy=6°; **P ≤ 0.01). Acrylic, originally hydrophilic with θy=31°, demonstrated enhanced hydrophilicity after exposure to triclosan

232 (θy=0°; ***P ≤ 0.001) and L-AMB (θy=8°; ***P ≤ 0.001). Conversely, tyrosol (θy=47°;
233 ***P ≤ 0.001) and farnesol (θy=74°; ***P ≤ 0.001) led to a reduction in surface
234 hydrophilicity.

235 HDPE, initially possessing a hydrophilic surface (θy=11°; ***P ≤ 0.001), displayed decreased
236 hydrophilicity after impregnation with triclosan (θy=58°; ***P ≤ 0.001), L-AMB (θy=53°;
237 ***P ≤ 0.001), tyrosol (40°; ***P ≤ 0.001), and farnesol (θy=52°; ***P ≤ 0.001). Nylon 6,
238 with an initial hydrophilic contact angle of θy=8°, experienced reduced hydrophilicity upon
239 exposure to L-AMB (θy=36°; ***P ≤ 0.001), tyrosol (θy=14°; **P ≤ 0.01), and farnesol (29°;
240 ***P ≤ 0.001).

241 PTFE, originally possessing a hydrophobic surface with θy=125°, demonstrated decreased
242 hydrophobicity with triclosan (θy=116°; **P ≤ 0.01), L-AMB (θy=95°; **P ≤ 0.01), and
243 tyrosol (θy=101°; **P ≤ 0.01). Conversely, farnesol changed the surface property to a
244 hydrophilic state (θy=75°; ***P ≤ 0.001). Silicone, initially hydrophobic with θy=107°,
245 exhibited decreased surface hydrophobicity with L-AMB (θy=96°; *P ≤ 0.05) and tyrosol
246 (θy=95°; *P ≤ 0.05). However, farnesol changed the surface property to a hydrophilic state
247 (θy=58°; ***P ≤ 0.001).

248 uPVC, with a hydrophilic original surface (θy=57°), demonstrated decreased hydrophilicity
249 with triclosan (θy=66°; **P ≤ 0.01) but increased hydrophilicity with tyrosol (θy=6°;
250 ***P ≤ 0.001). Notably, L-AMB (θy=91°; ***P ≤ 0.001) induced a change in the surface
251 property to a hydrophobic state.

252 The results showed that among the surfaces, uPVC surface turned hydrophobic after
253 impregnating with L-AMB. While hydrophobic surfaces (PTFE and silicone) impregnated
254 with farnesol became hydrophilic.

Microscopic comparison of *A. fumigatus* biofilm formed on unimpregnated PTFE and farnesol-impregnated PTFE surfaces in transmission flow cell

PTFE is a hydrophobic surface that attracts *A. fumigatus* conidia. A microscopic comparison of *A. fumigatus* biofilm formed on unimpregnated PTFE and farnesol-impregnated PTFE surfaces in a transmission flow cell demonstrates that the quantity of hyphal interwoven structures on the untreated PTFE surface is greater than that on the farnesol-impregnated PTFE surface under similar conditions (Fig. 3).

Screening *A. fumigatus* biofilm formation on agents impregnated uPVC surfaces in transmission flow cell

Impregnating uPVC with L-AMB resulted in a change to a hydrophobic state. The screening of *A. fumigatus* biofilm formation on triclosan, L-AMB, tyrosol, and farnesol-impregnated uPVC surfaces in a transmission flow cell is illustrated in Figure 4.

Photomicrographic images of the *A. fumigatus* biofilm on uPVC surfaces revealed that both the tyrosol-impregnated and untreated surfaces were highly hydrophilic, repelling the conidia and preventing the formation of biofilm. Additionally, the hyphal network appeared less dense with reduced conidiation of *A. fumigatus* on triclosan- and farnesol-coated uPVC surfaces compared to L-AMB-coated surfaces at 48 hours. In contrast, the L-AMB-impregnated uPVC attracted conidia and created an ideal substrate for their proliferation.

Investigating the effect of the agents on dynamic nature of the conidia surfaces

The examination of *A. fumigatus* conidial surface hydrophobicity in response to triclosan, L-AMB, farnesol, and tyrosol MIC_{50}s was carried out using the MATH assay (Fig. 5). The results from the assay indicate that upon treatment with the agents, the conidia surfaces exhibited clear hydrophilicity, with minimal distribution into the organic phase and predominant localization in the aqueous phase (HI < 0.7). Consequently, except for PTFE and silicone, characterized by hydrophobic surfaces, an anticipated repellent interaction is expected between the conidia treated with the agents and the hydrophilic surfaces, namely glass, acrylic, HDPE, nylon 6, and uPVC.

Discussion

In comparison with the broth medium, the observed reductions in protein levels within *A. fumigatus* mycelium across all test groups, following treatments with triclosan, L-AMB, farnesol, and tyrosol on both the inoculum and agar plates suggest a substantial influence of substrate on fungal growth dynamics. It is noteworthy that treating the inoculum with these agents yields a more pronounced antifungal effect compared to supplementing the substrate (agar) with them. Further research is needed to determine whether the decrease in mycelia protein quantity is related to the total amount of mycelia formed.

This experiment provides valuable insights into the inhibitory effects of triclosan, farnesol, and tyrosol on *A. fumigatus* when grown on PDA. The observed reductions in protein levels signify disruptions in fungal physiology, showcasing the potential of these compounds as effective antifungal agents.

Given that L-AMB is amphoteric, it was predicted that surfaces impregnated with L-AMB would become more hydrophobic as a result of L-AMB molecules adhering with their polar (hydrophilic) heads to surfaces that had opposing charges. Our findings demonstrated that L-AMB impregnating on HDPE, Nylon 6, and uPVC increased their hydrophobicity compared to their unimpregnated states. However, L-AMB impregnating on glass and acrylic surfaces reduced their hydrophobicity. The hydrophilic (anhydride) and hydrophobic (alkyl) moieties on the polymer surfaces cause chemical heterogeneity, which affects WCA measurements [42]. The heterogeneity of the surfaces may be the reason why L-AMB did not improve the hydrophobicity of acrylic and glass surfaces.

uPVC impregnated with L-AMB promoted the interaction between hydrophobic conidia and the formation of biofilms on uPVC surfaces. On the other hand, impregnating L-AMB onto the surface of uPVC appears to be a promising method for inhibiting the development of

hydrophilic microorganisms. The presence of TiO_2 in uPVC and its reaction with L-AMB's polar bonds may play a role in changing the property of uPVC from hydrophilic to hydrophobic. However, further exploration is warranted to determine whether the quantity of TiO_2 is sufficient for its interaction with antifungals to be considered significant.

Farnesol-impregnated hydrophobic surfaces, PTFE and silicone, underwent a transition into hydrophilic surfaces, thereby disrupting the interaction potential for conidia attachment during dynamic growth. This could be attributed to molecular attraction between farnesol and PTFE or to the orientation of farnesol alcohol groups toward the outside, interacting with water molecules in the medium through hydrogen bonding. Consequently, the surfaces became more hydrophilic than hydrophobic. Microscopic analysis was used to analyse attachments of the hydrophobic conidia, to PTFE, which is hydrophobic, as well as when PTFE was impregnated with farnesol, which made the surface hydrophilic. It was discovered that an extensive, firmly adherent mycelial growth had formed on the un impregnated PTFE surface, and the hyphae was completely embedded in the EPS. However, there were no EPS structures on the farnesol-impregnated PTFE surface.

PTFE is a fluorocarbon solid with a high molecular weight that is entirely composed of carbon and fluorine. The fluorine atoms completely encase the PTFE molecule on its surface. Fluorine atoms are highly electronegative. Nevertheless, the symmetrical conformation of the polymer backbone effectively neutralizes the dipole forces of the C-F bonds, leading to a net zero dipole moment [16].

If farnesol's effect on nonpolar surfaces is independent of the chemical composition of the surfaces, it could be used as a general impregnating agent to reduce hydrophobic microorganism attachment to hydrophobic surfaces.

Conclusion

The results of this study demonstrate notable alterations in surface properties among various materials including medical implants such as catheters, mechanical heart valves or pacemakers. Hydrophobic surfaces, PTFE and silicone, exhibited a shift towards hydrophilicity when impregnated with farnesol. Conversely, uPVC surfaces, initially hydrophilic, were transformed into hydrophobic surfaces by L-AMB. The conidia surface of *A. fumigatus* displayed a dynamic response, transitioning from hydrophobic to hydrophilic characteristics in the presence of triclosan, L-AMB, tyrosol, and farnesol. The results provided in this study form a foundation that can be harnessed for practical applications. To validate the relevance of these findings under real-life scenarios, further investigations *in vivo* are warranted.

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Conflict of interest

All authors declare that they have no conflicts of interest.

Figure Captions

Fig. S1 Angle values for the treated surfaces with triclosan, L-AMB, tyrosol, and farnesol. Hydrophobic surfaces have the property of repelling water (contact angle ≥90°). Error bars represent SEM for n = 3 replicates.

Fig. 1 Total protein concentration of (A) *A. fumigatus* mycelia in test groups treated with triclosan, L-AMB, tyrosol, and farnesol MIC_{50}s along with the untreated control group, cultivated in RPMI-1640 medium, assessed at 40 h of incubation. (B) Total protein quantification under two conditions: inoculum treated with agents at their MIC_{50}s levels and added on PDA vs. agar treated with the selected agents at MIC_{50}s, followed by the addition of an untreated inoculum. Significance levels are denoted as *P ≤ 0.05, **P ≤ 0.01, and ***P ≤ 0.001. Error bars represent SEM for n = 3 replicates

Fig. 2 Water droplet contact angle measurements on different surfaces

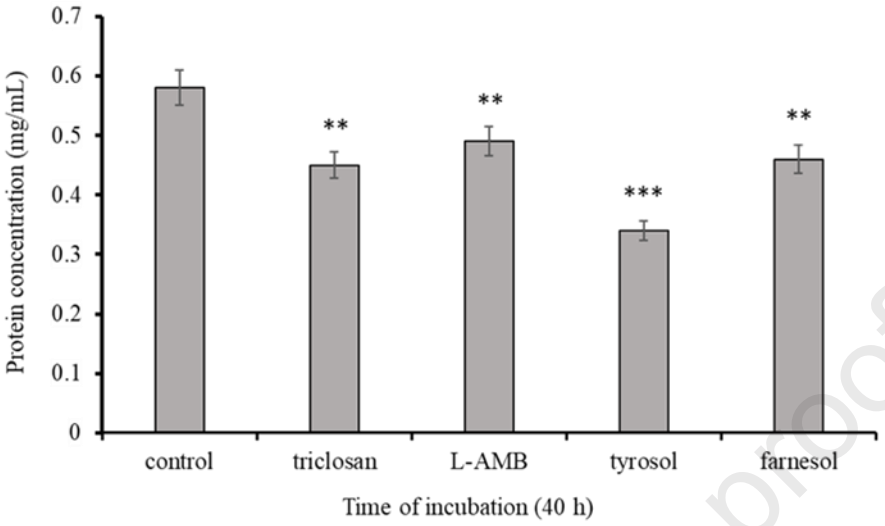
Fig. 3 Flow cell device analysis of (A) an un impregnated PTFE surface; B) a PTFE surface impregnated with farnesol and (100X magnification)

Fig. 4 Microscopic analysis of uPVC surfaces impregnated with triclosan, L-AMB, tyrosol, and farnesol in a transmission flow-cell under dynamic conditions. Unimpregnated uPVC surface was used as control (100X magnification)

Fig. 5 MATH assay analysis of the conidial hydrophobicity after treatment with the agents. The treated samples have been normalised relative to the control, where the control represents untreated fungus

Fig. 1

(A)



(B)

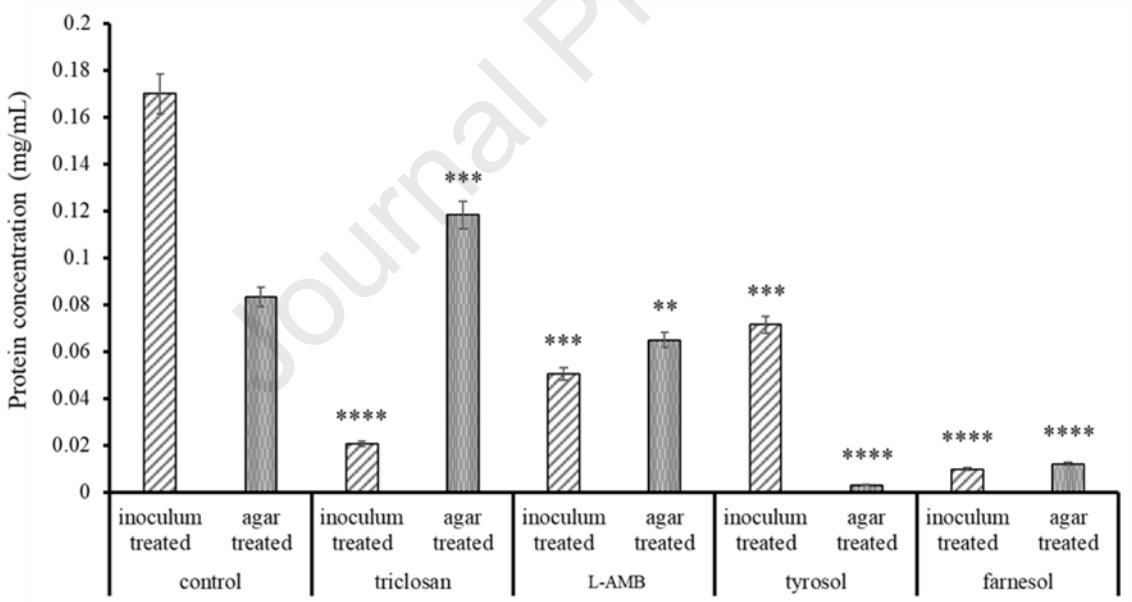


Fig. 2

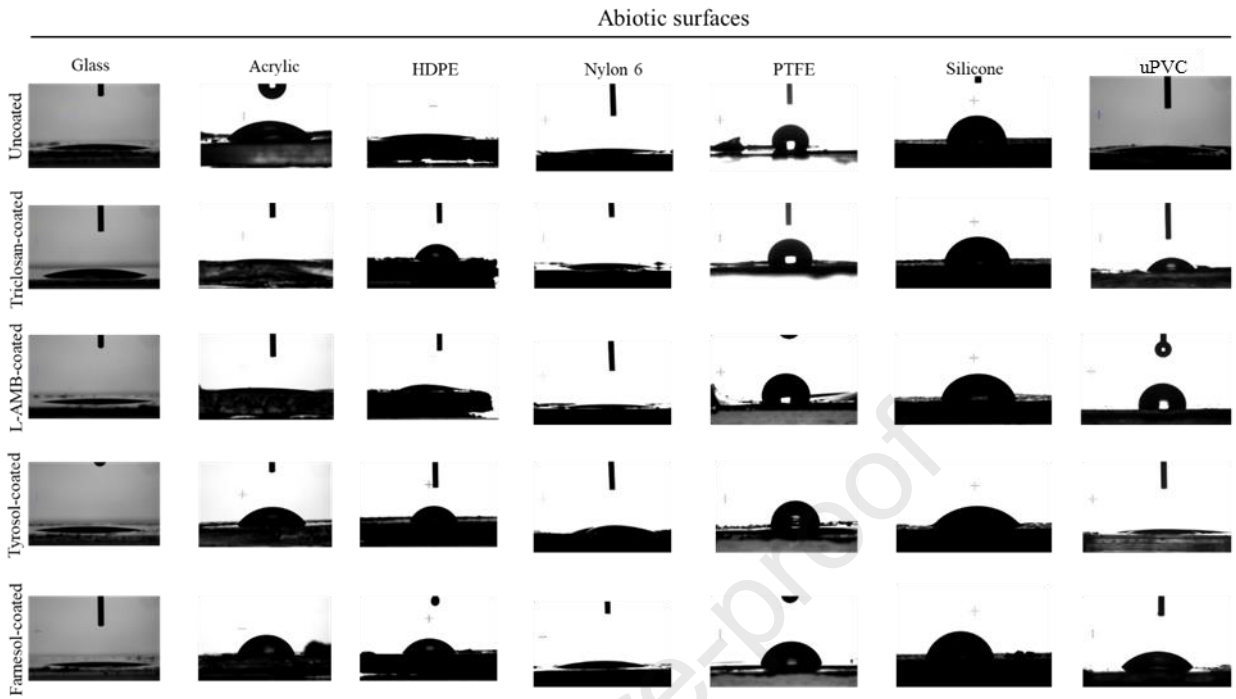
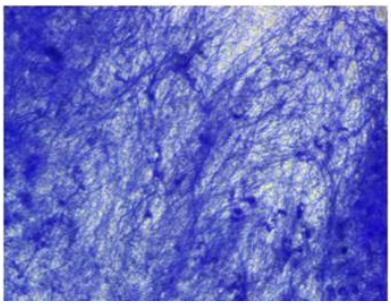


Fig. 3

(A)



(B)

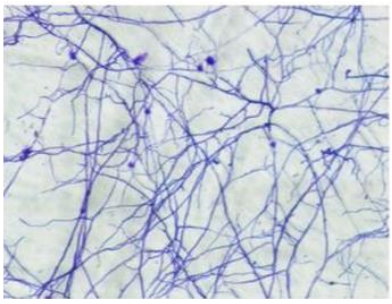


Fig. 4

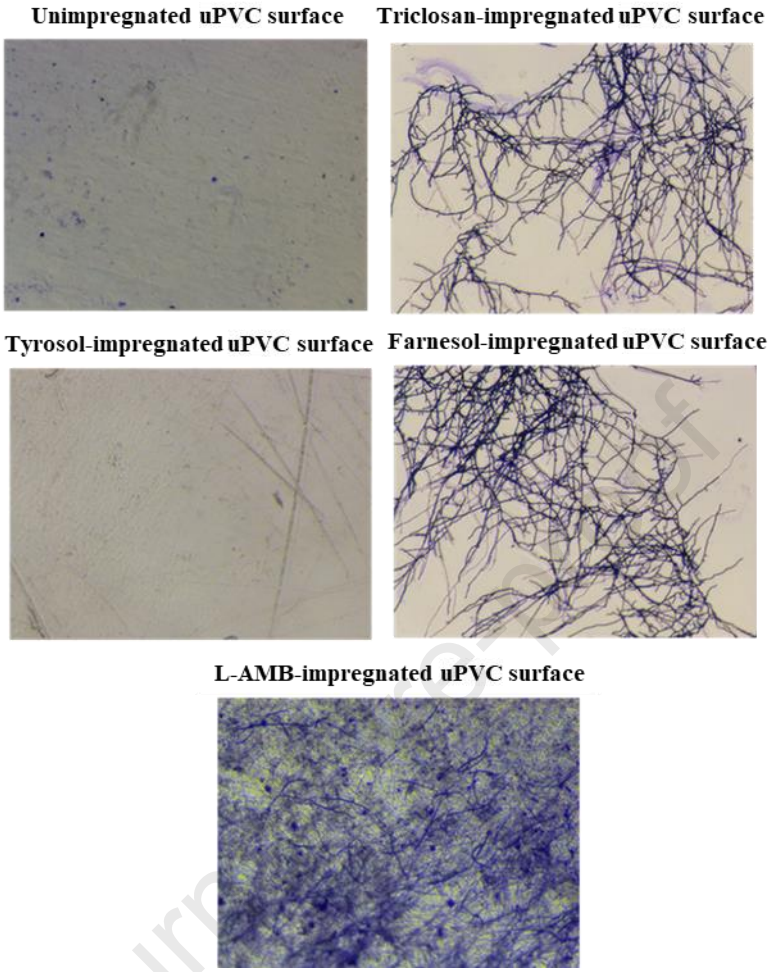
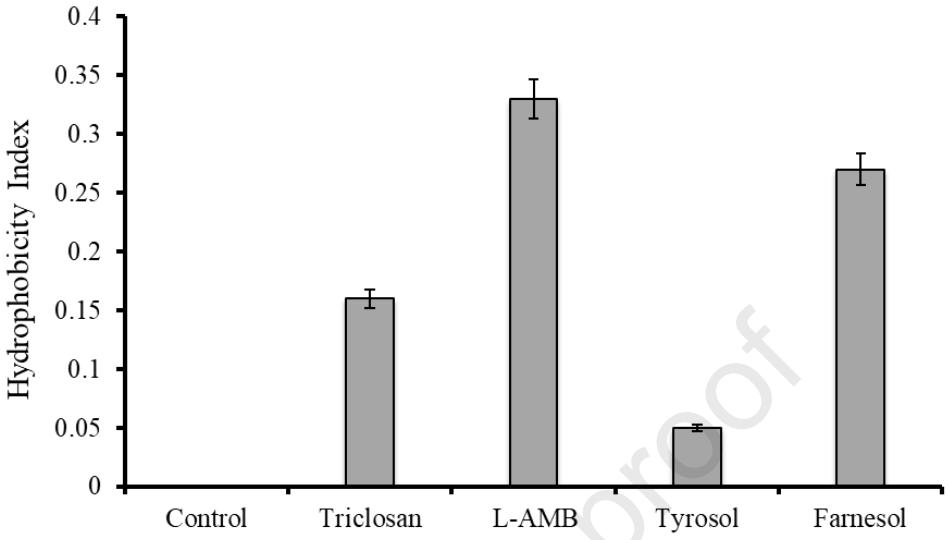


Fig. 5



- Coating hydrophobic surfaces (PTFE and silicone) with farnesol transforms them into hydrophilic.

- L-AMB changes uPVC surface from hydrophilic to hydrophobic.

- Antifungals influence the interaction between *Aspergillus fumigatus* conidia and abiotic surfaces.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: