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The Role of Biofilm Formation in *Candida* spp. Pathogenesis

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A B S T R A C T

Biofilms are defined as structured communities of microorganisms that are embedded in a self-produced matrix of extracellular polymeric substances and are typically attached to biological and non-biological surfaces. The formation of biofilms is a survival strategy adopted by microorganisms to withstand various environmental stresses, and biofilms can act as a reservoir for persistent sources of infection. Biofilm-forming microorganisms are known to be involved in a wide range of human infections that are challenging to treat and have severe health consequences. *Candida* spp. is one of the microorganisms capable of forming biofilms. As a commensal, *Candida* spp. can be found on the skin, mucosal tissues, upper respiratory tract, genitourinary tract, and gastrointestinal tract. In healthy individuals, the growth of *Candida* spp. is controlled by the immune system and the presence of other microbiota. However, when one of these barriers is disrupted, *Candida* spp. can behave as pathogens, causing superficial to systemic infections. Notably, *Candida* spp. biofilms often exhibit characteristics that render them resistant to several antifungal drugs, which has significant clinical implications and contributes to higher mortality rates. Therefore, this paper will primarily discuss the role of biofilms in modulating the pathogenicity of *Candida* spp

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INTRODUCTION

Most microorganisms are not found in a free-floating (planktonic) form; instead, they tend to associate with one another or exist as part of biofilm communities attached to either biological or non-biological surfaces. Research suggests that more than 99% of microorganisms in natural environments inhabit micro-ecosystems as surface-attached biofilms. Biofilms are structured communities of microbial cells that adhere to surfaces—or sometimes occur at air-liquid interfaces—encased within a self-produced extracellular matrix and exhibiting characteristics distinct from their planktonic forms. The development of biofilms represents an adaptive survival mechanism that enables microorganisms to endure various environmental stresses, such as temperature changes, desiccation, UV radiation, antimicrobial agents, and host immune responses. Consequently, many biofilm-forming microorganisms have been linked to persistent human infections that are difficult to treat and pose serious public health challenges. The National Institutes of Health (NIH) estimates that approximately 80% of infections in the United States are biofilm-related.

Among these microorganisms, *Candida* species—such as *C. albicans, C. parapsilosis, C. tropicalis, C. krusei,* and *C. auris*—are well-known for their ability to form biofilms within mammalian hosts. Infections caused by *Candida* spp. rank as the sixth most common type of hospital-acquired infection, according to data from the Centers for Disease Control and Prevention (CDC). The formation of *Candida* biofilms has significant clinical implications, often correlating with increased mortality, as many diseases caused by these fungi are associated with biofilm development. Therefore, biofilm formation is considered a crucial virulence factor in the pathogenesis of candidiasis.

Biofilm formation begins when planktonic microorganisms attach to a surface that provides nutrients or other advantages. Once attached, the cells regulate the expression of genes responsible for matrix production, initiating biofilm development. Biofilms are highly organized sessile microbial structures that create a stable environment, allowing other opportunistic pathogens to thrive and serving as reservoirs for persistent infections. Clinically, two major consequences of *Candida* biofilm formation are their enhanced resistance to antifungal agents and protection against the host immune system, both of which complicate patient management.

Mature biofilm cells possess biological characteristics distinct from those of their planktonic counterparts, even within the same strain. Biofilms demonstrate markedly higher resistance to antimicrobial compounds and are more capable of evading phagocytosis and other host immune mechanisms. As a result, biofilm-forming pathogens are capable of causing chronic infections, prolonged inflammation, and tissue damage. These adaptations complicate diagnostics, reduce therapeutic effectiveness, increase healthcare costs, and elevate mortality rates.

In general, antimicrobial treatments are more effective against actively growing planktonic cells. Disinfectants or biocides that efficiently kill free-living cells often fail to eradicate biofilm-associated microorganisms. Unfortunately,

most current microbial control strategies are designed to target planktonic cells, as the significance of biofilms has only recently gained broad recognition.

This paper aims to examine the role of biofilms in the pathogenicity of *Candida* species and to discuss current and emerging strategies for preventing and managing biofilm-associated *Candida* infections.

THEORETICAL REVIEW

Candida spp.

The genus *Candida* comprises approximately 150 species, of which around 10% are pathogenic to humans. These fungi are opportunistic organisms that typically reside as commensal members of the normal human microbiota. In healthy individuals, Candida species colonize various anatomical sites, including the skin, mucosal surfaces, the upper respiratory tract, the gastrointestinal tract, and the genitourinary system. Under certain predisposing conditions—such as immunosuppression or disruption of host barriers—these commensals can shift from a benign state to invasive pathogens, resulting in tissue invasion and infection. Infections caused by *Candida* spp. are frequently asymptomatic; however, under specific host conditions involving altered immunity, inflammatory responses, or dysbiosis, colonization may progress to overt disease. Common infection sites include the bloodstream, mucous membranes, skin, nails, and urinary tract. In systemic infections, *Candida* spp. can induce acute inflammatory responses and occasionally generate abscesses composed of polymorphonuclear and mononuclear cells.

Among the various species, *Candida albicans* remains the predominant etiological agent of invasive candidiasis. Nevertheless, in the last two decades, there has been a notable emergence of non-albicans *Candida* species such as *C. glabrata, C. parapsilosis, C. krusei, C. tropicalis*, and *C. auris*. The increasing incidence of infections caused by these species has been attributed to the widespread use of azole antifungal agents and the growing dependence on intravascular devices, both of which promote the selection of more resistant and biofilm-forming strains like *C. parapsilosis* and *C. glabrata*. These species differ markedly in their virulence potential and antifungal susceptibility profiles.

Candida spp. are well-known for their capacity to form biofilms—a complex, multicellular growth form that enhances persistence and pathogenicity. Several studies have identified multiple determinants influencing *Candida* biofilm formation, among which cell surface hydrophobicity (CSH) plays a significant role. CSH, an intrinsic physicochemical property of the fungal cell wall, is considered a crucial non-biological factor governing adhesion and surface colonization. Comparative analyses have demonstrated substantial interspecies variation in hydrophobicity and adhesion capabilities. *C. parapsilosis* typically exhibits the highest hydrophobicity, followed by *C. tropicalis*, suggesting a strong link between surface hydrophobicity and biofilm-forming potential. Conversely, *C. glabrata* displays limited biofilm formation, whereas *C. albicans* forms robust biofilms, correlating with its high virulence. Hydrophobic strains are generally associated with enhanced pathogenic potential compared to their hydrophilic counterparts.

General Characteristics of Biofilms

Biofilms are structured microbial communities that adhere to biotic or abiotic surfaces and are encased within a self-produced extracellular polymeric substance (EPS) matrix. This matrix primarily comprises polysaccharides, proteins, lipids, and nucleic acids, which collectively provide structural integrity and protection to the embedded cells. Analytical studies of the EPS have revealed that biofilms behave as viscoelastic hydrogels capable of withstanding physical and mechanical stress.

The EPS matrix not only provides mechanical stability but also serves as a reservoir for nutrients, which are retained and utilized by microbial populations within the biofilm. Water molecules are efficiently bound within the matrix through hydrogen bonding with hydrophilic polysaccharides, thereby maintaining hydration and nutrient diffusion. Comparative investigations have shown that biofilms formed under dynamic (flowing) conditions exhibit greater structural complexity, thicker extracellular matrices, and higher biomass than those developed under static environments.

Biofilm morphology may vary from single-layered to multilayered architectures, depending on the nature of cell–surface and cell–cell interactions. In monolayer biofilms, adhesion to the substrate predominates, whereas multilayered biofilms form when microorganisms adhere both to surfaces and to each other through intercellular aggregation. Biofilms can develop from a single microbial species or from polymicrobial consortia in which constituent cells undergo phenotypic and genetic alterations, including modified growth rates and transcriptional patterns. Biofilm development typically follows a sequential process initiated by microbial attachment to a surface. The formation of biofilms is frequently observed on medical devices such as catheters and implants, where they contribute to persistent infections.

Microorganisms embedded within biofilms on polymeric surfaces exhibit enhanced reproductive capacity, metabolic cooperation, and functional diversity relative to their planktonic counterparts. This cooperative metabolic behavior underlies the increased pathogenic potential observed in biofilm-associated populations. Moreover, biofilm-dwelling microorganisms demonstrate elevated resistance to antimicrobial agents and facilitate horizontal gene transfer, thereby promoting the evolution of more virulent and drug-resistant strains.

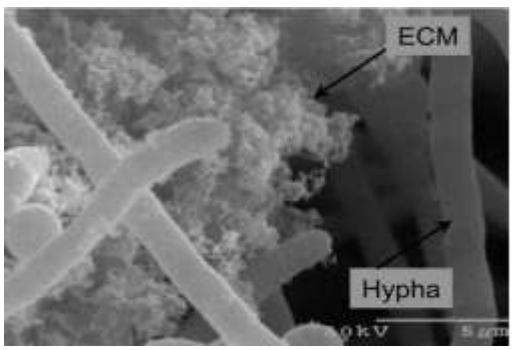

Figure 1. Visualization of *Candida albicans* biofilm using Scanning Electron Microscope (SEM) in vitro.

The development of biofilms in *Candida* species—most extensively characterized in *C. albicans*—is a highly coordinated and dynamic process that can be broadly divided into four distinct phases: attachment (adhesion), proliferation, maturation, and dispersion. In the initial attachment stage, planktonic yeast cells adhere to a biotic or abiotic surface, forming a basal monolayer that serves as the foundation for subsequent biofilm architecture. This adhesion is mediated by various surface adhesins and cell wall proteins that facilitate firm anchorage to the substrate.

The proliferation phase follows, marked by vigorous cell division and the initiation of morphogenetic transitions from yeast to filamentous forms, including hyphae and pseudohyphae. These filamentous structures interconnect to form a three-dimensional network that enhances the structural integrity and mechanical resilience of the developing biofilm.

During the maturation stage, cells within the biofilm engage in quorum sensing—a cell-to-cell communication process mediated by autoinducer molecules that regulate the expression of biofilm-associated genes. This phase is characterized by the extensive production of extracellular polymeric substances (EPS), primarily composed of polysaccharides, proteins, lipids, and extracellular DNA (eDNA), which serve to stabilize and reinforce the biofilm matrix. In C. *albicans*, the hyphal scaffold is encapsulated by an EPS layer that functions as a cohesive "biological adhesive," binding the entire community into a unified structure. The intricate interplay between carbohydrates, lipids, proteins, and eDNA endows the EPS with its viscoelastic and adhesive properties. Experimental studies have highlighted the crucial role of eDNA in maintaining matrix stability and facilitating intercellular signaling.

As the biofilm matures, it evolves into a multilayered structure reaching thicknesses of up to 10 µm. Concurrently, *C. albicans* biofilms release yeast cells with an elongated morphology—commonly referred to as "dispersal cells"—which act as propagules capable of colonizing new surfaces and initiating secondary infections.

The final dispersion phase marks the transition of sessile cells back to a planktonic lifestyle. During this stage, biofilm-associated enzymes—particularly saccharolytic hydrolases—degrade the polysaccharide components of the EPS, thereby releasing cells from the biofilm matrix. This process enables microbial dissemination and the establishment of new biofilms on distant surfaces, effectively completing the biofilm life cycle.

Under experimental conditions, the entire biofilm developmental sequence typically occurs within 24–48 hours, with mature biofilms often attaining a thickness of several hundred micrometers. This highly organized structural complexity is thought to represent an optimized spatial configuration that facilitates efficient nutrient diffusion, metabolic exchange, waste removal, and the formation of microniches throughout the biofilm ecosystem. Figure 2 schematically depicts the transition of *Candida* spp. between planktonic and biofilm-associated states.

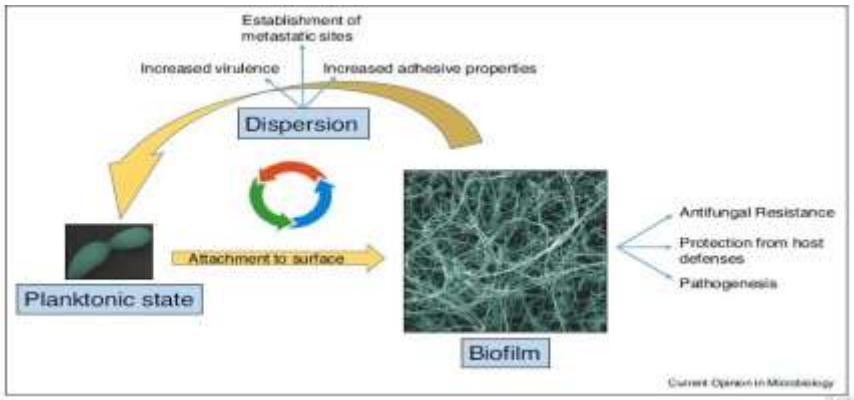

Figure 2. Transition of *Candida* spp. from planktonic to biofilm state and its main implications for pathogenesis.

Biofilm Dispersion and Factors Influencing Biofilm Formation

Once established, *Candida* biofilms act as persistent infection reservoirs that facilitate microbial survival and dissemination to secondary host sites. Recent studies indicate that cells dispersed from mature biofilms predominantly originate from the apical layer of hyphal structures and exhibit a distinct phenotype characterized by elongated yeast morphology. The frequency of dispersal is modulated by environmental parameters, particularly the available carbon source and the pH of the surrounding medium; glucose has been shown to enhance the formation of lateral yeast cells. Compared to age-matched planktonic yeast cells, dispersed *Candida* cells demonstrate increased adhesion to endothelial surfaces, heightened cytotoxicity, enhanced filamentation, more robust biofilm formation, elevated fluconazole resistance, and greater virulence in murine models of hematogenously disseminated candidiasis.

Multiple extrinsic and intrinsic factors govern *Candida* biofilm formation, including fluid dynamics, substrate characteristics, species composition, gene expression, and metabolic activity. Hydrodynamic flow plays a vital role in nutrient exchange and profoundly influences the architecture and stability of the biofilm matrix. Moreover, the physicochemical properties of the substrate—such as material composition and surface roughness—significantly affect microbial attachment, cell morphology, and biofilm biomass. The specific *Candida* species and strains involved are also critical determinants, with *C. albicans* generally producing thicker and more complex biofilms than non-*albicans Candida*. Several metabolic enzymes, such as alcohol dehydrogenase, glyceraldehyde 3-phosphate dehydrogenase, and pyruvate kinase, as well as regulatory genes including *ALS3*, *ACE2*, *NRG1*, *UME6*, and *EFG1*, have been implicated in modulating biofilm development and structural differentiation.

Patterns of Biofilm Formation on Mucosal and Abiotic Surfaces

Candida species exhibit remarkable adaptability in colonizing both mucosal and abiotic surfaces, including medical devices such as venous and urinary catheters. Biofilm formation has also been observed in patients with vulvovaginal candidiasis using copper intrauterine devices (Cu-IUDs). The transcription factor **Bcr1** is a key regulator of biofilm development in these contexts. Although *Bcr1* is not required for hyphal morphogenesis, it serves as a

positive regulator of hypha-specific adhesins, particularly *Als3* and *Hwp1*. Functional studies employing gene deletion and overexpression have demonstrated that these adhesins substantially contribute to biofilm formation in catheter-associated models. On mucosal surfaces, *Bcr1* plays an essential role in mediating biofilm-associated infections by regulating adhesin expression during epithelial cell and neutrophil interactions.

Distinct differences have been reported between biofilms formed on mucosal versus abiotic surfaces, as well as between in vitro and in vivo systems. In *C. albicans* in vitro biofilm models, the initial attachment phase occurs within approximately 11 hours and is followed by microcolony formation. The intermediate developmental phase (12–30 hours) involves EPS secretion and the establishment of a bilayer structure consisting of yeast cells, germ tubes, and early hyphae. Subsequent maturation (38–72 hours) results in the accumulation of a dense EPS matrix and the formation of a complex network of intertwined yeast and hyphal cells. Finally, the dispersal phase marks the release of cells responsible for infection spread. In vivo timelines differ substantially: in rat central vein models, biofilm formation is detectable within 8 hours, with full maturation achieved at 24 hours for *C. albicans* and after approximately 48 hours for *C. glabrata*.

Genetic Regulation of Biofilm Formation

The development of *Candida* biofilms is intricately controlled at the molecular level through a network of transcriptional and signaling pathways. Over recent decades, molecular investigations have elucidated the central role of morphogenetic transitions, adhesion mechanisms, and quorum sensing in biofilm development. Mutational analyses have revealed that transcription factor mutants such as *Δefg1* and *Δtec1* exhibit defective biofilm formation due to impaired filamentation. Likewise, *Bcr1* has been identified as a master regulator of biofilm formation, primarily by governing the expression of hyphal adhesins *Als3* and *Hwp1*, which mediate attachment and structural cohesion within the biofilm.[4] *Hwp1*, a substrate for human transglutaminase, facilitates covalent binding between fungal and host epithelial cells, while the agglutinin-like sequence (*ALS*) gene family encodes cell surface proteins critical for adhesion and biofilm formation.

Transcriptomic studies comparing *C. albicans* biofilms and planktonic cultures have revealed broad shifts in gene expression associated with metabolism, signaling, and cell wall remodeling. Nobile et al. identified a core transcriptional network comprising nine regulators—*Bcr1, Brg1, Efg1, Ndt80, Rob1, Tec1, Flo8, Gal4,* and *Rfx2*—that orchestrate normal biofilm development. Collectively, these regulators modulate approximately 1,000 genes, accounting for about 15% of the *C. albicans* genome. Many of these transcription factors also control the yeast-to-hyphae transition, underscoring the tight link between morphogenesis and biofilm growth.

The **Zap1** (zinc-responsive activator protein, also known as *Csr1*) transcription factor further contributes to biofilm regulation by modulating farnesol accumulation—a known inhibitor of hyphal formation—and regulating

the expression of zinc transporters (*ZRT1, ZRT2*) and other zinc-responsive genes. Deletion of *ZAP1* results in reduced farnesol accumulation and impaired biofilm formation, whereas overexpression of *ZRT2* alone fails to restore normal farnesol levels, suggesting that *Zap1* functions as a positive regulator of both farnesol production and cell signaling in *C. albicans* biofilms.

RNA-seq analyses comparing biofilm hyphae, dispersed cells, and planktonic yeast cells demonstrate that dispersed cells, despite retaining a yeast-like morphology, share over 60% of their transcriptional profile with parental hyphae, confirming their distinct hybrid phenotype. These dispersed cells upregulate genes involved in adhesion (*ALS5, ALS6, ECM33*), antifungal resistance (*MDR1, QDR1, ERG* family), nutrient acquisition (*ZRT1, ZRT2, ZAP1*), and virulence (*SAP* family), collectively enhancing their infectivity and persistence relative to planktonic cells.

Furthermore, the **hypha-to-yeast transition** is crucial for dispersal, as the released cells—termed *lateral yeast cells*—emerge from the lateral septa of biofilm hyphae. This process is governed by **PES1**, an essential gene conserved across eukaryotes. Both in vitro and in vivo studies, including catheter-associated biofilm models, have demonstrated that suppression of *PES1* expression effectively blocks the production of lateral yeast cells. Consequently, dispersed cells represent highly virulent, drug-resistant entities that enable the spread of biofilm-derived infections to distal sites. Targeting the regulatory pathways responsible for lateral yeast dispersal may therefore represent a promising therapeutic approach to limit biofilm persistence and reduce systemic dissemination.

METHODOLOGY

This study is a systematic literature review aimed at identifying and analyzing the role of biofilm formation in the pathogenesis of *Candida* spp. This method was used to collect, select, and synthesize various relevant research findings from published scientific sources.

Literature Search Strategy

The literature search was conducted online through several scientific databases, including PubMed, ScienceDirect, and Google Scholar. The selected articles were scientific publications issued between 2013 and 2025. The keywords used included the following combination:
"Candida" AND "biofilm formation" AND "pathogenesis" OR "virulence factors"

Logical operators (AND, OR) were applied to broaden or narrow the search results as needed. In addition, a manual search was conducted by reviewing the reference lists of relevant articles to ensure completeness of the sources.

Inclusion and Exclusion Criteria

The inclusion criteria in this review included:

- Original research articles or review papers discussing biofilm formation in *Candida* spp.

- Studies highlighting the relationship between biofilm formation and mechanisms of pathogenesis, virulence factors, or antifungal resistance.
- Articles written in English or Indonesian and published in indexed journals.

The exclusion criteria included:

- Articles focusing solely on non-*Candida* species.
- Case reports, opinion papers, or articles without experimental data.
- Publications not available in full-text format.

Literature Selection Process

The initial search yielded **210 articles**. After screening based on titles and abstracts, **87 articles** were considered potentially relevant. Following a thorough full-text review, **28 articles** met all the inclusion criteria and were selected for the final analysis. The selection and screening process were conducted systematically in accordance with the *Preferred Reporting Items for Systematic Reviews and Meta-Analyses* (**PRISMA**) guidelines to ensure transparency and methodological rigor.

Data Analysis

The selected articles were analyzed using a thematic analysis approach. The main information extracted included:

- Mechanisms of biofilm formation in *Candida* spp.,
- Genetic and environmental factors influencing biofilm formation,
- The relationship between biofilm formation and antifungal resistance, and
- The implications of biofilm on virulence and the pathogenesis of *Candida* infections.

Data from various sources were compared and synthesized to obtain a comprehensive understanding of the contribution of biofilm formation to the pathogenic process of *Candida* spp

RESEARCH RESULTS

Antifungal Resistance

Candida biofilm resistance represents a multifactorial phenomenon, involving several mechanisms operating simultaneously throughout different stages of biofilm growth. Three main antifungal classes are typically employed in the treatment of candidiasis—azoles, polyenes, and echinocandins. Cells of *C. albicans* within biofilms display remarkably high resistance to azoles and polyenes, particularly when biofilms are formed under flow conditions, which further enhance resistance levels. Biofilms are intrinsically resistant to fluconazole and other azole derivatives, while the antifungal efficacy of polyenes is achieved only at concentrations that are often toxic and clinically unsafe. Prolonged use of azoles and polyenes frequently results in the emergence of resistant strains. In fact, biofilm-associated cells have been reported to be up to 1000-fold more resistant to azoles than their planktonic counterparts, rendering these drugs largely ineffective for biofilm-associated infections. Furthermore, biofilms exhibit a 2–20-fold increase in resistance to echinocandins, a

phenomenon linked to point mutations in *FKS1*, which encodes β-1,3-glucan synthase—an enzyme essential for cell wall carbohydrate synthesis.

The extracellular matrix (ECM) of biofilms is a key determinant of resistance, functioning as a physical and chemical barrier that traps antifungal molecules and prevents their penetration. The matrix, composed mainly of extracellular polymeric substances (EPS), impedes antifungal diffusion and drug access to intracellular targets. Mature biofilms secrete ECM enriched with β-1,3-glucan, which can bind and sequester antifungal drugs. Biofilms of other *Candida* species, such as *C. glabrata*, *C. parapsilosis*, and *C. tropicalis*, also exhibit matrix-mediated sequestration mechanisms.

Among the available antifungal agents, echinocandins appear to possess superior anti-biofilm activity. Recent findings have also identified extracellular DNA (eDNA) as a matrix component contributing to drug resistance. Treatment of *C. albicans* biofilms with DNase enhances the activity of echinocandins and polyenes, although it does not affect fluconazole susceptibility.[17] This potentiating effect of DNase treatment has also been observed in bacterial biofilms and is thought to be related to horizontal gene exchange.[18] Other contributing factors to biofilm resistance include high cell density, overexpression of efflux pumps, altered sterol composition in cell membranes, and the presence of persister cell populations capable of surviving extreme antifungal exposure.[15]

Two primary classes of efflux pumps contribute to antifungal resistance in *Candida* spp.: the ATP-Binding Cassette (ABC) transporter superfamily—represented by *CDR1* and *CDR2*—and the Major Facilitator (MF) superfamily, including *MDR1*. In planktonic cells, these pumps are usually upregulated in response to antifungal exposure; however, in biofilms, efflux pumps are upregulated within the first hours of adhesion and remain active throughout biofilm maturation, even without antifungal presence. Elevated levels of *MDR1* and *CDR1* transcripts have been observed in 24-hour-old *C. albicans* biofilms compared to planktonic cultures of equivalent age. Deletion mutants lacking *MDR1*, *CDR1*, or *CDR2* exhibit hypersensitivity to fluconazole, although this effect diminishes as biofilms mature, indicating that efflux pumps play a more prominent role in early-stage resistance.

Alterations in sterol biosynthesis also contribute significantly to antifungal resistance. Disruptions in the ergosterol synthesis pathway are associated with increased resistance to azoles and polyenes, particularly amphotericin B. Studies show that the sterol content in biofilm cell membranes contains substantially lower ergosterol levels compared to planktonic cells, especially during later stages of biofilm development—approximately half the ergosterol concentration found in early-phase cells. This reduced ergosterol dependency may compromise the efficacy of antifungal drugs targeting ergosterol.

The high cell density of biofilms further enhances antifungal resistance. Research indicates that azole resistance increases with cell density, implying that biofilm compactness contributes to reduced drug susceptibility, although this characteristic is not unique to biofilm-associated cells.

Another crucial resistance mechanism involves the formation of persister cells—dormant subpopulations located deep within the biofilm matrix that exhibit tolerance to multiple antifungal classes, including azoles and amphotericin B. Persister cells survive antifungal exposure and serve as a reservoir for infection regrowth. Transcriptional analyses indicate that these cells differentially regulate genes involved in ergosterol biosynthesis (*ERG1*, *ERG25*) and β-1,6-glucan production (*SKN1*, *KRE1*), suggesting alterations in cell wall and membrane composition. The formation of persister cells appears to be species- and strain-dependent, with *C. albicans* and *C. krusei* frequently forming such cells, unlike *C. glabrata*.

Biofilm development and adhesion are also linked to stress response activation, which enhances drug resistance. The MAPK Mkc1p pathway, central to cell wall integrity, has been shown to regulate biofilm formation and fluconazole resistance; *C. albicans* mutants lacking *MKC1* are defective in both processes. Additional pathways, including those mediated by calcineurin—a Ca^{2+}/calmodulin-activated phosphatase crucial for homeostasis and virulence—also play roles in resistance. Inhibition of calcineurin enhances fluconazole efficacy against *C. albicans* biofilms. Moreover, Hsp90, a molecular chaperone that stabilizes client proteins, regulates matrix glucan synthesis through its association with calcineurin and Mkc1. Disruption of this pathway compromises matrix formation and biofilm resistance, underscoring Hsp90's central role in antifungal tolerance mechanisms.

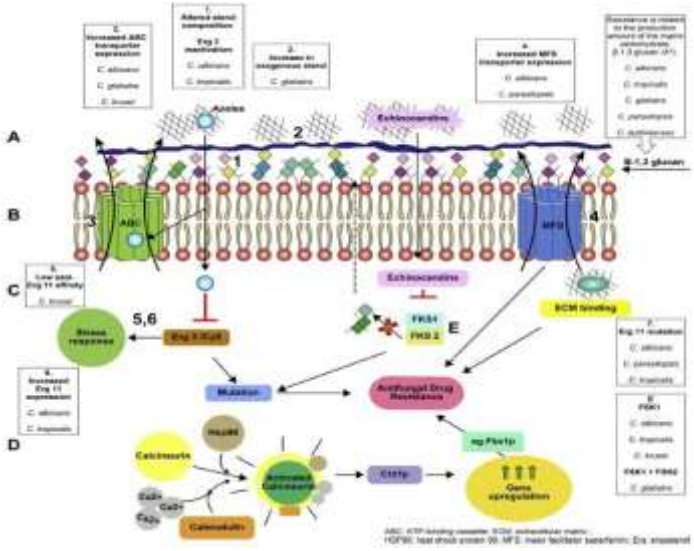

Figure 3. Illustration of the molecular mechanisms underlying *Candida* spp. biofilm resistance to antifungal drugs.

Immunity to Biofilms

In general, the host immune system exhibits a markedly reduced ability to eliminate microbial biofilms. This is primarily because biofilm-associated cells can rapidly alter their surface antigens through dynamic gene expression, thereby evading immune detection. Additionally, host immune responses to biofilms often result in collateral tissue damage. The release of proinflammatory

cytokines at biofilm sites fails to eradicate the biofilm but instead contributes to inflammation and destruction of surrounding tissues.

Although the precise mechanisms by which *Candida albicans* biofilms evade host immunity are not yet fully understood, it is hypothesized that the surface components of the biofilm matrix differ significantly from those of the fungal cell wall. These structural and compositional differences likely alter interactions with pattern recognition receptors (PRRs) on host immune cells, leading to impaired immune recognition and response. Cells embedded within biofilms are also protected from killing by neutrophils, macrophages, and monocytes—key immune effectors in antifungal defense.

In vivo studies demonstrate that mature *C. albicans* biofilms can be recognized and surrounded by neutrophils; however, these neutrophils often remain functionally inactive. Compared to planktonic cells, *Candida* biofilms suppress the release of neutrophil extracellular traps (NETs) and inhibit the generation of reactive oxygen species (ROS), both of which are critical for pathogen clearance. The β-glucan component of the biofilm extracellular matrix plays a major role in this resistance by preventing neutrophil activation. Furthermore, *Candida* biofilms impair macrophage chemotaxis and inhibit monocyte phagocytosis of biofilm-associated cells, leading to reduced production of proinflammatory cytokines such as TNF-α. Since TNF-α is essential for activating phagocytes and orchestrating antifungal immunity, its suppression facilitates immune evasion. Hyphal forms within biofilms further contribute to immune evasion by penetrating epithelial barriers and enabling fungal escape from within phagocytic cells via physical disruption of host cell membranes.

Several biofilm-associated proteins have also been implicated in modulating host immunity. Secreted proteins such as Pra1, Gpd2, and members of the secreted aspartyl protease (Sap) family are upregulated during biofilm formation and are capable of inhibiting complement activation. Another protein, Msb2, which is highly expressed in biofilms, binds and neutralizes host antimicrobial peptides, further contributing to immune resistance. In addition, deletion of the key transcriptional regulator *BCR1*—essential for biofilm development—renders *C. albicans* significantly more susceptible to leukocyte-mediated killing, underscoring its role in immune protection.

Although molecular-level evidence is still limited, cumulative findings strongly support that *C. albicans* biofilms interact with the host immune system in a manner distinct from planktonic cells, resulting in an overall phenotype that is more resistant to immune clearance (Figure 4).

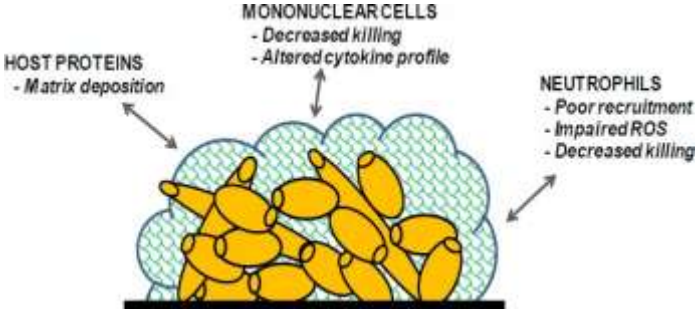

Figure 4. Host response to *Candida* spp. biofilms.

DISCUSSION

Strategies for Controlling and Inhibiting Biofilms

Currently, there is no specific treatment available for infections caused by microbial biofilms, making their management particularly challenging. The resistance of biofilms to standard antifungal drugs is multifactorial. In addition to providing physical protection against immune defenses and antifungal agents, cells embedded within biofilms exhibit intrinsic drug resistance through mechanisms such as upregulation of efflux pumps and alterations in metabolic activity. These distinctive features make the development of effective therapies against biofilm-associated infections a significant clinical challenge.

A deeper understanding of the molecular mechanisms underlying biofilm formation and stabilization is essential for developing novel therapeutic agents that specifically target biofilms. For instance, elucidating the molecular basis of cell-to-cell and cell-surface adhesion could facilitate the creation of strategies aimed at preventing biofilm initiation or disrupting mature biofilm structures. Likewise, identifying the molecular determinants of biofilm dispersal could lead to pharmacological approaches that either block the release of cells from biofilms or promote their dispersal to enhance eradication. Furthermore, understanding microbial interactions with host immune components—such as biofilm formation on mucosal epithelial tissues—and identifying virulence-inhibiting factors in *Candida* spp. may open new avenues for therapeutic intervention.

Two promising therapeutic approaches have emerged: lock therapy and catheter coating. Lock therapy aims to eradicate biofilms on indwelling medical devices, particularly catheters, before they come into contact with patients. This strategy involves instilling high concentrations of antimicrobial agents into the catheter lumen for several hours or days, allowing the agent to act locally and minimize systemic toxicity. For example, studies on silicone catheters infected with *C. albicans* and *C. glabrata* have shown that micafungin (5–15 mg/L), caspofungin (5–25 mg/L), and posaconazole (10 mg/L) effectively reduce *Candida* biofilms. However, limitations exist—such as reduced efficacy observed with lipid formulations of amphotericin B (1,000 mg/L).

The second approach involves modifying catheter coatings with antimicrobial compounds such as minocycline-rifampin, chlorhexidine, or silver sulfadiazine, which have been shown to decrease bloodstream infections associated with central venous catheters. Recent advances also include the use of nanomaterials or nanoparticles as coatings to further inhibit microbial colonization. While these technologies have primarily targeted bacterial biofilms, silane-based coating systems on titanium and zirconia surfaces have shown potential in reducing *Candida albicans* biofilm formation on implants.

In parallel, extensive research is being conducted to identify new natural and synthetic compounds with antifungal potential. Various bioactive molecules—such as plant-derived phenylpropanoids and terpenoids, and phenazines produced by *Pseudomonas aeruginosa*—have demonstrated inhibitory effects on the yeast-to-hypha transition and biofilm formation in *C. albicans*. Synthetic peptides, such as KSL-W, have also exhibited potent antifungal activity by impairing growth, hyphal development, and biofilm formation.

Another promising strategy involves developing non-adhesive or anti-fouling surface coatings to prevent microbial attachment (Figure 5). This can be achieved by incorporating nanoparticles, antibiotics, or enzymes such as dispersin and DNase to disrupt biofilm integrity. Surface modification using natural polymers like chitosan hydrogel has also shown remarkable efficacy. In vivo studies using rat catheter models demonstrated that chitosan coatings significantly inhibited biofilm formation by *C. albicans*, *C. parapsilosis*, *C. glabrata*, *C. tropicalis*, *C. krusei*, and *C. guilliermondii*.

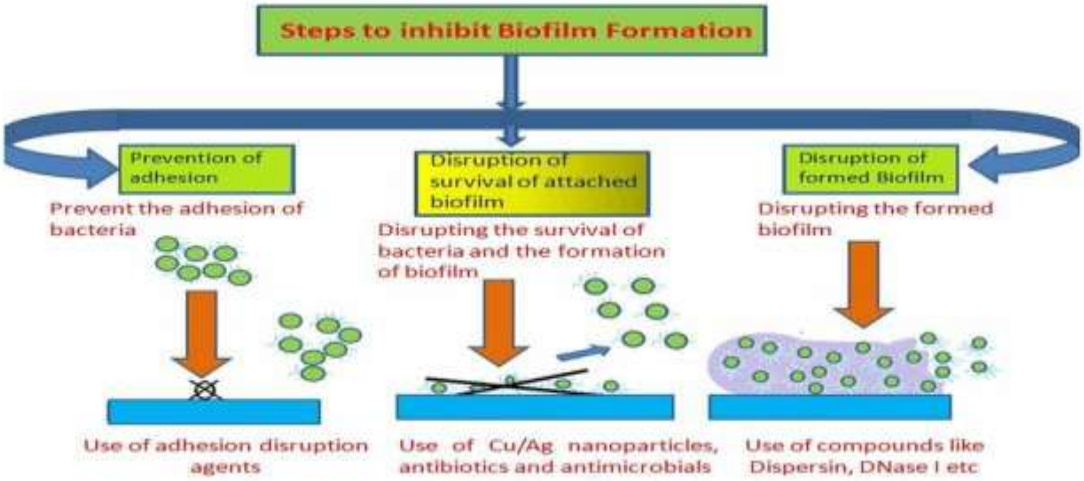

Figure 5. Strategies for preventing biofilm formation

CONCLUSION AND RECOMMENDATION

Based on the review presented earlier, it can be concluded that biofilm formation is a natural property of *Candida* spp., and various studies have recognized biofilms as contributing to the pathogenicity of *Candida* spp. Some roles of biofilms in pathogenicity include increased resistance to antifungal agents, ability to evade the host immune system, and support for more persistent infections. From a clinical perspective, biofilm-related infections that are resistant to antifungals have implications for limited treatment options, which can increase morbidity and mortality. Therefore, preventive measures against biofilm formation are needed, such as using non-adhesive coatings to inhibit *Candida* spp. cell attachment to abiotic surfaces. As a therapeutic approach, exploring compounds that specifically target *Candida* spp. biofilms is an effective step.

FURTHER STUDY

Further studies should focus on developing innovative strategies to prevent and control *Candida* spp. biofilm formation. Future research could explore the molecular mechanisms underlying biofilm development and antifungal resistance to identify novel therapeutic targets. Investigations into natural or synthetic compounds that can disrupt biofilm integrity or inhibit cell adhesion are also essential. Additionally, studying the effectiveness of surface modifications, such as non-adhesive or antimicrobial coatings, in clinical settings can provide practical insights for preventing biofilm-associated infections. Finally, integrating genomic, proteomic, and metabolomic approaches may offer

a deeper understanding of *Candida* spp. biofilm behavior and contribute to the development of more effective antifungal therapies.

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