Candida albicans biofilm formation and its clinical consequences

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Over the last several years there has been increasing recognition in the microbiology field that biofilms constitute the predominant mode of growth for most microorganisms in their natural habitats [1,2]. Different from planktonic (free floating) organisms, biofilms can be defined as structured microbial communities, attached to a biotic or abiotic surface, and are most frequently encapsulated within a matrix of self-produced exopolymeric material. Most importantly, the same is also true for the relative minority of microorganisms that are pathogenic to humans and, according to the CDC, today it is estimated that approximately 65% of all treated infections are associated with microbial biofilm formation on the surface of tissues, organs or medical devices. Biofilm formation carries important clinical implications, as sessile cells typically display increased levels of resistance to most antibiotics and also to host defence mechanisms. In addition, the protective structure of a biofilm provides invading cells with a safe sanctuary in which they are able to withstand adverse environmental conditions. In essence, biofilms act as reservoirs for persistent sources of infection. Thus, the net effect is that microbial biofilms negatively impact the health of a growing number of patients, which ultimately translates to a soaring financial burden on our healthcare system [3].

Candida albicans is no exception to this rule, and this often benign commensal of humans is now the fungal species most frequently associated with formation of biofilms affecting different types of immunosuppressed patients [4]. There is little doubt that different manifestations of candidiasis, including oropharyngeal candidiasis, denture stomatitis, endocarditis and catheter-related candidemia and

candiduria, among others, are intimately associated with the formation of biofilms on host surfaces and/or implantable medical devices [5,6]. A variety of biomaterials used in clinical practice are able to support biofilm formation by *Candida* and, ironically, the increase in candidiasis in recent years (now the third or fourth most common nosocomial infection in US hospitals and abroad) has been virtually concomitant with the increase in use of a broad range of medical implant devices, mostly in immunocompromised patients [7,8]. The increasing awareness of the importance of *Candida* biofilms is reflected by the number of publications on this topic: a simple PubMed search using the terms ‘*Candida*’ and ‘biofilm’ returned 549 articles, with over 90% of these articles published in the last 10 years.

But how does *C. albicans* form a biofilm and what are the most important characteristics linked to biofilm formation? In order to answer these fundamental questions, multiple groups of investigators have developed different models of *C. albicans* biofilm formation, with varying degrees of sophistication, both *in vitro* and most recently *in vivo*. Using these models and now armed with state of the art analytical techniques (including advanced microscopy techniques, genomics and proteomics), researchers have been able to shed some light on the structural characteristics and molecular mechanisms governing biofilm formation by this opportunistic pathogenic fungus [4]. From these studies it is now clear that these biofilms are not a simple accumulation of cells, but rather highly structured microbial communities, postulated to represent an optimal spatial arrangement to facilitate the influx of nutrients and disposal of waste products. In general, most investigators in the field agree

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that *C. albicans* biofilm development encompasses different phases, including initial adherence, colonization, proliferation, maturation and ultimately dispersion so that the 'biofilm lifecycle' can be repeated all over again [4,9,10]. Mature *C. albicans* biofilms typically consist of an intricate network of yeasts, hyphae and pseudohyphae within ramifying water channels and are encased within exopolymeric material. They exhibit a rather complex 3D architecture, likely indicative of a high degree of specialization reminiscent of what is found in primitive tissue. Our current understanding of the mechanisms controlling *C. albicans* biofilm formation at the molecular level is still somewhat limited; however, in recent years studies by multiple groups of investigators have begun to unravel some of the major driving forces behind the transition to this biofilm lifestyle [4,11]. Besides some insights into biofilm metabolism, these studies have revealed a pivotal role for morphogenetic conversions (the ability to reversibly switch between yeast and filamentous forms in response to different environmental stimuli), adhesive interactions and quorum-sensing mechanisms in *C. albicans* biofilm development, as well as some very important implications in mating. Two very interesting reviews published in this issue of *Future Microbiology* [12,13] provide readers with up-to-date information on the Ras/cAMP/PKA signaling pathway that regulates filamentation and virulence in *C. albicans* and on the role of farnesol as a quorum-sensing molecule: both of these phenomena are critical for the *C. albicans* biofilm mode of growth. For example, the *C. albicans* Δ efg1 mutant strain is locked in the yeast form and forms only a rudimentary (monolayer) biofilm, thus pointing to the Efg1p regulator protein (a main component of the Ras/cAMP/PKA signaling pathway) as a key factor in *C. albicans* biofilm formation [14]. Moreover, *HWP1* and *ALS3*, whose expression is under the control of Efg1p, encode hypha-specific cell-wall proteins that play complementary adhesive functions critical in *C. albicans* biofilm development [15]. Likewise, farnesol and other autoregulatory molecules also control *C. albicans* biofilm formation via quorum-sensing mechanisms [16,17].

As mentioned, *C. albicans* biofilm formation carries important negative clinical implications, mostly owing to the fact that cells in biofilms are recalcitrant to antifungal therapy. As such, biofilm formation is now widely considered one of the major virulence attributes of *C. albicans* and a key contributing factor to the

unacceptably high mortality rates associated with candidiasis. A plethora of articles have now been published reporting that fungal biofilms show intrinsic resistance to azole derivatives and display high levels of resistance against polyenes, two of the most common classes of antifungal agents. Contrary to these observations, echinocandins, a new class of antifungal agents targeting cell-wall glucan, seem to display excellent antibiofilm activity at therapeutic concentrations [4,18]. Antifungal drug resistance of *C. albicans* cells within biofilms is likely multifactorial and, among other mechanisms, may be due to: metabolic and physiological state of sessile fungal cells; elevated cellular density within the biofilm; the protective effect of the biofilm matrix, including presence of glucans, which may bind to molecules of certain antifungal agents (azoles); differential expression of genes linked to resistance, including those encoding efflux pumps; differences in sterol composition of the cell-wall membrane; and presence of a subpopulation of 'persister' cells [4].

In conclusion, sessile *C. albicans* cells within biofilms possess distinct developmental properties and phenotypic characteristics that are in stark contrast to planktonic cells. Because of this, infections associated with *C. albicans* biofilm formation represent an escalating problem in healthcare and negatively impact the health of an increasing number of individuals as progress in modern medicine prolong the lives of severely ill patients. The increased recognition of the role that biofilms play during infection by the research and medical community should, without any doubt, lead to major advances in the diagnosis, prevention and treatment of biofilm-associated candidiasis in the near future.

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