Rev Iberoam Micol. 2014;31(1):22–29

Revista Iberoamericana de Micología

www.elsevier.es/reviberoammicol

Mycologic Forum

Highlights in pathogenic fungal biofilms

Janaina De Cássia Orlandi Sardi[a,c], Nayla De Souza Pitangui[a,c], Gabriela Rodríguez-Arellanes[b], Maria Lucia Taylor[b], Ana Maria Fusco-Almeida[a,d], Maria José Soares Mendes-Giannini[a,*,d]

[a] Department of Clinical Analysis, Laboratory of Clinical Mycology, Faculty of Pharmaceutical Sciences, Universidade Estadual Paulista (UNESP), Araraquara, São Paulo, Brazil
[b] Fungal Immunology Laboratory, Department of Microbiology and Parasitology, School of Medicine, National Autonomous University of Mexico (UNAM), Mexico City, Mexico

ARTICLE INFO

Article history:
Received 19 August 2013
Accepted 27 September 2013
Available online 16 November 2013

Keywords:
Biofilm
Quorum sensing
Pathogenic fungi
Adhesins
Anti-biofilm therapy

ABSTRACT

A wide variety of fungi have demonstrated the ability to colonize surfaces and form biofilms. Most studies on fungal biofilms have focused on *Candida albicans* and more recently, several authors have reported the involvement of other genera of yeasts and *Candida* species, as well as of filamentous fungi in the formation of biofilms, including: *Cryptococcus neoformans*, *Cryptococcus gattii*, *Rhodotorula* species, *Aspergillus fumigatus*, *Malassezia pachydermatis*, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, *Pneumocystis* species, *Coccidioides immitis*, *Fusarium* species, *Saccharomyces cerevisiae*, *Trichosporon asahii*, Mucorales and *Blastoschizomyces*. There is a current interest in describing the particular characteristics of the biofilm formation by of these fungi. A major concern is the control of biofilms, requiring knowledge of the biofilm mechanisms. However, our knowledge of these microbial communities is limited, due to the complexity of these systems and metabolic interactions that remain unknown. This mini-review aims to highlight recently discovered fungal biofilms and to compare them with the current knowledge on biofilms.

This manuscript is part of the series of works presented at the "V International Workshop: Molecular genetic approaches to the study of human pathogenic fungi" (Oaxaca, Mexico, 2012).

Aspectos sobresalientes en la formación de biopelículas por hongos patógenos

Palabras clave:
Biopelículas
Quorum sensing
Patógenos fúngicos
Adhesinas
Tratamiento anti-biopelículas

RESUMEN

Una amplia variedad de hongos poseen la capacidad para colonizar superficies y formar biopelículas (biofilms). La mayoría de los estudios efectuados sobre biopelículas de hongos han prestado atención a *Candida albicans* y, más recientemente, varios autores han descrito la implicación de otros géneros de levaduras y especies de *Candida*, al igual que de hongos filamentosos, en la formación de biopelículas, incluidos *Cryptococcus neoformans*, *Cryptococcus gattii*, especies de *Rhodotorula*, *Aspergillus fumigatus*, *Malassezia pachydermatis*, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, especies de *Pneumocystis*, *Coccidioides immitis*, especies de *Fusarium*, *Saccharomyces cerevisiae*, *Trichosporon asahii*, mucorales y *Blastoschizomyces*. En la actualidad suscita interés la descripción de las características particulares de la formación de biopelículas de estos hongos. Una preocupación importante es el control de las biopelículas, que requiere una comprensión de los mecanismos de su formación. Sin embargo, nuestros conocimientos sobre estas comunidades microbianas son limitados debido a la complejidad de estos sistemas y a las interacciones metabólicas que aún no conocemos. Esta revisión tiene como objetivo poner de relieve las biopelículas fúngicas descubiertas recientemente y compararlas con los conocimientos actuales disponibles sobre ellas.

Este artículo forma parte de una serie de estudios presentados en el «V International Workshop: Molecular genetic approaches to the study of human pathogenic fungi» (Oaxaca, México, 2012).

* Corresponding author.
E-mail address: gianninimj@gmail.com (M.J.S. Mendes-Giannini).
[c] Equal contribution to the development of the review.
[d] Supervisors.

1130-1406/$ – see front matter
http://dx.doi.org/10.1016/j.riam.2013.09.014

It is estimated that 95% of the microorganisms found in nature are attached in biofilms. According to Costerton et al.,[21] a biofilm can be defined as a complex structured community of microorganisms, surrounded by an extracellular matrix of polysaccharides, adhered to each other at a surface or interface. This three-dimensional structure may become integrated naturally into any solid surface in contact with non-sterile water.[139] Hence, these structures started to have great importance in diverse human activities. McCoy et al.[80] were the first to describe the formation of biofilms in pipes. From this study, greater attention was given by researchers to this topic, after all the negative aspects of biofilm formation, and led the scientific community to seek alternatives to eliminate harmful biofilms that would cause damage to equipments through biocorrosion, product contamination,[59] and represent significant losses to industries globally. If on one hand the biofilms can cause serious damage, on the other side they can be used in numerous bioprocesses. Examples include production of vinegar,[10] citric acid,[114] pharmaceutical applications through the production of secondary metabolites,[96] and biological processes for extracting metals from ores.[109] Recognition of biofilms, from the 1980s on, contributed to recognize numerous persistent infectious diseases persistent as being caused by biofilms.[22] Some infections caused by the use of medical devices in hospital environments such as catheters, are also related to biofilms.[32] The extracellular polymers (EPS) matrix, which holds the biofilm cohesive, is also responsible for the persistence of biofilm-related infections,[20] and protects microorganisms from disinfectants. Besides, resistance to UV radiation and dehydration (EPS matrix hydrated) has been demonstrated.[14,139] This report aims to review the advances in fungal biofilms and in adhesins genes involved in biofilm formation, quorum sensing (QS), as well as to cover some new therapeutic strategies against fungal biofilms.

Fungal biofilms

Infections associated with the formation of biofilms are recognized as a significant and growing clinical problem; therefore, research in mycology has been increasingly focused on in biofilm phenotyping.[57] Recent advances in molecular techniques and confocal microscopy have shown that the formation of biofilms is the natural and preferred form of fungal growth and a major cause of persistent human infections. Microorganisms in biofilms grow in multicellular communities and produce an extracellular matrix that provides protection against from host defense mechanisms and antifungal drugs.[22]

A wide variety of fungi have demonstrated the ability to colonize surfaces and form biofilms. Most studies on fungal biofilms have focused on *Candida albicans* and more recently, several authors have reported the involvement of other genera of yeasts and *Candida* species as well as of filamentous fungi in the formation of biofilms, including: *Cryptococcus neoformans*, *Cryptococcus gattii*, *Rhodotorula* species, *Aspergillus fumigatus*, *Malassezia pachydermatis*, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis* (unpublished data), *Pneumocystis* species, *Coccidioides immitis*, *Fusarium* species, *Saccharomyces cerevisiae*, *Trichosporon asahii*, Mucorales, and *Blastoschizomyces*.[13,25,26,28,31,75,88,99,104,110,122,137]

There is growing interest in uncovering the true participation of fungal biofilms in human disease. These formations play an important role in the development of infections, since microorganisms that grow in biofilms exhibit unique phenotypic characteristics when compared to their planktonic counterparts.[104] These characteristics include increased resistance to host defense mechanisms and antibiotic therapy.[78]

The adherence of a biofilm to the host may trigger an acute fungemia and/or disseminated infection. This occurs when cell clusters are dispersed from the initial biofilm and occupy a niche not previously colonized.[106] A recent study developed by Uppuluri et al.[133] demonstrated that cells that detach from a biofilm have a greater association with mortality as compared to planktonic microorganisms. In fact, over 65% of human infections involve the formation of biofilms, which is related to the increasing use of biomaterials in medical practice and the increasing number of immunocompromised patients.[19,107] In addition, more than 500,000 deaths per year are caused by biofilm-associated infections.[89]

As a result, biofilms have important and, often, deleterious effects on human health. Fungal biofilm formation on catheters and prostheses contributes to the development of nosocomial infections.[135] According to Kojic et al.,[63] the persistence of fungal infections occurs due to the ability of a fungus to form biofilms on a wide variety of medical devices and because of persisting cells representing an important mechanism of resistance.[115] Once infected, the in vivo eradication of a biofilm usually requires the administration of toxic concentrations of antimicrobials, and the recommended treatment includes removal of the contaminated device; however, this is a difficult and costly procedure that can result in medical complications.[43] Therefore, fungal biofilms have become a major clinical and economic problem.

Multidrug tolerance is caused by a small subpopulation of microbial cells termed persisters that become a reservoir from which recurrence of infection may be developed. These cells are responsible for an important mechanism of resistance in chronic infections extensively studied in bacteria,[7,106,115] which have attracted some attention recently in the context of fungal biofilms.[9] In *C. albicans* biofilms, a small subset of yeast cells have been described that is highly resistant to amphotericin B, following adhesion, and this is independent of the upregulation of efflux pumps and cell membrane composition. *C. albicans* persisters were detected only in biofilms and not in diverse planktonic populations.[65] When a biofilm was killed with amphotericin B and reinoculated with cells that survived, a new biofilm was produced with a new subpopulation of persisters; this suggests that these cells were not mutants but phenotypic variants of the wild type. The basis of this drug resistance is not clear and involves different mechanisms, including the presence of a small number of persisters, which are cells that survive high doses of an antimicrobial agent. Unlike bacterial persisters, *C. albicans* persisters have so far been observed only in biofilms and not in planktonic populations. Identification of important cellular components that are responsible for the occurrence of persisters in fungal biofilms could open the way to the rational design of antibiofilm agents.[68,115]

Recent findings have reported the involvement of new fungal genera and species in the formation of pathogenic biofilms and it is important to look for the role they can play in infections. There is a current interest in describing the particular characteristics of biofilm formation of the species *Rhodotorula*, *A. fumigatus*, *M. pachydermatis* and the dimorphic fungi *H. capsulatum*, *Coccidioides* spp., and *Paracoccidioides* spp.[37,89,99,105,106]

It was also recently demonstrated that *Rhodotorula* species are able to form biofilms. The increase in invasive infections caused by emerging pathogens such as *Rhodotorula* is related to the increased occurrence of degenerative and malignant diseases in different populations, the growing number of patients who undergo organ transplantation therapies that include immunosuppression, broad-spectrum antibiotics and invasive medical procedures[131]; and the use of implantable medical devices, such as central venous catheters, which facilitate the formation of biofilms by these pathogens, causing fungemia followed by eye infections, peritonitis, and meningitis.[29,116,131,132] Nunes et al.[94] studied various isolates of *Rhodotorula* species and noted that this genus is able to form biofilms, which could play a role in the pathogenesis

of infections caused by these species. Canabarro et al.[12] isolated *Rhodotorula* sp. in association with *C. albicans* subgingival biofilms from patients with severe chronic periodontitis.

Recent reports describe the growth of biofilm structures for the filamentous fungus *A. fumigatus*.[60,106] This species is responsible for approximately 90% of cases of invasive aspergillosis, a severe infectious disease characterized by high mortality rates.[51,105] *Aspergillus* colonization and biofilm formation predominantly occurs in patients with genetic functional lung abnormalities, such as cystic fibrosis or chronic obstructive pulmonary disease.[52,86] Biofilms of *Aspergillus* can affect diverse biomaterials, such as catheters, prostheses, cardiac pacemakers, heart valves and breast implants.[35,61] In addition, a spherical mass of hyphae, called aspergilloma, can form in the respiratory tract[106] or the urinary tract.[67,79] All clinical antifungal drugs are significantly less effective under the biofilm or spherical hyphae conditions, suggesting that there is need for high dosages or antifungal combination therapy for better penetration of drugs in biofilms.[87]

Another pathogen that has received growing attention is the fungus *M. pachydermatis*, capable of forming in vitro biofilms on devices commonly used in the medical practice, including polystyrene microplates and polyurethane catheters.[13] *M. pachydermatis* is a commensal yeast found on the skin and mucosa of healthy dogs and cats,[11] but has become an important pathogen of human fungemia in intensive care units[5] and has been isolated from preterm neonates, children and adults. These infections are directly associated with the formation of biofilms on catheters in patients receiving parenteral nutrition with lipid formulations.[18,24]

Recently, an in vitro study demonstrated the efficiency of *H. capsulatum* to form biofilms on abiotic surfaces.[99] *H. capsulatum* is the causative agent of histoplasmosis, a systemic fungal disease that has become a major health problem in Latin America and worldwide.[93] High concentrations of this fungus are found in areas with bird and bat droppings, such as caves, chicken coops, or even urban buildings.[1,56,123] A study by Pitangui et al.[99] determined the pattern of infection of *H. capsulatum* in epithelial cells, characterized as a compact mass of yeast cells, which possibly leads to the formation of a complex three-dimensional architecture of biofilms and promotes the internalization of yeast into host cells. A previous study by Suarez-Alvarez et al.[124] described the profile of *H. capsulatum* yeast adhesion on different bat organs. That study also noted that the yeasts are found in clusters in the lung parenchyma, spleen, liver, and intestine. Recent advances in high-throughput methods for the investigation of biofilms opened the possibility of starting an "omics" approach to study these complex structures in the next decade. Additionally, in vivo studies are needed to define the true role and growth regulation of *H. capsulatum* biofilms.

Paracoccidioidomycosis is a systemic mycosis of great relevance in Latin America, especially in Brazil, which has the highest concentration of endemic areas, as more than 80% of the reported cases occurred in this country. The causative agents are the dimorphic fungi *P. brasiliensis* and *P. lutzii*.[4,72] These fungi have several virulence factors that can cause harm to the host. The adhesion, colonization and characteristics of these fungi enable them to withstand the hostile environments of the host and are correlated with the development of disease.[39,84] Adhesion is a widely distributed phenomenon that is shared by many microorganisms, enabling them to colonize in their habitats. Many fungi, especially pathogenic fungi, are able to adhere to host tissues, which is the first step in the process of biofilm formation. The present authors were able to demonstrate biofilm formation by *P. brasiliensis*. Those experiments were performed in vitro, with the fungus forming biofilms at low oxygen tensions (unpublished data).

Davis et al.[28] described recurrent coccidioidal meningitis and *C. immitis* biofilm was found on the tip of the ventricle-peritoneal shunt tubing despite the patient's taking an adequate dosage of fluconazole.

Quorum sensing in fungal biofilms

The regulation of the expression of virulence genes is a crucial step in pathogenesis and in microorganism adaptation to host tissues.[2] QS is a mechanism of microbial communication dependent on cell density that can regulate several behaviors in bacteria such as secretion of virulence factors, biofilm formation, competence and bioluminescence.[2] It is a major mechanism of microbial communication and QS occurs by the continuous release and monitoring of hormone-like molecules called auto-inducers or QS molecules.[138] QS has been observed in many bacterial species regulating the most diverse processes, including secretion of virulence factors, biofilm formation, and antibiotic production; now, it is believed that the same occurs in fungi.[2,46,85] In pathogenic microbes, the coordinated expression of virulence factors during infection of a host probably constitutes a significant survival advantage by enhancing the chances of establishing infection and escaping the immune response.[138] Several molecules have been described as belonging to QS. Lipids, such as sphingolipids, farnesol and oxylipins are signaling molecules in pathogenic fungi.[121] Recently, aromatic alcohols phenylethanol and tryptophol molecules were identified as quorum-sensing in *S. cerevisiae*. These compounds, which are also produced by *C. albicans*, showed growth on *S. cerevisiae* pseudohyphae at relatively low concentrations.[16]

Farnesol and tyrosol are QS molecules in *C. albicans*. The primary mechanism of regulation of QS is the production of auto-inducers that are released into the external environment, where they accumulate and concomitant measurement of their concentration is achieved through its interaction with its receptor, which may be as much as being in intracellular cell surface.[48] In bacteria, these inducers have been widely studied and they are related to various cellular processes, such as antibiotic production, sporulation ability, and expression of virulence genes, DNA transfer and formation of biofilms.[50] Shirtliff et al.[120] have shown that 40 mM or 100 mM farnesol concentrations are able to induce high regulation of *C. albicans* protein involved in protection against oxidative stress. Sharma et al.[119] demonstrated that farnesol can modulate the action of drugs on planktonic cells of *C. albicans*. Ramage et al.[108] evaluated the effects of farnesol on biofilm development and observed that farnesol inhibits the formation of hyphae when added in the initial phase of biofilm formation and, hence, can compromise the structure. Other studies have shown detrimental effects of farnesol on many microorganisms, including fungi and bacteria, such as *Staphylococcus aureus*, *S. cerevisiae*, *Aspergillus* species, *P. brasiliensis* and *Mycobacterium smegmatis*.[58,117] The high density of microorganisms in biofilms led to speculate that detection of QS plays a specific and important role in the physiology of biofilms. In other bacteria, QS detection can function in the dispersion of individual organisms from biofilm.[98,103] It seems, therefore, that the morphogenesis in *C. albicans* is under control of antagonistic tyrosol and farnesol.[17] Both farnesol and tyrosol in biofilms have been studied to emphasize the morphological aspect. The same can happen with other fungi, potentially increasing the efficacy of drugs, leading to new strategies for the treatment of fungal infections.[30]

Genes involved in the formation of fungal biofilms

It is clear that the current knowledge on fungal biofilms has increased significantly and much of that knowledge has been gained through in vitro and in vivo studies of *Candida* biofilms.[66] Through research focused on the biofilm of *C.*

albicans, the molecular characteristics of fungal biofilm development were elucidated.[40,107] The increased amount of studies on *Candida* biofilms is partly because this pathogen is associated with infections of several medical devices, leading to high mortality (approaching 40%).[41] Recently, the transcriptional network that governs the development of biofilms for *C. albicans* was identified. This network consists of six master transcription regulators (EFG1, TEC1, BCR1, NDT80, ROB1, and BRG1) and approximately 1000 target genes, whose expression is controlled by these regulators. The six master regulators were identified by screening a library of approximately 165 mutant transcripts during in vitro biofilm formation and observing that the mutants had changed during the event. Six deletion mutants that produced defects in biofilm formation were identified, three are new (ROB1, BRG1 and NDT80) and three were previously known to play a role in the development of biofilms (BCR1, TEC1 and EFG1). All six identified genes were associated with defects in both in vitro and in vivo biofilm formation.[41] Banerjee et al.[6] studied the role of UME6 and found it to be a regulator of hyphae in *C. albicans* biofilms. Another study, performed by Taff et al.,[126] demonstrated that three enzymes were related to the production of extracellular polysaccharides, encoded by genes BGL2, PHR1, and XOG1. It has been shown that these enzymes are essential for the delivery of β-1,3-glucan for the matrix of the biofilm biomass and accumulation of a mature, extracellular matrix. Through the construction of mutants, researchers have demonstrated an increased biofilm susceptibility to commonly used antifungals, such as fluconazole. These investigators have proposed that the discovery of inhibitors of these enzymes provide promising anti-biofilm effects. The use of molecular biology tools has helped to unravel the "mystery" of microbial biofilms. Much has been discovered; however, despite advances in technology and arrays to evaluate enzymes and proteins, a way to completely eliminate biofilms has yet to be discovered.

Adhesins in fungal biofilm

Adherence is a precondition for colonization and an essential step in the establishment of infection. Adherence is mediated through a large number of differentially regulated cell wall-bound adhesins. Studies with *Candida* spp. and *P. brasiliensis* have shown that these fungi have great potential for adherence to epithelial cells.[8,83]

Among several groups of genes involved in biofilm formation, it was found that the family ALS (agglutinin-like sequence), present in *C. albicans*, *Candida tropicalis* and *Candida glabrata*, plays a key role in this process and encodes proteins having the characteristics of adhesin glycoproteins on the cell surface.[38] It has been shown that ALS genes exhibit increased expression during the formation of biofilm.[95] The family present in *C. albicans* ALS includes eight genes (ALS1–ALS7 and ALS9) encoding many surface glycoproteins.[23,53] Molecular studies on the expression of ALS genes showed that they are differentially expressed and regulated as a function of cell physiological processes, such as the growth stage and cell morphology, i.e., yeast or predominantly in the form of hypha and pseudo-hypha.[54,55] ALS1, encoding cell surface glycoproteins, exhibits high expression in *C. albicans* biofilm cells.[23] Gene ALS3 also showed high expression, however, it is apparently associated with the production of *C. albicans* hyphae.[23,55] Nailis et al.[90] compared gene expression of ALS1 and ALS3 among cells of *C. albicans* biofilm formed on the surface of silicone and on suspended cells (planktonic) and found a significant increase in the expression of ALS1 biofilm cells, and decreased expression of ALS3. Moreover, Nobile et al.[92] concluded, after several tests with mutants als1/als1 als3/als3 that ALS3 and ALS1 are essential for biofilm formation in vivo and reduced expression of these proteins entails the formation of a fragile biofilm, whereas their functions are compatible with biofilm structure and biochemical property. Zhao et al.[140] demonstrated that the decrease in ALS2 protein expression resulted in the reduction of biofilm biomass, suggesting that ALS2 contributes to the later stages of biofilm development and not to the adhesion stage. In an experimental model of catheter infection in vivo, ALS1 and AlS3 also had redundant functions, and other highly expressed genes of the family – ALS5, ALS6, ALS7, and ALS9 – were able to partially or completely replace the absence of ALS1 and/or ALS3, facilitating the development of biofilm in such an experimental model, whereas ALS2 and ALS4 were unable to do so, and all ALS genes could be replaced by ALS3 or ALS1 models in vivo and in vitro.

C. albicans adheres to epithelial cells in culture, mainly through EAP1 adhesion. EAP1 is a member of a family of up to 23 putative adhesin-encoding genes present in this yeast genome. EAP1 expression in vitro is controlled both positively and negatively; in addition, it presents high cell-to-cell heterogeneity, which depends on Sir-mediated silencing. EPA6 also encode functional adhesions in *C. glabrata*.[64]

Some molecules of *H. capsulatum* have been identified as ligands of extracellular matrix components. McMahon et al.[81] reported that a 50-kDa protein present in the fungus cell walls is able to bind to laminin, an extracellular matrix component of host lung cells. This protein is an essential step in the pathogenesis of the fungus, once in the alveolar macrophages yeasts inhibit the production of proinflammatory cytokines, facilitating infection.[127] There are no studies demonstrating *H. capsulatum* adhesins related to biofilm formation.

Pathogenic fungi such as *Paracoccidioides* spp., have multiple factors that can cause damage to the host and contribute to the virulence phenotype. Adhesion, colonization and characteristics of fungi enable them to resist the hostile environments of the host and are correlated to disease development.[3,27,33,49,82] Further, this protein has virulence potential with high affinity for laminin, thereby increasing the capacity of the fungi to invade and destroy tissues.[134] Adherence of *Paracoccidiodies* to epithelial cells is also greatly reduced in the presence of anti-gp43.[47] Gp43 also interacts with fibronectin, another component of the extracellular matrix.[82] Other adhesion molecules in *P. brasiliensis* have also been described, such as a 30-kDa adhesion molecule, with the ability to bind to laminin, and are expressed in *P. brasiliensis*, isolates with high adhesion capacity. Enolase is a cytoplasmic enzyme most abundantly expressed in many microorganisms.[97] Thus, for many years enolase was seen as a soluble glycolytic enzyme, present exclusively in the cytoplasm. In 2009, Donofrio et al.,[33] demonstrated that enolase from *P. brasiliensis* (*Pb*Eno) is a fibronectin-binding protein and genetic and proteomic evidences support its localization on the cell surface.[69,71] Studies conducted in the Clinical Mycology Laboratory, UNESP, Araraquara, Brazil, have shown an increase of some adhesins of *P. brasiliensis* in hypoxic conditions, precisely the condition that occurs in biofilm formation (unpublished data).

Antibiofilm strategies

Fungal biofilm resistance mechanisms include extracellular matrix, efflux pump activity, persisters, cell density, overexpression of drug targets, stress responses, and the general physiology of the cell.[106] Thus, to increase the efficiency of new treatment strategies against bacterial and fungal infections, factors that lead to biofilm growth inhibition, biofilm disruption, or biofilm eradication are being sought. These factors could include enzymes, sodium salts, metal nanoparticles, antibiotics, acids, chitosan derivatives, or plant extracts. Biofilm formation almost always leads to a large increase in resistance to antimicrobial agents (up to 1000-fold decrease in susceptibility) in comparison with planktonic cultures

grown in conventional liquid media.[22] Many studies have focused on the search for natural or synthetic products for various fungal biofilms, but biofilms of *Candida* species are the most studied.

Studies performed by Pires et al.[100] showed the presence of biofilms in the fluid pathways of hemodialysis machines. The impacts of four biocides used for the disinfection of hemodialysis systems were tested against *Candida parapsilosis* and *Candida orthopsilosis* biofilms generated by isolates obtained from a hydraulic circuit, and collected in a hemodialysis unit. Acetic acid was shown to be the most effective agent against *Candida* biofilms. Strategies for effective disinfection procedures used for hemodialysis systems should also seek to kill and inhibit biofilms. On the other hand, some natural products have been tested against *C. orthopsilosis* and *C. parapsilosis* on planktonic and biofilm conditions and could be natural anticandidal agents that can be effectively utilized for the control of the yeasts.[101,102] In the Clinical Mycology Laboratory, UNESP, has been consolidating a platform for the development of antifungal and bioreagents. This platform is based on FAPESP programs, such as the Biota-FAPESP, the BIOPROSPECTA, and also in SISBIOTA – CNPq. Among natural substances evaluated that deserves highlighting lies maytenin with antifungal potential against several fungal species.[45]

Another promising strategy is the antifungal activity of silver nanoparticles. Silver (Ag) has been well known for its antimicrobial characteristics, and has a long history of applications in medicine with a well-tolerated tissue response.[91,115] In the hope of inhibiting biofilm formation, thereby reducing the chance of microbial infections and rejection, AgNP has been used for lining of medical implants with titanium.[42,70] Recently, Sun et al.[125] reported the antibiofilm activity of terpinen-4-ol-loaded lipid nanoparticles against *C. albicans* biofilms and this compound (10 μg/ml) eradicated formed biofilms.

Studies with antibodies have been performed by several authors to test their effects on diverse fungal and bacterial organisms. The latest therapeutic treatment of *Cryptococcus* biofilms suggests that monoclonal antibodies (MAbs) are potentially useful in clinical treatment.[77] Martinez et al.[74] demonstrated that alpha radiation, guided by MAb, effectively impairs fungal biofilm formation. Other authors have found that administering a prophylactic dose of antibodies specific to biofilms, immediately after insertion of a medical device, is effective in managing biofilm formation.[76]

Another important therapeutically promise is photodynamic therapy (PDT), widely used for species of *Candida* biofilms. Several authors have associated light emitting diode with other substances.[15,111] There are two major types of cellular damage: DNA damage and the destruction of cellular membranes and organelles. Recent studies have shown that the antimicrobial effect can be obtained with the use of photosensitizers belonging to different chemical groups. Junqueira et al.[62] assessed the PDT on biofilms of *Candida* spp., *Trichosporon mucoide*, and *Kodamaea ohmeri*.

Because the biofilm matrix is composed of DNA, proteins, and extracellular polysaccharides, recent studies have indicated that the disruption of the biofilm structure could be achieved via degradation of individual biofilm compounds by several enzymes such as DNAse, lactonases, α-amylases, and lyase.[128]

Research methodology used recently in biofilms

Infections associated with biofilm formation are resistant to conventional antifungal therapy and due to the high morbidity and mortality caused by these formations there is an urgent need to use new technologies and innovative therapies for success in eradicating these infections.[113] In this sense, the ease of working with new models, in vivo approaches of "omics" techniques of molecular biology and nano science are innovative avenues of research that have paved the way for new lines of study in the search for antifungal targets.

In vitro biofilm models are needed to elucidate mechanisms of development of biofilms. Nevertheless, results of testing in vitro of biofilm formation by clinical isolates do not always agree with results in vivo.[112] In this context, invertebrate models become useful to visualize infection, determining the true role of biofilms in infectious processes and how these formations directly affect the health of the host.[113] A recent review published by Edwards and Kjellerup[34] highlights the advances in cell–cell interactions and the understanding how host immune system reacts to biofilm formation in five invertebrate models: *Lemna minor* (duckweed), *Arabidopsis thaliana* (thale cress), *Dictyostelium discoideum* (slime mold), *Drosophila melanogaster* (common fruit fly), and *Caenorhabditis elegans* (roundworm). These models were described and assessed for their relevance to infections associated with polymicrobial biofilm formation. According to the authors, it is possible to use each of these models to investigate the peculiar characteristics of such biofilm, however *C. elegans* is presented as the most complete model to elucidate virulence factors, host innate immune function and to visualize the infection. Some authors have adopted *C. elegans* as a model to determine the toxicity and antifungal activity of fungicidal compounds, aimed at the discovery of new targets for the treatment of biofilms of *C. albicans*.[36,130] Thus, the nematode *C. elegans* has given rise to promising perspectives for innovative human therapies.

Concomitantly, another important branch of research should be stressed, "omics" approaches have been widely exploited by pharmaceutical and biotechnology companies for the development of safer and more effective drugs. Currently, there is great interest in the search for effective drugs against novel targets and, in this context, we highlight proteins by proteomic analysis, since the identification of a target protein essential to cell survival can provide important information for the treatment of mycoses.[44,136] The proteome of *C. albicans* in planktonic and biofilm cultures is well documented by several authors.[73,118,129] In this sense, our group in Brazil has noted that there is a different pattern of proteins when comparing *H. capsulatum* in planktonic and biofilm cultures. Using mass spectrometry more than 40 proteins, belonging to different functional groups, were differentially expressed and identified between the biofilm and dispersed cells, and the three main functional groups include proteins involved in the metabolism of amino acids, nuclear proteins, and translation protein (unpublished data). Additionally, our group has been working on the standardization of methodologies that aim to characterize the differential transcriptional profile exhibited by fungi shaped biofilm and planktonic conditions through transcriptomics analysis. In addition, we aim at identifying the secreted molecules and metabolites, generated during biofilm formation, using secretomic and metabolomics analyses, respectively. These techniques should allow targeting cellular receptors for biofilm disruption in the interaction with host cells.

Conclusions

Biofilms control is necessary and has been the subject of many investigations in the fields of biotechnology and public health, as biofilms are present in many situations, from human disease to industry. A major concern is the control of biofilms, for which knowledge of biofilm mechanisms is essential. However, information of microbial communities is scarce, due to the complexity of these systems and to metabolic interactions that remain unknown. For this reason, advances in high-throughput methods have allowed the interaction of systems, combining genomics, transcriptomics, proteomics, and metabolomics to elucidate the real function and ecology of these complex formations.

Conflict of interest

None declared.

Acknowledgements

The authors acknowledge receipt of grant from Brazilian organizations: FAPESP No. 2011/12734-3, 2012/01270-9, BIOTA-2003/02176-7, Bioprospecta-2004/07932-7, and CNPq-Sisbiota-563311/2010-0. This paper constitutes partial fulfillment of the Bilateral Collaboration Agreement between UNAM-Mexico (Ref: 15090-563-24-V-04) and UNESP-Brazil (Ref: 000528/04/01/2005), and of the project DGAPA-UNAM (PAPIIT IN204210).

References

1. Aidé MA. Histoplasmosis. J Bras Pneumol. 2009;35:1145–51.
2. Albuquerque P, Casadevall A. Quorum sensing in fungi: a review. Med Mycol. 2012;50:337–45.
3. Andreotti PF, Monteiro da Silva JL, Bailão AM, Soares CM, Benard G, Soares CP, et al. Isolation and partial characterization of a 30 kDa adhesin from *Paracoccidioides brasiliensis*. Microbes Infect. 2005;7:875–81.
4. Arantes TD, Theodoro RC, Da Graça Macoris SA, Bagagli E. Detection of *Paracoccidioides* spp. in environmental aerosol samples. Med Mycol. 2013;51:83–92.
5. Ashbee HR, Leck AK, Puntis JW, Parsons WJ, Evans EG. Skin colonization by *Malassezia* in neonates and infants. Infect Control Hosp Epidemiol. 2002;23:212–6.
6. Banerjee M, Uppuluri P, Zhao XR, Carlisle PL, Vipulanandan G, Villar CC, et al. Expression of UME6, a key regulator of *Candida albicans* hyphal development, enhances biofilm formation via Hgc1- and Sun41-dependent mechanisms. Eukaryot Cell. 2013;12:224–32.
7. Behrends V, Ryall B, Zlosnik JE, Speert DP, Bundy JG, Williams HD. Metabolic adaptations of *Pseudomonas aeruginosa* during cystic fibrosis chronic lung infections. Environ Microbiol. 2013;15:398–408.
8. Biasoli MS, Tosello ME, Magaró HM. Adherence of *Candida* strains isolated from the human gastrointestinal tract. Mycoses. 2002;45:465–9.
9. Bink A, Vandenbosch D, Coenye T, Nelis H, Cammue BP, Thevissen K. Superoxide dismutases are involved in *Candida albicans* biofilm persistence against miconazole. Antimicrob Agents Chemother. 2011;55:4033–7.
10. Boletti FT. Manufactor of vinegar. Microbiology: a textbook on microorganisms general and applied. CE Marshall; 1921.
11. Cafarchia C, Otranto D. The pathogenesis of *Malassezia* yeasts. Parassitologia. 2008;50:65–7.
12. Canabarro A, Valle C, Farias MR, Santos FB, Lazera M, Wanke B. Association of subgingival colonization of *Candida albicans* and other yeasts with severity of chronic periodontitis. J Periodontal Res. 2012;48:428–32.
13. Cannizzo FT, Eraso E, Ezkurra PA, Villar-Vidal M, Bollo E, Castellá G, et al. Biofilm development by clinical isolates of *Malassezia pachydermatis*. Med Mycol. 2007;45:357–61.
14. Ceri H, Olson M, Morck D, Storey D, Read R, Buret A, et al. The MBEC Assay System: multiple equivalent biofilms for antibiotic and biocide susceptibility testing. Methods Enzymol. 2001;337:377–85.
15. Chen CM, Lee JA, Huang TC. Construction of a light-emitting diode fluorescence detector for high-performance liquid chromatography and its application to fluorometric determination of L-3-hydroxybutyrate. Biomed Chromatogr. 2012;26:256–60.
16. Chen H, Fink GR. Feedback control of morphogenesis in fungi by aromatic alcohols. Genes Dev. 2006;20:1150–61.
17. Chen H, Fujita M, Feng Q, Clardy J, Fink GR. Tyrosol is a quorum-sensing molecule in *Candida albicans*. Proc Natl Acad Sci U S A. 2004;101:5048–52.
18. Chryssanthou E, Broberger U, Petrini B. *Malassezia pachydermatis* fungaemia in a neonatal intensive care unit. Acta Paediatr. 2001;90:323–7.
19. Cos P, Toté K, Horemans T, Maes L. Biofilms: an extra hurdle for effective antimicrobial therapy. Curr Pharm Des. 2010;16:2279–95.
20. Costerton JW, Geesey GG, Cheng KJ. How bacteria stick. Sci Am. 1978;238:86–95.
21. Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM. Microbial biofilms. Annu Rev Microbiol. 1995;49:711–45.
22. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science. 1999;284:1318–22.
23. Cuéllar-Cruz M, López-Romero E, Villagómez-Castro JC, Ruiz-Baca E. *Candida* species: new insights into biofilm formation. Future Microbiol. 2012;7:755–71.
24. Curvale-Fauchet N, Botterel F, Legrand P, Guillot J, Bretagne S. Frequency of intravascular catheter colonization by *Malassezia* spp. in adult patients. Mycoses. 2004;47:491–4.
25. Cushion MT, Collins MS, Linke MJ. Biofilm formation by *Pneumocystis* spp. Eukaryot Cell. 2009;8:197–206.
26. D'Antonio D, Parruti G, Pontieri E, Di Bonaventura G, Manzoli L, Sferra R, et al. Slime production by clinical isolates of *Blastoschizomyces capitatus* from patients with hematological malignancies and catheter-related fungemia. Eur J Clin Microbiol Infect Dis. 2004;23:787–9.
27. da Silva Neto BR, de Fátima da Silva J, Mendes-Giannini MJ, Lenzi HL, de Almeida Soares CM, Pereira M. The malate synthase of *Paracoccidioides brasiliensis* is a linked surface protein that behaves as an anchorless adhesin. BMC Microbiol. 2009;9:272.
28. Davis LE, Cook G, Costerton JW. Biofilm on ventriculo-peritoneal shunt tubing as a cause of treatment failure in coccidioidal meningitis. Emerg Infect Dis. 2002;8:376–9.
29. De Almeida GM, Costa SF, Melhem M, Motta AL, Szeszs MW, Miyashita F, et al. *Rhodotorula* spp. isolated from blood cultures: clinical and microbiological aspects. Med Mycol. 2008;46:547–56.
30. Derengowski LS, De-Souza-Silva C, Braz SV, Mello-De-Sousa TM, Báo SN, Kyaw CM, et al. Antimicrobial effect of farnesol, a *Candida albicans* quorum sensing molecule, on *Paracoccidioides brasiliensis* growth and morphogenesis. Ann Clin Microbiol Antimicrob. 2009;8:13.
31. Di Bonaventura G, Pompilio A, Picciani C, Iezzi M, D'Antonio D, Piccolomini R. Biofilm formation by the emerging fungal pathogen *Trichosporon asahii*: development, architecture, and antifungal resistance. Antimicrob Agents Chemother. 2006;50:3269–76.
32. Donlan RM. Biofilms and device-associated infections. Emerg Infect Dis. 2001;7:277–81.
33. Donofrio FC, Calil AC, Miranda ET, Almeida AM, Benard G, Soares CP, et al. Enolase from *Paracoccidioides brasiliensis*: isolation and identification as a fibronectin-binding protein. J Med Microbiol. 2009;58:706–13.
34. Edwards S, Kjellerup BV. Exploring the applications of invertebrate host-pathogen models for in vivo biofilm infections. FEMS Immunol Med Microbiol. 2012;65:205–14.
35. Escande W, Fayad G, Modine T, Verbrugge E, Koussa M, Senneville E, et al. Culture of a prosthetic valve excised for streptococcal endocarditis positive for *Aspergillus fumigatus* 20 years after previous A fumigatus endocarditis. Ann Thorac Surg. 2011;91:e92–3.
36. Ewbank JJ, Zugasti O. *C. elegans*: model host and tool for antimicrobial drug discovery. Dis Model Mech. 2011;4:300–4.
37. Figueredo LA, Cafarchia C, Desantis S, Otranto D. Biofilm formation of *Malassezia pachydermatis* from dogs. Vet Microbiol. 2012;160:126–31.
38. Filler SG. *Candida*–host cell receptor–ligand interactions. Curr Opin Microbiol. 2006;9:333–9.
39. Filler SG, Sheppard DC. Fungal invasion of normally non-phagocytic host cells. PLoS Pathog. 2006;2:e129.
40. Finkel JS, Mitchell AP. Genetic control of *Candida albicans* biofilm development. Nat Rev Microbiol. 2011;9:109–18.
41. Fox EP, Nobile CJ. A sticky situation: untangling the transcriptional network controlling biofilm development in *Candida albicans*. Transcription. 2012;3:315–22.
42. García-Contreras R, Argueta-Figueroa L, Mejía-Rubalcava C, Jiménez-Martínez R, Cuevas-Guajardo S, Sánchez-Reyna PA, et al. Perspectives for the use of silver nanoparticles in dental practice. Int Dent J. 2011;61:297–301.
43. Garsin DA, Willems RJ. Insights into the biofilm lifestyle of enterococci. Virulence. 2010;1:219–21.
44. Gomase VS, Kale KV, Tagore S, Hatture SR. Proteomics: technologies for protein analysis. Curr Drug Metab. 2008;9:213–20.
45. Gullo FP, Sardi JC, Santos VA, Sangalli-Leite F, Pitangui NS, Rossi SA, et al. Antifungal activity of maytenin and pristimerin. Evid Based Complement Alternat Med. 2012;2012:3407–87.
46. Gupta RK, Chhibber S, Harjai K. Quorum sensing signal molecules cause renal tissue inflammation through local cytokine responses in experimental UTI caused by *Pseudomonas aeruginosa*. Immunobiology. 2013;218:181–5.
47. Hanna SA, Monteiro da Silva JL, Giannini MJ. Adherence and intracellular parasitism of *Paracoccidioides brasiliensis* in Vero cells. Microbes Infect. 2000;2:877–84.
48. Hense BA, Kuttler C, Müller J, Rothballer M, Hartmann A, Kreft JU. Does efficiency sensing unify diffusion and quorum sensing? Nat Rev Microbiol. 2007;5:230–9.
49. Hernández O, Almeida AJ, Tamayo D, Torres I, Garcia AM, López A, et al. The hydrolase PbHAD32 participates in the adherence of *Paracoccidioides brasiliensis* conidia to epithelial lung cells. Med Mycol. 2012;50:533–7.
50. Hogan DA. Talking to themselves: autoregulation and quorum sensing in fungi. Eukaryot Cell. 2006;5:613–9.
51. Hogan LH, Klein BS, Levitz SM. Virulence factors of medically important fungi. Clin Microbiol Rev. 1996;9:469–88.
52. Horré R, Symoens F, Delhaes L, Bouchara JP. Fungal respiratory infections in cystic fibrosis: a growing problem. Med Mycol. 2010;48:S1–3.
53. Hoyer LL, Green CB, Oh SH, Zhao X. Discovering the secrets of the *Candida albicans* agglutinin-like sequence (ALS) gene family—a sticky pursuit. Med Mycol. 2008;46:1–15.
54. Hoyer LL, Payne TL, Hecht JE. Identification of *Candida albicans* ALS2 and ALS4 and localization of als proteins to the fungal cell surface. J Bacteriol. 1998;180:5334–43.
55. Hoyer LL, Scherer S, Shatzman AR, Livi GP. *Candida albicans* ALS1: domains related to a *Saccharomyces cerevisiae* sexual agglutinin separated by a repeating motif. Mol Microbiol. 1995;15:39–54.
56. Huhn GD, Austin C, Carr M, Heyer D, Boudreau P, Gilbert G, et al. Two outbreaks of occupationally acquired histoplasmosis: more than workers at risk. Environ Health Perspect. 2005;113:585–9.
57. Jabra-Rizk MA, Falkler WA, Meiller TF. Fungal biofilms and drug resistance. Emerg Infect Dis. 2004;10:14–9.

58. Jabra-Rizk MA, Shirtliff M, James C, Meiller T. Effect of farnesol on *Candida dubliniensis* biofilm formation and fluconazole resistance. FEMS Yeast Res. 2006;6:1063–73.
59. Jass J, O'Neill JG, Walker JT. Direct biofilm monitoring by a capacitance measurement probe in continuous culture chemostats. Methods Enzymol. 2001;337:63–70.
60. Jayshree RS, Shafiulla M, George J, David JK, Bapsy PP, Chakrabarti A. Microscopic, cultural and molecular evidence of disseminated invasive aspergillosis involving the lungs and the gastrointestinal tract. J Med Microbiol. 2006;55:961–4.
61. Jeloka TK, Shrividya S, Wagholikar G. Catheter outflow obstruction due to an aspergilloma. Perit Dial Int. 2011;31:211–2.
62. Junqueira JC, Jorge AO, Barbosa JO, Rossoni RD, Vilela SF, Costa AC, et al. Photodynamic inactivation of biofilms formed by *Candida* spp., *Trichosporon mucoides*, and *Kodamaea ohmeri* by cationic nanoemulsion of zinc 2,9,16,23-tetrakis(phenylthio)-29H, 31H-phthalocyanine (ZnPc). Lasers Med Sci. 2012;27:1205–12.
63. Kojic EM, Darouiche RO. *Candida* infections of medical devices. Clin Microbiol Rev. 2004;17:255–67.
64. Kucharíková S, Tournu H, Lagrou K, Van Dijck P, Bujdáková H. Detailed comparison of *Candida albicans* and *Candida glabrata* biofilms under different conditions and their susceptibility to caspofungin and anidulafungin. J Med Microbiol. 2011;60:1261–9.
65. LaFleur MD, Kumamoto CA, Lewis K. *Candida albicans* biofilms produce antifungal-tolerant persister cells. Antimicrob Agents Chemother. 2006;50:3839–46.
66. Lebeaux D, Ghigo JM. Management of biofilm-associated infections: what can we expect from recent research on biofilm lifestyles? Med Sci (Paris). 2012;28:727–39.
67. Lee SW. An aspergilloma mistaken for a pelviureteral stone on nonenhanced CT: a fungal bezoar causing ureteral obstruction. Korean J Urol. 2010;51:216–8.
68. Lewis K. Persister cells: molecular mechanisms related to antibiotic tolerance. Handb Exp Pharmacol. 2012;211:121–33.
69. López-Villar E, Monteoliva L, Larsen MR, Sachon E, Shabaz M, Pardo M, et al. Genetic and proteomic evidences support the localization of yeast enolase in the cell surface. Proteomics. 2006;6:S107–18.
70. Lu X, Zhang B, Wang Y, Zhou X, Weng J, Qu S, et al. Nano-Ag-loaded hydroxyapatite coatings on titanium surfaces by electrochemical deposition. J R Soc Interface. 2011;8:529–39.
71. Marcos CM, de Fátima da Silva J, de Oliveira HC, Moraes da Silva RA, Mendes-Giannini MJ, Fusco-Almeida AM. Surface-expressed enolase contributes to the adhesion of *Paracoccidioides brasiliensis* to host cells. FEMS Yeast Res. 2012;12:557–70.
72. Marques-da-Silva SH, Rodrigues AM, de Hoog GS, Silveira-Gomes F, Camargo ZP. Occurrence of *Paracoccidioides lutzii* in the Amazon region: description of two cases. Am J Trop Med Hyg. 2012;87:710–4.
73. Martínez-Gomariz M, Perumal P, Mekala S, Nombela C, Chaffin WL, Gil C. Proteomic analysis of cytoplasmic and surface proteins from yeast cells, hyphae, and biofilms of *Candida albicans*. Proteomics. 2009;9:2230–52.
74. Martinez LR, Bryan RA, Apostolidis C, Morgenstern A, Casadevall A, Dadachova E. Antibody-guided alpha radiation effectively damages fungal biofilms. Antimicrob Agents Chemother. 2006;50:2132–6.
75. Martinez LR, Casadevall A. *Cryptococcus neoformans* biofilm formation depends on surface support and carbon source and reduces fungal cell susceptibility to heat, cold, and UV light. Appl Environ Microbiol. 2007;73:4592–601.
76. Martinez LR, Casadevall A. Specific antibody can prevent fungal biofilm formation and this effect correlates with protective efficacy. Infect Immun. 2005;73:6350–62.
77. Martinez LR, Christaki E, Casadevall A. Specific antibody to *Cryptococcus neoformans* glucuronoxylomannan antagonizes antifungal drug action against cryptococcal biofilms in vitro. J Infect Dis. 2006;194:261–6.
78. Martinez LR, Fries BC. Fungal biofilms: relevance in the setting of human disease. Curr Fungal Infect Rep. 2010;4:266–75.
79. Martinez-Pajares JD, Martinez-Ferriz MC, Moreno-Perez D, Garcia-Ramirez M, Martin-Carballido S, Blanch-Iribarne P. Management of obstructive renal failure caused by bilateral renal aspergilloma in an immunocompetent newborn. J Med Microbiol. 2010;59:367–9.
80. McCoy WF, Bryers JD, Robbins J, Costerton JW. Observations of fouling biofilm formation. Can J Microbiol. 1981;27:910–7.
81. McMahon JP, Wheat J, Sobel ME, Pasula R, Downing JF, Martin WJ. Murine laminin binds to *Histoplasma capsulatum*. A possible mechanism of dissemination. J Clin Invest. 1995;96:1010–7.
82. Mendes-Giannini MJ, Andreotti PF, Vincenzi LR, da Silva JL, Lenzi HL, Benard G, et al. Binding of extracellular matrix proteins to *Paracoccidioides brasiliensis*. Microbes Infect. 2006;8:1550–9.
83. Mendes-Giannini MJ, Monteiro da Silva JL, de Fátima da Silva J, Donofrio FC, Miranda ET, Andreotti PF, et al. Interactions of *Paracoccidioides brasiliensis* with host cells: recent advances. Mycopathologia. 2008;165:237–48.
84. Mendes-Giannini MJ, Soares CP, da Silva JL, Andreotti PF. Interaction of pathogenic fungi with host cells: molecular and cellular approaches. FEMS Immunol Med Microbiol. 2005;45:383–94.
85. Miller MB, Bassler BL. Quorum sensing in bacteria. Annu Rev Microbiol. 2001;55:165–99.
86. Moss RB. Allergic bronchopulmonary aspergillosis and *Aspergillus* infection in cystic fibrosis. Curr Opin Pulm Med. 2010;16:598–603.
87. Mowat E, Butcher J, Lang S, Williams C, Ramage G. Development of a simple model for studying the effects of antifungal agents on multicellular communities of *Aspergillus fumigatus*. J Med Microbiol. 2007;56:1205–12.
88. Mowat E, Williams C, Jones B, McChlery S, Ramage G. The characteristics of *Aspergillus fumigatus* mycetoma development: is this a biofilm? Med Mycol. 2009;47:S120–6.
89. Müller FM, Seidler M, Beauvais A. *Aspergillus fumigatus* biofilms in the clinical setting. Med Mycol. 2011;49:S96–100.
90. Nailis H, Coenye T, Van Nieuwerburgh F, Deforce D, Nelis HJ. Development and evaluation of different normalization strategies for gene expression studies in *Candida albicans* biofilms by real-time PCR. BMC Mol Biol. 2006;7:25.
91. Nam KY. In vitro antimicrobial effect of the tissue conditioner containing silver nanoparticles. J Adv Prosthodont. 2011;3:20–4.
92. Nobile CJ, Schneider HA, Nett JE, Sheppard DC, Filler SG, Andes DR, et al. Complementary adhesin function in *C. albicans* biofilm formation. Curr Biol. 2008;18:1017–24.
93. Nosanchuk JD, Zancopé-Oliveira RM, Hamilton AJ, Guimarães AJ. Antibody therapy for histoplasmosis. Front Microbiol. 2012;3. http://dx.doi.org/10.3389/fmicb.2012.00021.
94. Nunes JM, Bizerra FC, Ferreira RC, Colombo AL. Molecular identification, antifungal susceptibility profile, and biofilm formation of clinical and environmental *Rhodotorula* species isolates. Antimicrob Agents Chemother. 2013;57:382–9.
95. O'Connor L, Lahiff S, Casey F, Glennon M, Cormican M, Maher M. Quantification of ALS1 gene expression in *Candida albicans* biofilms by RT-PCR using hybridisation probes on the LightCycler. Mol Cell Probes. 2005;19:153–62.
96. Okita WB, Kirwan DJ. Simulation of secondary metabolite production by immobilized living cells: penicillin production. Biotechnol Prog. 1986;2:83–90.
97. Pancholi V. Multifunctional alpha-enolase: its role in diseases. Cell Mol Life Sci. 2001;58:902–20.
98. Parsek MR, Fuqua C. Biofilms 2003: emerging themes and challenges in studies of surface-associated microbial life. J Bacteriol. 2004;186:4427–40.
99. Pitangui NS, Sardi JC, Silva JF, Benaducci T, Moraes da Silva RA, Rodríguez-Arellanes G, et al. Adhesion of *Histoplasma capsulatum* to pneumocytes and biofilm formation on an abiotic surface. Biofouling. 2012;28:711–8.
100. Pires RH, da Silva JD, Martins CH, Fusco Almeida AM, Soares CP, Mendes-Giannini MJ. Effectiveness of disinfectants used in hemodialysis against both *Candida orthopsilosis* and *C. parapsilosis* sensu stricto biofilms. Antimicrob Agents Chemother. 2013;57:2417–21.
101. Pires RH, Lucarini R, Mendes-Giannini MJ. Effect of usnic acid on *Candida orthopsilosis* and *C. parapsilosis*. Antimicrob Agents Chemother. 2012;56:595–7.
102. Pires RH, Montanari LB, Martins CH, Zaia JE, Almeida AM, Matsumoto MT, et al. Anticandidal efficacy of cinnamon oil against planktonic and biofilm cultures of *Candida parapsilosis* and *Candida orthopsilosis*. Mycopathologia. 2011;172:453–64.
103. Puskas A, Greenberg EP, Kaplan S, Schaefer AL. A quorum-sensing system in the free-living photosynthetic bacterium *Rhodobacter sphaeroides*. J Bacteriol. 1997;179:7530–7.
104. Ramage G, Mowat E, Jones B, Williams C, Lopez-Ribot J. Our current understanding of fungal biofilms. Crit Rev Microbiol. 2009;35:340–55.
105. Ramage G, Rajendran R, Gutierrez-Correa M, Jones B, Williams C. *Aspergillus* biofilms: clinical and industrial significance. FEMS Microbiol Lett. 2011;324:89–97.
106. Ramage G, Rajendran R, Sherry L, Williams C. Fungal biofilm resistance. Int J Microbiol. 2012. http://dx.doi.org/10.1155/2012/528521.
107. Ramage G, Saville SP, Thomas DP, López-Ribot JL. *Candida* biofilms: an update. Eukaryot Cell. 2005;4:633–8.
108. Ramage G, Saville SP, Wickes BL, López-Ribot JL. Inhibition of *Candida albicans* biofilm formation by farnesol, a quorum-sensing molecule. Appl Environ Microbiol. 2002;68:5459–63.
109. Rawlings DE, Dew D, du Plessis C. Biomineralization of metal-containing ores and concentrates. Trends Biotechnol. 2003;21:38–44.
110. Reynolds TB, Fink GR. Bakers' yeast, a model for fungal biofilm formation. Science. 2001;291:878–81.
111. Ribeiro DS, Frigerio C, Santos JL, Prior JA. Photoactivation by visible light of CdTe quantum dots for inline generation of reactive oxygen species in an automated multipumping flow system. Anal Chim Acta. 2012;735:69–75.
112. Rohde H, Frankenberger S, Zähringer U, Mack D. Structure, function and contribution of polysaccharide intercellular adhesin (PIA) to *Staphylococcus epidermidis* biofilm formation and pathogenesis of biomaterial-associated infections. Eur J Cell Biol. 2010;89:103–11.
113. Römling U, Balsalobre C. Biofilm infections, their resilience to therapy and innovative treatment strategies. J Intern Med. 2012;272:541–61.
114. Sakurai A, Imai H, Takenaka Y, Sakakibara M. Simulation of citric acid production by rotating disk contactor. Biotechnol Bioeng. 1997;56:689–96.
115. Sardi JC, Scorzoni L, Bernardi T, Fusco-Almeida AM, Mendes Giannini MJ. *Candida* species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. J Med Microbiol. 2013;62:10–24.
116. Savini V, Sozio F, Catavitello C, Talia M, Manna A, Febbo F, et al. Femoral prosthesis infection by *Rhodotorula mucilaginosa*. J Clin Microbiol. 2008;46:3544–5.
117. Semighini CP, Hornby JM, Dumitru R, Nickerson KW, Harris SD. Farnesol-induced apoptosis in *Aspergillus nidulans* reveals a possible mechanism for antagonistic interactions between fungi. Mol Microbiol. 2006;59:753–64.

118. Seneviratne CJ, Wang Y, Jin L, Abiko Y, Samaranayake LP. *Candida albicans* biofilm formation is associated with increased anti-oxidative capacities. Proteomics. 2008;8:2936–47.
119. Sharma M, Prasad R. The quorum-sensing molecule farnesol is a modulator of drug efflux mediated by ABC multidrug transporters and synergizes with drugs in *Candida albicans*. Antimicrob Agents Chemother. 2011;55:4834–43.
120. Shirtliff ME, Krom BP, Meijering RA, Peters BM, Zhu J, Scheper MA, et al. Farnesol-induced apoptosis in *Candida albicans*. Antimicrob Agents Chemother. 2009;53:2392–401.
121. Singh A, Del Poeta M. Lipid signalling in pathogenic fungi. Cell Microbiol. 2011;13:177–85.
122. Singh R, Shivaprakash MR, Chakrabarti A. Biofilm formation by zygomycetes: quantification, structure and matrix composition. Microbiology. 2011;157:2611–8.
123. Smith JA, Kauffman CA. Pulmonary fungal infections. Respirology. 2012;17:913–26.
124. Suárez-Alvarez RO, Pérez-Torres A, Taylor ML. Adherence patterns of *Histoplasma capsulatum* yeasts to bat tissue sections. Mycopathologia. 2010;170:79–87.
125. Sun LM, Zhang CL, Li P. Characterization, antibiofilm, and mechanism of action of novel PEG-stabilized lipid nanoparticles loaded with terpinen-4-ol. J Agric Food Chem. 2012;60:6150–6.
126. Taff HT, Nett JE, Zarnowski R, Ross KM, Sanchez H, Cain MT, et al. A *Candida* biofilm-induced pathway for matrix glucan delivery: implications for drug resistance. PLoS Pathog. 2012;8:e1002848.
127. Tagliari L, Toledo MS, Lacerda TG, Suzuki E, Straus AH, Takahashi HK. Membrane microdomain components of *Histoplasma capsulatum* yeast forms, and their role in alveolar macrophage infectivity. Biochim Biophys Acta. 2012;1818:458–66.
128. Taraszkiewicz A, Fila G, Grinholc M, Nakonieczna J. Innovative strategies to overcome biofilm resistance. BioMed Res Int. 2013;2013:1–13. http://dx.doi.org/10.1155/2013/150653.
129. Thomas DP, Bachmann SP, Lopez-Ribot JL. Proteomics for the analysis of the *Candida albicans* biofilm lifestyle. Proteomics. 2006;6:5795–804.
130. Thevissen K, Pellens K, De Brucker K, François IE, Chow KK, Meert EM, et al. Novel fungicidal benzylsulfanyl-phenylguanidines. Bioorg Med Chem Lett. 2011;21:3686–92.
131. Tuon FF, Costa SF. *Rhodotorula* infection. A systematic review of 128 cases from literature. Rev Iberoam Micol. 2008;25:135–40.
132. Unal A, Koc AN, Sipahioglu MH, Kavuncuoglu F, Tokgoz B, Buldu HM, et al. CAPD-related peritonitis caused by *Rhodotorula mucilaginosa*. Perit Dial Int. 2009;29:581–2.
133. Uppuluri P, Chaturvedi AK, Srinivasan A, Banerjee M, Ramasubramaniam AK, Köhler JR, et al. Dispersion as an important step in the *Candida albicans* biofilm developmental cycle. PLoS Pathog. 2010;6:e1000828.
134. Vicentini AP, Gesztesi JL, Franco MF, de Souza W, de Moraes JZ, Travassos LR, et al. Binding of *Paracoccidioides brasiliensis* to laminin through surface glycoprotein gp43 leads to enhancement of fungal pathogenesis. Infect Immun. 1994;62:1465–9.
135. Vlamakis H. The world of biofilms. Virulence. 2011;2:431–4.
136. Walgren JL, Thompson DC. Application of proteomic technologies in the drug development process. Toxicol Lett. 2004;149:377–85.
137. Walsh TJ, Schlegel R, Moody MM, Costerton JW, Salcman M. Ventriculoatrial shunt infection due to *Cryptococcus neoformans*: an ultrastructural and quantitative microbiological study. Neurosurgery. 1986;18:373–5.
138. Williams P. Quorum sensing, communication and cross-kingdom signalling in the bacterial world. Microbiology. 2007;153:3923–38.
139. Xavier JB, Picioreanu C, Rani SA, van Loosdrecht MC, Stewart PS. Biofilm-control strategies based on enzymic disruption of the extracellular polymeric substance matrix—a modelling study. Microbiology. 2005;151:3817–32.
140. Zhao X, Oh SH, Yeater KM, Hoyer LL. Analysis of the *Candida albicans* Als2p and Als4p adhesins suggests the potential for compensatory function within the Als family. Microbiology. 2005;151:1619–30.