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Review

Strength in numbers: antifungal strategies against fungal biofilms

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ABSTRACT

Pathogenic fungi have the capacity to form tenacious biofilm structures that are notoriously unresponsive to antifungal therapies. Fungal biofilms are ubiquitous, located all over the human host, including the oral cavity, respiratory tract, gastrointestinal tract, urinary tract, wounds and upon biomedical devices. This latter category represents one of the greatest hurdles in clinical management, where the presence of inert substrates such as a catheter provides a reservoir for fungal biofilm development. Here, *Candida albicans* is the most adept at forming biofilms and is the principal nosocomial fungal pathogen based on its high rates of mortality, which are often associated with the biofilm lifestyle. This review will summarise some of the key fungal biofilm-forming organisms and their clinical significance and will discuss current and novel strategies to manage these hard-to-treat infections based on in vitro and in vivo studies.

1. Introduction

Microbes, if unchallenged, have the capacity to flourish and persist as individual planktonic cells within their respective environments; however, this is seldom the case as it is suggested that 80% of bacteria have a preference for social integration rather than living as free-floating cells. Bacterial and fungal micro-organisms have a social predilection to exist within complex and dense communities of interspersed cells. These populations are defined as biofilms, which are characteristically heterogeneous, encased within an extracellular matrix (ECM) that is highly recalcitrant to antimicrobial factors [1]. Within the clinical realm, these infections are highly important and are often overlooked despite the devastating consequences if not managed appropriately.

Microbial biofilms are governed by the notion of strength in numbers and this underlying concept makes them an important and bourgeoning area of research. None more so than with fungal biofilms, where in recent years there has been a growing appreciation that pathogenic fungal species have the innate ability to form biofilms that impact clinical practice [2]. *Candida albicans* represents the paradigm of these fungal biofilm-forming organisms and has now been extensively studied and characterised. This polymorphic fungus exists in yeast and filamentous morphologies, helping to create and support a structurally complex and dense biofilm. The process is initiated by the adhesion of yeast cells via adhesins followed by microcolony formation, subsequently spreading into one another during maturation to form an intertwined meshwork of hyphae interspersed with budding yeast cells covered with a thick layer of ECM [3]. For further information on the characteristics of fungal biofilm formation, please refer to the following reviews [4–6]. Throughout the development of these biofilms, a series of key resistance factors participate in maintaining the survival and propagation of the biofilm, which may ultimately lead to clinical failure. This review will explore the breadth and scope of fungal biofilms and provide new insights into their management through conventional and novel antifungal therapies.

2. Fungal biofilms are ubiquitous

Whilst fungal biofilms are arguably at their most dangerous amongst the myriad of tubes and devices attached to patients within the intensive care unit (ICU), they are however not limited to this environment. They are clinically ubiquitous, ranging from denture-related stomatitis in the oral cavity to complex polymicrobial biofilms of traumatic wounds [2]. They are also located within the upper and lower airways as well as the gastrointestinal and genitourinary tracts. The breadth and scope of these infections is vast, as a closer anatomical survey reveals numerous examples of important fungal biofilms residing on the compromised human host.

The oral cavity, for example, represents an opportunistic environment for these structures, particularly residing upon dentures [7]. Denture-induced stomatitis, a candidal disease, is associated with biofilms forming on the surface adjacent to the oral mucosa [8]. Positive correlations have been demonstrated between the

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severity of this disease and a biofilm phenotype [9]. It is also possible that mixed *Candida* species biofilms synergise with one another to elicit infection [10]. Moving down into the airways, paranasal sinus fungus balls have been described [11,12], which have been likened to fungal biofilms [13,14]. For example, infection of a zygomatic implant with *Aspergillus fumigatus* within the maxillary sinus has been reported [15]. *Candida albicans* and *Candida glabrata* have also been described in the context of voice prostheses biofilms [16,17]. These are clinically important as they restrict airflow [18] and impede speech, swallowing and respiration [19]. Ventilator-associated pneumonia (VAP) is also implicated, where it has been shown that *Candida* spp. isolated alone or in combination from respiratory secretions in those with suspected VAP are associated with increased mortality compared with patients with bacteria isolated [20]. Methods to prevent these biofilm infections include care bundles that include infection control measures such as oral decontamination with chlorhexidine, dramatically cutting the rates of VAP in the intensive care setting [21,22].

Deeper into the lower airways, filamentous moulds such as *A. fumigatus* can cause a spectrum of respiratory diseases, including aspergillomas, invasive aspergillosis and allergic bronchopulmonary aspergillosis [23]. Numerous intertwined hyphae are often observed histologically from clinical specimens in the form of a complex multicellular structure [24], which is indicative of a biofilm phenotype [13,14]. An aspergilloma has the most apparent biofilm characteristics, whereas aspergillary bronchitis is characterised by bronchial casts containing mycelia forming compact masses [25]. The evidence is therefore credible that *Aspergillus* spp. form medically important biofilms [5,26]. For example, a patient group synonymous with biofilm infections, i.e. cystic fibrosis patients, has been shown to harbour numerous fungi in addition to *A. fumigatus*, including other *Aspergillus* spp., *Candida* spp., *Scedosporium* spp. and *Exophiala* spp. [27–30].

The mucosa of the gastrointestinal tract is heavily laden with microbial biofilm communities [31]. *Candida* spp. colonisation of the gastrointestinal tract is common, accounting for 30–80% in normal healthy adults [32]. It is therefore not surprising that *C. albicans* and *Candida tropicalis* have been shown to colonise percutaneous endoscopy gastronomy tubes and contribute to degradation of the polyurethane [33,34]. This may lead to diarrhoea or possibly cause translocation of microbes across the epithelial barrier leading to sepsis. It has also been suggested that *Candida* colonisation may enhance inflammation in the gastrointestinal tract [35]. Mucosal fungal biofilms are also found within the urinary tract, where it is suggested that 75% of woman experience vulvovaginal candidiasis at some point in their life. *Candida* spp. have been associated with pyelonephritis, cystitis and prostatitis [36,37], with biofilms formed by these species detected on ureteral stents [38]. These are a significant risk factor in the ICU for healthcare-associated fungal infections [39]. Whilst relatively rare, aspergillomas can be detected within the urinary tract [40,41].

Evidence is also slowly emerging that pathogenic fungal species play a role in wound infections [42], and whilst generally uncommon, various factors have led to an increased awareness, and even the development of novel diagnostics [43]. These infections are increasingly common in combat trauma [44,45]. Moreover, molecular analysis of 915 chronic wound infections, including pressure ulcers, diabetic foot ulcers, non-healing surgical wounds and venous leg ulcers, showed that 208 (23%) contained pathogenic fungi, of which *Candida* spp. were the most abundant [46].

3. Nosocomial fungal biofilms

The hospital environment represents a significant reservoir for fungal biofilms, particularly given that many within the patient population possess temporary and permanent biomaterials. This is confounded by the use of broad-spectrum antibiotics, immunosuppression due to chemotherapy and radiotherapy, and disruption of mucosal barriers owing to surgery [47]. These factors explain why *Candida* spp. are so important in the hospital setting and why they are reported as the fourth most common bloodstream infection [48]. This statistic translates to unduly high mortality rates from invasive candidiasis, which is exemplified in a recent study reporting that 63.5% of 224 hospitalised patients with septic shock and with a positive blood culture for *Candida* spp. died [49]. This study is of particular interest as it showed quite clearly that if adequate source control and antifungal therapy were administered within 24 h then the mortality rate was reduced to 52.8%, whereas if these were not implemented within 24 h then this rose significantly to 97.6% ($P < 0.001$). Multivariate logistic regression analysis showed that delayed antifungal treatment led to an adjusted odds ratio (OR) of 33.75, with a 95% confidence interval (CI) of 9.65–118.04 ($P = 0.005$). These figures make for compelling reading and suggest that not only do we have diagnostic failings in detecting these infections, but that the nature of the infection may be a critical factor, i.e. biofilm lifestyle. This is verified by a recent meta-analysis from seven prospective randomised clinical trials where it was reported that removal of a central venous catheter (CVC) is associated with decreased mortality (OR = 0.50, 95% CI 0.35–0.72; $P = 0.0001$) [50]. Judging from these data, biofilm-related fungal disease is more problematic than is currently appreciated.

Intravascular catheters frequently become colonised with *Candida* spp., especially in the ICU. Upon these biomaterials they have the capacity to develop into adherent biofilm structures glued intraluminally and extraluminally to the specific catheter substrate. From here they are able to disperse into the bloodstream resulting in candidal sepsis and dissemination to distal organs. The importance of biofilm-mediated disease is clearly apparent when laboratory and clinical data are considered in combination. For example, it has been demonstrated that cells detaching from biofilms are more cytotoxic than their planktonic counterparts, which also significantly increases mortality within a murine model of infection [51]. Moreover, a retrospective clinical investigation performed using multivariate analysis to analyse the risk factors associated with patients with candidaemia reported in addition to inadequate antifungal therapy (OR = 2.35; $P = 0.03$) and Acute Physiology and Chronic Health Evaluation (APACHE) III scores (OR = 1.03; $P < 0.001$), that biofilm formation (OR = 2.33; $P = 0.007$) was an independent predictor of mortality [52]. Both *C. albicans* ($P < 0.001$) and *Candida parapsilosis* ($P = 0.007$) were shown to correlate with increased mortality. Furthermore, an interesting prospective case-controlled follow-up study showed that candidal sepsis caused by isolates with the capacity to form biofilms could be independently predicted by the presence of CVCs (OR = 6.44, 95% CI 3.21–12.92), urinary catheters (OR = 2.40, 95% CI 1.18–4.91) and total parenteral nutrition (OR = 5.21, 95% CI 2.59–10.48) [53]. Subsequent analysis revealed that hospital mortality, costs of antifungal therapy, and post-candidal sepsis hospital length of stay for those who survived were significantly greater in those patients with an isolate able to form biofilms. Moreover, those patients with high biofilm-forming isolates treated with antifungal agents considered highly active against biofilms, e.g. echinocandins and liposomal polyenes, had significantly shorter hospital length of stay than those treated with azoles. Overall, the presence of biofilm-forming isolates equated to an increased risk for hospital death compared with non-biofilm-forming isolates (OR = 1.77). Interestingly, in this study it was reported that *C. albicans* biofilm production was significantly less frequent (26.2%, $n = 122$) than non-*albicans Candida* spp. (61.1%; $n = 85$) ($P < 0.001$) [53], an observation also reported elsewhere [54]. Indeed, one of the first documented episodes of biofilm-related disease was associated with the non-*albicans* species *C. glabrata*