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Fungal biofilms: From bench to bedside

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ABSTRACT

Biofilms cause recurrent invasive infections that are difficult to eradicate because of their high resistance to antimicrobials and host defence mechanisms. Fungal biofilm-related infections are associated with high mortality rates. Although current guidelines recommend catheter removal for catheter-related bloodstream infections due to *Candida* species, several studies have shown that the efficacy of the antifungal lock technique. The use of combinations of antifungal agents may improve the management of biofilm-related fungal infections and prevent the emergence of resistance associated with monotherapy. Since the presence of mixed bacterial-fungal biofilm infections is very prevalent, a combination of antibacterial and antifungal agents should be considered.

Key words: *Candida*, biofilm, antifungal, lock technique

Biopelículas fúngicas: Del laboratorio a la práctica clínica

RESUMEN

Las infecciones relacionadas con biopelículas fúngicas se asocian con altas tasas de mortalidad. La infección asociada a catéteres son un ejemplo. Aunque las guías actuales recomiendan la retirada del catéter para tratar estas infecciones, varios estudios han demostrado la eficacia de la técnica de sellado antifúngico. El uso de combinaciones de agentes antifúngicos puede mejorar el pronóstico de las infecciones fúngicas relacionadas con biopelículas y prevenir la aparición de resistencias. Las infecciones asociadas a biopelículas mixtas (bacteria-hongo) son cada vez más frecuentes y requieren de un abordaje específico para conseguir su erradicación.

Palabras clave: *Candida*, biofilm, antifúngicos, técnica de sellado

INTRODUCTION

A biofilm is a community of microorganisms embedded in a self-produced matrix of extracellular polymeric substance (EPS) that can adhere to biotic or abiotic surfaces and so facilitate survival in a large number of environments, including medical devices. Biofilm-associated organisms are responsible for more than 60% of all microbial infections in humans. Biofilms cause recurrent invasive infections that are difficult to eradicate because of their high resistance to antimicrobial treatments and host defence mechanisms and their excellent ability to adhere to biomaterials [1].

In many cases, colonization precedes infection. The main involved microorganisms are usually commensal flora, including bacteria and fungi. Gram-positive cocci, mainly coagulase-negative staphylococci, are involved in more than 70% of foreign body related infections. The fungal pathogen most commonly associated with biofilm infections is *Candida albicans* and the resulting infection is associated with a high mortality. Other biofilm-forming *Candida* species include *C. parapsilosis*, *C. tropicalis*, *C. krusei* and *C. glabrata*. *Cryptococcus neoformans*, *Coccidioides immitis*, *Aspergillus* spp., *Fusarium* spp., *Blastoschizomyces capitatus*, *Malassezia pachydermatis*, *Pneumocystis* spp.,*Trichosporon asahii*, *Rhizopus* spp. and *Rhizomucor* spp. are also described as causative agents of biofilm-related fungal infections [2]. Common sites for fungal infections are the oral cavity, lungs (mainly in ventilated patients), burn wounds, the lower reproductive tract, skin and intravascular catheters, the gastrointestinal tract and at insertion sites of urinary catheters.

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C. albicans biofilm, which is the fungal biofilm model that has been best studied, comprises two main kinds of cell: small oval yeast-form cells (blastospores) and long tubular hyphal cells. The formation of *C. albicans* biofilms involves four stages: First, *adherence* of the yeast-form cells to a substrate; second, initiation and proliferation of biofilm formation, in which the yeast cells proliferate across the surface producing elongated projections that grow into filamentous forms containing hyphae and pseudohyphae (*proliferation* stage); third, *maturation* into a complex, structured biofilm, in which the cells encased in the extracellular matrix display increased drug resistance. Finally, *dispersal* of the yeast-form cells from the biofilm to colonize the surrounding environment [3].

Studies of the regulation of fungal biofilm formation have gradually become much more important in recent years, with the discovery of the genes and regulatory mechanisms involved and the identification of molecules with potential quorum-sensing functions in biofilm maturation. Many biofilm genes encode cell wall proteins that can play a direct role in cell-substrate or cell-cell adherence. Other genes involved in biofilm formation encode predicted transcription factors or protein kinases. Several alcohol dehydrogenase and aryl-alcohol dehydrogenase genes also have an impact on biofilm formation [4].

An important factor involved in biofilm formation is the nature of the material to which the microbial cells adhere. In the case of human infections, this means the chemical composition of materials used in medical devices. *Candida* spp. has the ability to form biofilms on the surfaces of a variety of medical devices, such as those made of polymethyl methacrylate (PMMA), silicone, elastomer, polyurethane, polyvinyl chloride, polypropylene and polystyrene. Other important factors involved in biofilm formation include the conditioning film, *Candida* morphogenesis, the fungal strain, bacterial competition/cooperation and the location of the implanted medical device [5].

CLINICAL RELEVANCE OF BIOFILM FORMATION

Candida spp. can cause severe disseminated disease associated with high mortality, particularly in patients with implanted medical devices or compromised immune systems. Wisplinghoff et al. analyzed data from a prospective nationwide surveillance study carried out over a 7-year period in US hospitals that included 24,179 cases of nosocomial bloodstream infection (BSI) [6]. *Candida* spp., isolated mainly from patients being cared for in intensive care units (ICU), was the fourth causal agent detected (9%) and was associated with the highest crude mortality rate (39.2%). The mortality rate was even higher in patients admitted to the ICU (47.1%). *C. albicans* was the most common yeast isolated (54%), followed by *C. glabrata* (19%), *C. parapsilosis* (11%) and *C. tropicalis* (11%). The crude mortality rate was lowest for *C. albicans* infection (37%) and highest for *C. krusei* infection (59%). The rate of *Candida* spp. isolated from blood cultures increased from 8% to 12% over the 7-year period. In another study, Tumbarello et al. evaluated the risk factors for mortality in 294 hospitalized patients with *Candida* BSI, specifically testing to determine whether biofilm formation was a risk factor associated with a worse evolution [7]. More than 25% of these patients were infected by biofilm-forming isolates. The mortality rate in patients with BSI due to biofilm-positive isolates (70%) was significantly higher than in those due to biofilm-negative isolates (45.7%) and infection by biofilm-forming *Candida* spp. was an independent risk factor for mortality.

BIOFILMS AND ANTIFUNGAL RESISTANCE

Biofilms are often associated with high-level antimicrobial resistance. *Candida* spp. can develope antimicrobial resistance during treatment with antifungals via expression of the following mechanisms: changes in the fungal cell wall that reduce the absorption of the antifungal agent; changes in drug-target affinity; and increased overexpression of the membrane transport proteins that facilitate the efflux of antifungal drugs. The major genes that contribute to drug resistance are those encoding transport proteins that efflux multiple drugs. The *Candida* genome has gene families that encode the ATP-dependent transporters (ABC) and major facilitators (MDR), the CDR and MDR genes, respectively, which are regulated during the formation and development of the biofilm [5]. Nonetheless, the main survival mechanisms of biofilm cells against antifungals are the physical barrier to the entry of antifungals created by the extracellular matrix, and the increased cell density, enhanced stress response and decreased metabolic activity shown by the biofilm cells. In fact, biofilm-forming cells are able to survive in the presence of high concentrations of antimicrobial agents, even though the same cells are susceptible in the planktonic phase. This phenomenon is known as recalcitrance [8].

LOCAL ANTIFUNGAL TREATMENT

For catheter-related BSI due to *Candida* species, the Clinical Practice Guidelines for the Diagnosis and Management of Intravascular Catheter-Related Infection by Mermel et al. recommend catheter removal and treatment with antifungal therapy for 14 days after the first negative blood culture [9]. However, the antifungal lock technique has been considered an option in selected patients (haemodynamically stable patients with severe coagulopathy or with limited or no other options for vascular access) [8]. Several studies have shown the efficacy of antifungal agents as catheter lock solutions. Cateau et al investigated *in vitro* the optimal antifungal lock treatment details against *C. albicans* biofilms. Equinocandins significantly reduced the metabolic activity of *C. albicans*, suggesting that they could be good candidates for use in catheter lock solutions [10]. Successful results have also been obtained in animal models, with a catheter salvage rate of more than 80% (54/64), mainly using amphotericin B lipid complex as the antifungal agent. There are a limited number of published case reports describing the use of antifungal lock therapy in

various patient populations. Amphotericin B deoxycholate was the most commonly used antifungal agent, with a catheter salvage rate of 76.9% (10/13), followed by liposomal amphotericin associated with a 60% catheter salvage rate (3/5) [11]. *In vitro* studies showed anidulafungin to be a promising option in antifungal lock therapy [12].

OTHER THERAPEUTIC OPTIONS

The use of combinations of antifungal agents may improve the management of biofilm-related fungal infections and prevent the emergence of resistance associated with monotherapy. Some in vitro studies analyzed the activity of various antifungal combinations against *Candida* biofilms. The best results were obtained with the amphotericin B/posaconazole combination (synergistic against 100% of strains tested) compared with other combinations that yielded indifferent (amphotericin B/caspofungin, amphotericin B/fluconazole, fluconazole/caspofungin), or even antagonistic effects (voriconazole/micafungin) [13]. Promising results were also obtained from *in vitro* studies that analysed the activity of amphotericin B in combination with antibiotics (rifampicin or clarithromycin) against *C. albicans*, *C. parapsilosis*, *C. glabrata*, *C. krusei* and *C. tropicalis* biofilms [14].

MIXED BIOFILMS

Since the human microbiota is diverse and includes a variety of bacteria and fungi, multispecies or polymicrobial biofilms are often found. In the vast majority of cases, *C. albicans* interacts with bacteria in the specific niche where the biofilm infection originates, such as *S. epidermidis* in the skin or *Streptococcus* spp. in the oral cavity. Bacterial cells can bind directly to *C.* albicans hyphal cells in the biofilm, or they can be linked through the mediation of other factors such as fungal adhesion proteins, hyphal wall proteins or regulators of transcription. The bacterial-fungal interaction may be synergistic, with the mixed biofilm offering one or both species physical protection or enhancing the virulence of both species compared with the monomicrobial infection, or it can have an inhibitory effect, causing the destruction of one of the microbial agents [3]. In addition, the susceptibility of the microorganisms in a mixed biofilm may be higher than in the monomicrobial counterpart. These complex interactions are likely to have significant clinical implications, so that understanding the mechanisms involved in adhesion and signalling in fungal–bacterial interactions could lead to the development of novel therapeutic strategies for impeding microbial colonization and the development of polymicrobial disease [15].

CONCLUDING REMARKS

Biofilm infections are difficult to eradicate because of their high resistance to antimicrobial treatments, mainly due to efflux pumps and persister cells forming within biofilms. Not all antifungals have the same activity against *Candida* biofilms. Since the biofilm-forming capacity of *Candida* is a determinant of mortality, better knowledge of this form of fungal development is necessary to develop new therapeutic strategies. Further studies are needed to understand the complexity of polymicrobial biofilm infections and interspecies interactions.

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