**Candida albicans** biofilm development, modeling a host–pathogen interaction
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Medical device-associated infections involve the attachment of cells to a surface, production of an extracellular matrix and development of a mature biofilm. Many *Candida albicans* disease states involve biofilm growth. These infections have great impact on public health because organisms in biofilms exhibit dramatically reduced susceptibility to antifungal therapy. Progression to a mature biofilm is dependent on cell adhesion, extracellular matrix production and the yeast-to-hyphae transition. Numerous *in vitro* biofilm model systems have been successfully used to examine biofilm architecture, development, cell phenotypes and drug resistance. Although these studies have included a number of experimental variables to mimic infections in patients, it is difficult to accurately account for the multitude of host and infection-site variables that are probably important in humans. Recent studies have begun to explore *C. albicans* biofilms using animal biofilm infection models in order to more completely reflect the complexity of this host–fungal interaction.

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**Introduction**
*Candida albicans* is a major human fungal pathogen, causing both mucosal and deep tissue infections [1*]. Recent evidence suggests that the majority of disease produced by this pathogen is associated with biofilm growth [2**],3,4**,5]. The human host implications of *Candida* biofilms, the developmental process and the *in vitro* and *in vivo* models used to study this process are discussed here.

**Candida infection and medical device biofilm infection**
*C. albicans* is a dimorphic fungus that can be either a commensal or an opportunistic pathogen with the ability to cause a variety of infections, ranging from superficial to life-threatening [1*].

Many aspects of *C. albicans* biology have been studied during liquid growth, but the organism associates with surfaces in many of its disease states; most manifestations of candidiasis are associated with biofilm formation on a wide variety of implanted medical devices, which have become an essential component of patient care [1*,6*,7]. The vulnerability of these materials to infection is increasingly being appreciated. There are estimated to be more than 45 million medical devices implanted every year in the United States. Infection of these devices occurs in 1–60% of patients, and *Candida* species are responsible for up to 20% of these [2**]. At least 50% of all cases of hospital infections are associated with medical devices.

*C. albicans* can form biofilms on almost any medical device. The most commonly involved systemic devices include vascular and urinary catheters, joint prostheses, cardiac valves, artificial vascular bypass devices, pacemakers, ventricular assist devices and central nervous system shunts [2**,8**]. In addition there are many topical devices at risk, including contact lens and dentures.

**Candida biofilm development**
The ability of *C. albicans* to form biofilms on medical devices has a profound impact on its capacity to cause human disease [9]. Biofilms are structured microbial communities in which the cells bind tightly to a surface and become embedded in a matrix of extracellular polymeric substances produced by these cells (Figure 1) [4**,9,10]. The cellular communities formed on device surfaces have a characteristic architecture and phenotypic properties that are distinct from their planktonic counterparts. The most notable phenotype is a reduced susceptibility to the host immune system and to conventional antifungal drug therapy [11–17] — removal of the device is nearly always necessary to cure these infections [1*].

Biofilms are organized cellular communities under the control of signaling molecules rather than random accumulations of cells resulting from cell division [4**,18,19]. Fully developed biofilms exhibit a highly heterogeneous architecture composed of cellular and non-cellular elements [9]. They are made up of a mixture of host cells, yeast, pseudohyphae and hyphae, and the fungal-derived extracellular matrix, which is comprised of polysaccharides and proteins [2**,12,20]. Development occurs in three phases over a period of 24–48 h [4**,20,21]. First, the initial phase begins with adherence of single cells to
the substratum. Second, attached cells proliferate to form microcolonies and begin to deposit an extracellular matrix. Finally, once confluence of cells is achieved, the network of yeast cells transition to filamentous forms (pseudohyphae and hyphae) and become encased in the exopolymeric matrix. The resulting biofilm has a three-dimensional structure and is often more than several hundred microns deep. In the past few years, molecular studies have focused on adherence, filamentation and matrix production, in order to shed light on the driving forces behind these phases of biofilm development [10,12,22–27,28**].

**Candida biofilm model systems**

Various model systems have been used to investigate the properties of *Candida* biofilms in vitro. Hawser and Douglas [29] initially described a simple method, involving growth of adherent populations on the surfaces of small discs cut from catheters. Growth was monitored quantitatively by a colorimetric assay using the reduction of a tetrazolium salt and (3H) leucine incorporation. Biofilm architecture was examined using scanning electron and confocal microscopy. Subsequent in vitro model systems have included a variety of different plastics, glass slides, perfused biofilm fermenters, cylindrical cellulose filters, acrylic strips and disks, germanium substra, microtiter plates and tissue culture flasks [21,29,30,31*,32–34]. These models include biofilms formed under both static and nutrient media flow conditions. For rapid processing of large numbers of samples, biofilms have also been grown in 96-well microtiter plates.

The majority of the pioneering observations regarding biofilm architecture and associated cellular phenotypes have been described using these models. More recently, study of the impact of specific gene products on biofilm formation have relied on one or more in vitro models [22,25,28**,35**,36**,37]. Numerous experimental variables have been incorporated into these systems in order to mimic conditions in patients; including flow to simulate blood flow, coating of substrate surfaces with various host blood and salivary proteins, variation in nutrient media and use of common device materials. Each of these factors has been shown to impact the amount of extracellular matrix production and cell proliferation in *Candida* biofilms to varying extents.

**Flow**

Flow conditions — induced either by rocking in a single compartment model or through perfusion in a more elaborate system — favor development of more extensive matrix and enhanced cell proliferation [10,30]. Conditions of high flow are encountered within the circulatory system surrounding venous catheters and heart valves [38]. However, flow is probably not necessary for robust biofilm formation at all infection sites: low or intermittent flow would be expected at the sites of dentures, urinary catheters, and joint prostheses, all known to be associated with biofilm formation [2**,39].

**Host conditioning**

A ‘conditioning film’ is formed on the surface of the biomedical devices soon after implantation because of contact with surrounding body fluids such as urine, blood, saliva and synovial fluid [2**,8**,32,40,41*,42–45]. Study of the interactions between these devices and the host suggests that most host proteins randomly adsorb to the...
The relative susceptibility of these materials to biofilm formation probably results from a wide variety of variables. The two factors of demonstrated importance include the micro-topography (roughness) and hydrophobicity of the material [8**,46–48]: both surface roughness and increasing hydrophobicity correlate positively with Candida biofilm formation. Recent biomaterial engineering investigations have attempted to minimize these traits to reduce device susceptibility to these infections [8**]. However, following deposition of a host conditioning film, the properties of the underlying device material might be markedly masked, allowing organisms to produce a biofilm structure on almost all substrate materials examined to date. Yet another consideration that complicates the study of various device substrates is the recent evidence demonstrating that the human host protein response is able to vary markedly to different materials [8**]. Thus, the host conditioning film might be specific to the device material.

**Candida biofilm models in vivo**

The majority of fungal biofilm investigations have been undertaken using in vitro test systems. Studies from these in vitro models have provided numerous critical insights into biofilm formation and pathogenesis. However, it is the formation of biofilms in vivo that ultimately causes disease, and conditions encountered in vivo are quite distinct from standard in vitro biofilm culture conditions. As noted above, it is difficult to accurately duplicate the multitude of potentially relevant experimental variables encountered in the host.

For example, whereas several in vitro models have introduced flow, current models have not mimicked the flow characteristics found in the host vasculature [39]. In addition, despite attempts to mimic the conditioning of intravascular devices by pre-infection exposure to single serum components such as fibrinogen, continuous exposure to all of the potential host proteins has not been accomplished using current in vitro models [29]. Perhaps most importantly, the host immune system and infection site have not been accounted for in these in vitro systems.

Another variable that is difficult to accurately incorporate in vitro is the nutrient milieu in which these processes occur in patients. Whereas it is suggested that portions at least of the cell population within a biofilm mass are nutrient limited, it is anticipated that there would be a continuous gradient of both elements and carbon sources [10,45,49]. However, many in vitro models use a finite nutrient source, and most phenotypic and genotypic studies are undertaken following depletion of these resources [35**].

A variety of host cell types have been shown to adhere to medical devices. For example, platelets and red blood cells commonly coat portions of vascular catheters and heart valves [2**,38]. In vitro models have not examined the impact of these cells on initial adherence. Additional host cell types including neutrophils and macrophages have been identified within mature biofilm matrices [2**,20**]. Whether these cells become randomly entrapped within the net-like matrix, or are directed to this site by the host is unclear. Similarly it is unknown how the organisms in a biofilm respond to host cells and other secreted products [8**]. Examination of the impact of specific immune system components in biofilm formation and maintenance should be possible through use of either chemical depletion of components (e.g. neutrophils) or genetically defined animals.

Use of in vivo biofilm models to account for the multiplicity of potentially confounding host variables in the
study of Candida biofilms has, thus far, been limited to the vascular catheter model. Two models of central venous catheter biofilm infection have recently been developed to mimic vascular catheter infection in patients. These models are similar to patient infection, with respect to device material, anatomic location, vascular flow, host proteins and immune system exposure. Preliminary studies in these models have demonstrated their usefulness in corroborating the results of many experimental questions addressed using in vitro test systems. Encouragingly, comparison of results from the in vitro models demonstrates many similarities.

Not surprisingly, examination of growth dynamics in vivo are comparable to those observed in vitro, demonstrating a slow growth rate and maximal viable cell burden after 24 h incubation. In vivo biofilms are structurally similar to those described in laboratory model systems, with the exception of the numerous host cells including red blood cells, platelets, macrophages and neutrophils that are embedded in the matrix. The in vivo models have also been useful for examining antifungal drug efficacy. Both qualitative and quantitative measures of drug resistance have been developed. The general trends in biofilm drug resistance described in detail using in vitro systems have been similarly observed in vivo. For example, the instillation through the catheter of triazoles and amphotericin B to mature biofilms at concentrations more than 500-fold in excess of the planktonic MIC (minimum inhibitory concentration) result in no change in viable cell burden or architecture. However, both echinocandins and lipid-associated amphotericin B effectively eliminate a mature biofilm when similarly administered. This differential drug class activity is similar to that reported using in vitro models.

Several recent reports describe the global transcriptional response of C. albicans to biofilm growth using a variety of in vitro culture conditions. Similar studies should also be possible using the in vivo models. Initial studies have demonstrated that the cell burden in the catheter model is adequate to enable estimation of gene expression in response to the biofilm lifestyle. In the limited gene-set reported thus far, the results from the in vivo model have been congruent with those from a variety of in vitro systems. Transcripts from both of the ATP-binding cassette pumps, CDR1 and CDR2, are increased in the biofilm state. However, transcription of ERG11 (14 alpha demethylase) and MDR1 (major facilitator efflux pump) does not appear to be affected by biofilm growth in vivo when compared to planktonic cells. The model and assay tools should also be useful for phenotypic examination of the role of specific gene products on the ability to form biofilms and engender drug resistance in vivo.

Global gene expression studies have demonstrated differential expression of adhesins and secreted products, specific to the infection site. It is likely that many components of the Candida biofilm response will vary in different anatomic environments. Aside from the catheter models described above, the only other model systems that have been used to study Candida device-infection are the primate and rat denture models. The expense and ethical issues preclude high-throughput study in the primate model. Thus far, the rodent denture model has not been exploited to specifically examine the host-device biofilm process.

The general set of outcomes would indicate that our understanding of biofilm properties in vitro has clear relevance to the in vivo model. However, it is apparent that despite attempts to mimic host conditions, many details are different in vivo. To better understand and control biofilm infections, research must progress in a number of key areas. Among these is the development of new in vivo models to include host factors at different infection sites. Several infection site models have been used in the study of bacterial biofilms, including urinary catheters, endocarditis and joint arthroplasty. These models have been developed in less sentient rodents and should be useful for study of Candida biofilm infections.

Conclusion

Biomaterial infections are an increasingly alarming problem, and because of their intrinsic recalcitrance to conventional therapy, new methods of examining these infections must be explored. Fungal biofilm studies in vitro have shown that development involves a coordinated response of the adherence to the device surface, morphogenetic transition, matrix production and induction of drug resistance. In vivo biofilm models should be used to confirm the role of specific genes and regulatory pathways. Furthermore, these host-focused models will provide a means to explore the impact of a variety of host components and biofilm infection sites.

Acknowledgments

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


Host-microbe interactions: fungi


This is the first in vivo catheter Candida biofilm model.


