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Review Article

Candida biofilms in medical devices: Evolving trends

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The study of microorganisms since ages has been associated with their isolation and growth in pure culture and their subsequent identification down to their genes. This channel of study has been actively pursued in various scientific disciplines pertaining to anatomical, physiological and pathological aspects of microbes and the scientific research that is based on them. However, many microbes in their natural habitats are found in biofilm ecosystems

attached to surfaces and not as free-floating (planktonic) organisms.[1] Cells in these biofilms are embedded within a matrix of extracellular polymeric material and display an altered phenotype.[2] Biofilms are studied in a wide range of scientific disciplines including biomedicine, water engineering, and evolutionary biology.[3] They are universal, complex, interdependent communities of surface-associated microorganisms enclosed in an exopolysaccharide matrix occurring on any surface, particularly aquatic and industrial water systems as well as medical devices.[4] Biofilms represent the most prevalent type of microbial growth in nature and are crucial to the development of clinical infections.[5] They can serve as a nidus for disease and are often associated with high-level antimicrobial resistance of the associated organisms.[5] This is of particular significance since it is now estimated that a significant proportion of all human microbial infections involve biofilm formation.[1]

Recently, it has been estimated that some 65% of all human microbial infections involve biofilms.[2] Many of these are implant-related infections in which adherent microbial populations can be demonstrated on the surfaces of devices such as catheters, prosthetic heart valves, joint replacements and dental acrylic.[1,6] Biofilm microorganisms can also be detected in tissues taken from nondevice- related chronic infections such as native valve endocarditis.[2] Such infections may be caused by a single microbial species or by a mixture of bacterial or fungal species.[2]

Transplantation procedures, immunosuppression, the use of chronic indwelling devices, and prolonged intensive care unit stays have increased the prevalence of fungal disease. Fungi most commonly associated with such disease episodes are in the genus Candida, most notably *Candida albicans*, which causes both superficial and systemic disease.[3] Candida species are frequently found in the normal microbiota of humans, which facilitates their encounter with most implanted biomaterials and host surfaces. Devices such as stents, shunts, prostheses, implants, endotracheal tubes, pacemakers, and various types of catheters, to name a few, have all been shown to support colonization and biofilm formation by Candida.[1] *Candida albicans* remains the fungal species most commonly associated with biofilm formation[1]and the increase in Candida infections in the last decades has almost paralleled the increase and widespread use of a broad range of medical implant devices, mainly in populations with impaired host defences. Even with current antifungal therapy, mortality of patients with invasive candidiasis can be as high as 40%.[3]

Candidiasis is usually associated with indwelling medical devices (e.g., dental implants, catheters, heart valves, vascular bypass grafts, ocular lenses, artificial joints, and central nervous system shunts), which can act as substrates for biofilm growth. The role of bacterial biofilms in disease have been investigated in detail over a number of years and considerable literature is available on their structure and properties.[3] However, sufficient literature is hard to find on medically relevant fungal biofilms particularly in the prevailing scenario where immunocompromised conditions and nosocomial infections are on the rise. This review article aims to provide insights on various aspects of Candida biofilms, their role in pathogenesis, antifungal drug resistance and the recent advances on Candida biofilms.

Candida biofilms: Structure and formation

Adherence of fungal cells to biomaterial surfaces must first occur for colonization to take place. The initial attachment of Candida cells to biomaterials is mediated by both nonspecific factors (cell surface hydrophobicity and electrostatic forces) and by specific adhesins on the fungal surface recognizing ligands in the conditioning films, such as serum proteins (fibrinogen and fibronectin) and salivary factors.[1]

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Recent studies suggest that specific adherence events may also be mediated by cell surface proteins such as those encoded by members of the agglutinin-like(*ALS*) family of adhesin- producing genes and *EAP1*.[1] Various techniques such as fluorescence microscopy and confocal scanning laser microscopy have been used to study Candida biofilm formation on polymethylmethacrylate strips or silicone elastomer disks in vitro.[3]

C.albicans biofilm formation has three developmental phases: adherence of yeast cells to the device surface (early phase), formation of a matrix with dimorphic switching from yeast to hyphal forms (intermediate phase), and increase in the matrix material taking on a three-dimensional architecture (maturation phase).[7] Fully mature Candida biofilms have a mixture of morphological forms and consist of a dense network of yeasts, hyphae, and pseudohyphae in a matrix of polysaccharides, carbohydrate, protein, and unknown components. The formation and structure of Candida biofilms is influenced by the nature of the contact surface, environmental factors, Candida morphogenesis, and the Candida species involved.[7]

Additionally, Candida cells can also coaggregate and/or bind to bacteria.[1] Mature Candida biofilms exhibit a complex three-dimensional structure and display extensive spatial heterogeneity.[1] This structural complexity is thought to represent the optimal spatial arrangement to facilitate the influx of nutrients, the disposal of waste products, and the establishment of micro-niches throughout the biofilm. The overall architecture of the biofilm may vary depending on the substrate on which it is formed and its growth conditions.[1] Moreover, different strains of *C. albicans* and different Candida spp. differ in their capacities to form biofilms.[1]

The factors which affect Candida biofilm formation are diverse-
(i) The chemical nature of the contact surface has been shown to influence the magnitude of biofilm formation, which is increased on latex compared with polyvinyl chloride but substantially decreased on polyurethane and 100% silicone.[7]
(ii) High-glucose medium promotes the formation of biofilms, particularly of *C. parapsilosis*, reflecting its potential to cause device-related infections in patients receiving parenteral nutrition.[7] Cell surface hydrophobicity correlates positively with Candida biofilm formation, and gentle shaking also enhances biofilm formation. These conditions are also encountered in vivo (like in the circulation and urinary system), favouring biofilm formation when devices are inserted.[7]

(iii) The different morphological forms are important in biofilm formation, as evidenced by a study that compared biofilms formed by wild-type strains of *C. albicans* and two mutants incapable of yeast and hyphal growth, respectively. The wild-type mutant produced a distinct two-layer biofilm, the hypha-negative mutant produced only the basal layer, and the yeast-negative mutant produced only the outer layer, which was more easily detached from the catheter disks. This suggests that dimorphism might be necessary for biofilm architecture and structure and is a pivotal factor for the pathogenic potential of *C. albicans*.[7]

In addition, catheter materials in vivo rapidly adsorb host proteins which form a conditioning film on the catheter surface. Similarly, conditioning films of serum or saliva promoted biofilm formation on denture acrylic.[2]
Other factors which affect biofilm formation in vitro include liquid flow and presence of bacteria.[2]

Role of Candida biofilms in pathogenesis:
Biofilm formation in Candida could confer certain advantages like protection from the environment including antimicrobial agents, nutrient availability, metabolic cooperation and acquisition of new genetic traits.[4] Biofilms are notoriously difficult to eliminate and are a source of many recalcitrant infections.[4]

Different mechanisms may be at work for the intrinsic resistance of Candida biofilms. These include the following: (i) the high density of cells within the biofilm; (ii) the effects of the biofilm matrix; (iii) decreased growth rate and nutrient limitation; (iv) the expression of resistance genes, particularly those encoding efflux pumps; and (v) the presence of "persister" cells.[1] The ability of Candida to switch reversibly between yeast and filamentous forms is important (morphogenetic conversions) for its pathogenicity.[1]

Adherence to bioprosthetic surfaces and cell aggregation are precursors for biofilm formation and hence it is logical to assume that gene expression involved in the transition process from planktonic to biofilm growth will change. Studies by Chandra J et al[3] have demonstrated that expression of ALS genes are differentially regulated during the transition from a planktonic to a biofilm-associated organism. This represents a small number of transcriptional changes that are likely to occur during biofilm formation-regulation of genes encoding enzymes involved in carbohydrate biosynthesis during biofilm growth indicated by formation of extracellular material and increased expression of drug resistance genes such as CDR1, CDR2 and MDR. Another important aspect of biofilm formation in Candida is cell-cell

signalling particularly quorum sensing. This strategy of cell-cell communication benefits the biofilms' wellbeing by preventing unnecessary overpopulation and controlling competition for nutrients and has important implications in the infectious process, particularly for dissemination and for the establishment of distal sites of infection. It has been shown that farnesol acts as a quorum-sensing molecule that inhibits filamentation in *C. albicans*.[1] Preincubation of *C. albicans* cells with high concentrations of farnesol almost completely inhibited biofilm formation.

Furthermore, supernatants recovered from mature biofilms inhibited the filamentation of planktonic *C. albicans*, indicating that a morphogenetic autoregulatory compound, most likely farnesol, is produced in situ in biofilms.[1] In a recent study, Cao et al used a partial *C.albicans* cDNA microarray to analyze changes in the gene expression in *C. albicans* biofilms grown in the presence of farnesol.[1] As expected, some hyphal-formation-associated genes were differentially expressed in farnesol-treated biofilms, including *TUP1* (up-regulated). Other differentially expressed genes included some involved in drug resistance as well as genes encoding proteins with roles in cell wall maintenance (namely chitinases), iron transport, and heat shock/stress response.

Also, *CSH1*, which codes for a protein that has been associated with cell surface hydrophobicity, was down-regulated in farnesol-treated biofilms. Another quorum-sensing/autoregulatory molecule with a role in the growth and morphogenesis of *C. albicans* is tyrosol, which is found in conditioned medium from high-density cultures[1]; tyrosol abolishes the delay of growth after dilution and stimulates filamentation under conditions permissive for germ tube formation, but its role in biofilms has not been investigated. A recent study has shown that both quorum sensing and biofilm formation in *C. albicans* are regulated by the two-component signal transduction protein Chk1p.[1] Biofilm formation also has an important role to play in resistance to antifungal therapy which causes infections to persist. This is discussed later under antifungal drug resistance.

Clinical significance

Candida organisms are commensals, and to act as pathogens, interruption of normal host defences is necessary. Therefore, general risk factors for Candida infections include immunocompromised states, diabetes mellitus, and iatrogenic factors like antibiotic use, indwelling devices, intravenous drug use, and hyperalimentation fluids. There are several specific risk factors for particular non-albicans species: *C. parapsilosis* is related to foreign-body insertion, neonates, and hyperalimentation; *C. krusei* is related to azole prophylaxis and, along with *C. tropicalis*, to neutropenia and bone marrow transplantation; *C. glabrata* is related to azole prophylaxis, surgery, and urinary or vascular catheters; and *C. lusitaniae* is related to previous polyene use.[7]

The medical devices in use which are liable to Candida biofilm formation and subsequent infection include vascular catheters, joint prostheses, dialysis access devices, cardiac devices like prosthetic valves, pacemakers, cardioverter defibrillators, ventricular assist devices, intrauterine devices, central nervous system devices such as ventriculo-peritoneal shunts, urinary catheters and penile implants.[7]

Candidiasis associated with intravenous lines and bioprosthetic devices is especially problematic, since these devices can act as substrates for biofilm growth. Antifungal therapy alone is insufficient for cure; affected devices generally need to be removed.[5] Removal of these devices has serious implications in the case of infected heart valves, joint prostheses, and central nervous system shunts.[5]

Among prostheses, those for the knee and hip joint are at higher risk of Candida infection because of long duration of operations.[8] Risk factors for infection of prosthetic joints include prior surgery at the site of the prosthesis, rheumatoid arthritis, immunocompromised state, diabetes mellitus, poor nutritional status, obesity, psoriasis, and advanced age.[7] The surgically implanted device most commonly infected is the central venous catheter which is used for administration of fluids, nutrients and cytotoxic drugs. Patients with central venous catheters (CVC) are prone to primary bloodstream infections. Risk factors for CVC-related-infections include neutropenia for >8 days, hematologic malignancy, total parenteral nutrition, duration of site use, frequent manipulation of the catheter, improper insertion and maintenance of the catheter, and high APACHE II score.[7] C. *albicans* accounts for up to 63% of all cases of candidemia.[9] Maximal treatment of central venous catheter-related infections depends on the kind of catheter, the type of causative agent and the severity of illness.[10]
Fungal infections of haemodialysis access sites are rare; Candida accounts for 2.6 to 7% of peritoneal dialysis-related infections.[7] Use of polytetrafluoroethylene (PTFE) grafts used for permanent haemodialysis access and a larger number of graft revisions are independently associated with hemoaccess site infections.[7] Most patients with Candida infection of PTFE grafts received antecedent antibacterial agents.[7]

As many as 23% of peritoneal dialysis catheters become infected. Fungi reportedly cause up to 15% of peritonitis cases, and Candida spp. are the most common fungal isolates.[7] Recent reports indicate that about 2.6 to 7% of patients undergoing peritoneal dialysis develop Candida peritonitis. Non-albicans Candida spp. account for up to two-thirds of Candida isolates.[7] This infectious complication is associated with a high mortality (5 to 25 %) and morbidity, including prolonged hospital stay and recourse to hemodialysis.[7] Risk factors for fungal peritonitis include prior hospitalization, recent episodes of bacterial peritonitis, gastrointestinal disease, and treatment with antibiotics.[7]

Fungi are responsible for 2 to 10% of all cases of prosthetic valve endocarditis (PVE), and Candida accounts for up to 90% of these fungal infections.[7] Patients with prosthetic heart valves who develop nosocomial candidemia have a notable risk of developing Candida PVE, often months or years later (up to 690 days later). In a review of 44 cases of candidemia in patients with prosthetic heart valves, Candida PVE developed in 25% of such patients.[7]

Specific risk factors for fungal PVE include the presence of intravascular catheters, prior bacterial endocarditis, prolonged (more than 4 weeks) antibiotic treatment, total parenteral nutrition, intravenous drug use, disseminated fungal infection, prosthetic valve recipient, and immunosuppression.[7]

Candida infections of the urinary tract are strongly associated with the presence of a urinary catheter. The National Nosocomial Infections Surveillance (NNIS) data indicated that *C. albicans* caused 21% of catheter-associated urinary tract infections, in contrast to 13% of non-catheter associated infections.[7]
Risk factors for funguria include diabetes mellitus, urinary tract abnormalities, malignancy, and antibiotic use.[7]

The most commonly used devices of the central nervous system (CNS) are the ventriculoperitoneal shunts (VPS) which are made of silicone polymers. Obstruction and infections are the two most common complications, with infection occurring in 6 to 15% patients with these devices.[7] Risk factors for Candida shunt infections and meningitis include the use of broad-spectrum antibiotics, prior or concurrent bacterial meningitis, cerebrospinal fluid leakage, bowel perforation and/or abdominal surgery, steroids, and indwelling catheters. Candida is the causative agent in 1% of these infections.[7] The mortality of Candida VPS infections is estimated to be 9%.[7]

The use of intrauterine devices (IUDs) has been linked to pelvic inflammatory disease. IUDs removed from women have been shown to be severely contaminated with *Candida albicans.* Evidence for biofilms on IUDs has been proven by scanning electron microscopy and transmission electron microscopy.[11] In the case of penile implants, the reported overall rate of infection ranges from 1 to 9% and is higher (18%) in patients with reconstructive procedures or surgical revisions.[7] Bacteria account for the vast majority of cases of penile implant-related infections, whereas yeast infections are relatively rare. *C. albicans* has been reported to cause 5 to 9.2% of infections of penile implants.[7]
Known risk factors for infections of penile prostheses include urinary tract infection, spinal cord injury, insertion of an inflatable device, neurogenic bladder, diabetes mellitus, reimplantation, and revisions.[7]

Antifungal drug resistance of Candida:
Antifungal drug resistance is rapidly becoming a major therapeutic concern with the increase in immunocompromised conditions like AIDS, malignancy, steroid therapy. It has correspondingly led to a drastic increase in the incidence of opportunistic and systemic fungal infections. The most notable characteristic of microbial biofilms is their inherent ability to resist the action of antibiotics, antiseptics and other antimicrobial agents against them. Resistance of Candida biofilms to antifungal agents was first demonstrated in 1995. In this study, clinically important antifungal agents - amphotericin B, fluconazole, flucytosine, itraconazole and ketoconazole - were tested using a catheter disc assay. All of these agents showed much less activity against *C. albicans* biofilms than against planktonic cells. Biofilms of non-*C. albicans* species, such as *C. tropicalis* and *C. parapsilosis*, were also drug resistant.[2]

As mentioned earlier, several mechanisms may be responsible for drug resistance such as slow penetration of drug through the biofilm, expression of resistance genes and presence of persister cells. An alternative line of thought appears to be the slow growth of biofilms as being responsible for drug resistance. Cells in these biofilms appear to grow slowly because of the limited availability of nutrients, especially at the base of the biofilm. Growth rate has therefore been considered as an important modulator of drug activity in biofilms.[2,12] However, a study by Chandra et al,[3] related to the increase of antifungal resistance during biofilm development, showed that the progression of drug resistance was associated with increase in metabolic activity of the developing biofilm and was not a reflection of slower growth rate, which

indicates that drug resistance develops over time, coincident with biofilm maturation. This was the first report correlating the emergence of antifungal drug resistance with the development of biofilm.[4] Studies by Baillie et al used a perfused fermentor to generate *C. albicans* biofilms at different growth rates, and the susceptibility of the biofilm cells to amphotericin B was compared with that of planktonic organisms grown at the same rates in a chemostat. The results indicated that biofilms were resistant to the drug at all growth rates tested, whereas planktonic cells were resistant only at low growth rates. [12] A similar study by Al-Fattani et al showed that drug penetration of biofilms failed to completely kill the biofilm cells after prolonging incubation to 24 hours.[9]

Another alternative mechanism of drug resistance might be upregulation of genes coding for multidrug efflux pumps in biofilm cells. *C. albicans* possesses two different types of efflux pump: Adenosine triphosphate-binding cassette transporters and major facilitators, which are encoded by *CDR* and *MDR* genes, respectively. Recent work has shown that genes encoding both types of pump are indeed upregulated during biofilm formation and development. However, mutants carrying single or double deletion mutations in some of these genes were highly susceptible to fluconazole when they were growing planktonically but retained the resistant phenotype during biofilm growth. [12] Finally, mention must be made of the role of sterols in drug resistance. As is well known, sterol metabolism is the primary cellular process affected by the most widely employed antifungal drugs. Sterol analyses have revealed that ergosterol levels are significantly decreased in the intermediate and mature phases of biofilm growth compared to those in the early phases of development.[1] Hence, the diminished levels of ergosterol present in sessile *C. albicans* may reflect a physiological state more conducive to resistance in these cells. Taken together, all these observations and studies indicate that drug resistance seen with Candida biofilms is a complex phenomenon and involves multiple mechanisms.

Newer concepts

Several studies indicate that the Candida biofilm lifestyle leads to dramatically increased levels of resistance to the most commonly used antifungal agents.[1] Newer antifungal agents, such as the echinocandins(caspofungin and micafungin) and liposomal formulations of amphotericin B, have shown increased activity against Candida biofilms.[1,13] The echinocandins and their analogs, the pneumocandins, represent the newest class of antifungal drugs.[4] The potent antifungal activity of the echinocandins against Candida species was demonstrated by Cuenca-Estrella et al[4] and Quindos et al,[4] who evaluated the in vitro activity of LY303366, a semi-synthetic echinocandin B derivative, against 156 clinical isolates of Candida species and 36 *C. dubliniensis* clinical isolates, respectively. Results showed that LY303366 had potent activity against several Candida species including *C. albicans*, *C. tropicalis*, as well as *C. glabrata* and *C. krusei*, two species usually considered refractory to azoles. Similarly, 100% of the isolates were susceptible to the new antifungal drugs, indicating that echinocandins may provide new alternatives to fluconazole for treating *C. dubliniensis* infections.[4] A study by Bachmann et al[13] showed that even though combinations of Amphotericin B and Caspofungin showed in general an indifferent effect, the use of these two agents in combination against *C. albicans* biofilms may still benefit from the rapid killing by high concentrations of Amphotericin B and the more sustained effect of physiological concentrations of Caspofungin. This approach to therapy could be appealing in a clinical setting, particularly if biofilm resistance is due to the presence of a few "persister" cells able to withstand antimicrobial treatment.[13] The excellent invitro activity of echinocandins demonstrated against fluconazole-resistant Candida species strains indicates that the echinocandins are very promising as novel antifungal agents with important implications for the treatment of infections by these yeasts.[4] Their unique mode of action and their specificity to fungal cell walls result in minimal toxicity to mammalian cells.[4]

Another concept that holds considerable promise is the modification of biomaterial surfaces used in medical devices. Hydrogel technology is a process that applies biocompatible water absorbable polymer hydrogels to medical device surfaces. Most microorganisms find it difficult to adhere to hydrogel coated surface.[14]

A study by Chandra J et al[6] has identified 6% polyethylene oxide(6PEO) as a surface modifying agent which inhibits *C. albicans* biofilm formation. A polyetherurethane (Elasthane 80A[E80A]) was modified using 6PEO and used in the study to see Candida biofilm formation. There was no detectable biofilm formation on the E80A-6PEO surface and this was confirmed by confocal laser scanning microscopy. These results have significant implications in the clinical set-up for the design of novel biomaterials which inhibit biofilm formation.

Another approach that merits consideration is the use of non-toxic, anti-infective agents which will act as a chemical barrier against invading microorganisms. The use of silver on orthopaedic implants, silver sulfadiazine and chlorhexidine in a

polyurethane matrix on central venous catheters has been found to significantly reduce the frequency and qualitative levels of microbial colonization.[15] Shuford et al in their study[16] have demonstrated the effect of fresh garlic extract (FGE) on *C.albicans* biofilms. Though only one clinical strain was tested, FGE holds promise and merits further investigation for determination of the antifungal activity of FGE against *C. albicans* biofilms.

Conclusions

To conclude, biofilm formation needs to be recognized as an important virulence trait exhibited by Candida species. This ability to form biofilms is intricately linked with the ability of the organisms to adhere, colonize and subsequently cause infection in susceptible individuals. Biofilm formation helps the organism to evade host defences, exist as a persistent source of infection and develop resistance against antifungal drugs. Recent research studies have focused on the mechanisms behind morphogenetic conversions, differential gene expressions and cell-cell signalling which may hold the key to future therapeutic interventions. Newer drug strategies such as the use of amphotericin B formulations and echinocandins hold considerable promise in the treatment of invasive systemic Candida infections by enhancing retention of affected intravascular devices and obviating the need for valve surgery in Candida endocarditis.[4] More importantly, these antifungal drugs may be useful in management of biofilm infections by fungi and may have other clinical applications including those of oral diseases and prostheses rejection. Research on newer technologies have demonstrated that surface modifying agents (6PEO) having antibiofilm properties when incorporated in biomedical device materials can inhibit biofilm formation of Candida. In-depth knowledge of ultrastructure of microbial biofilms and the use of novel treatment therapies will lead to reduction in device-related infections caused by Candida.

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