Fungal Super Glue: The Biofilm Matrix and Its Composition, Assembly, and Functions

Kaitlin F. Mitchell, Robert Zarnowski, David R. Andes*

Departments of Medicine and Microbiology and Immunology, University of Wisconsin, Madison, Wisconsin, United States of America

* dra@medicine.wisc.edu

The Common Thread of Microbial Biofilms

Biofilms are arguably the most common state of microbial growth found in nature and in patients infected with pathogenic organisms. A canonical feature of prokaryotic and eukaryotic biofilms is their production of an extracellular matrix (Fig 1). The matrix provides a protective environment for biofilm cells, offering a three-dimensional framework for both surface adhesion and cell cohesion [1,2]. In addition, this extracellular material controls cell dispersion from the biofilm and provides a nutrient source for the community [3]. The physical barrier formed by the matrix is also clinically relevant, as it shields cells from environmental threats, including immune cells and antimicrobial drugs used for treatment [4,5]. This defensive characteristic has been demonstrated for biofilms formed by diverse fungal pathogens, including *Aspergillus, Candida, Cryptococcus*, and *Saccharomyces*, with *Aspergillus fumigatus* and *Candida albicans* being the best studied [2,5–8]. Biofilm-associated *Candida* infections are the fourth cause for nosocomial infections (predominantly infecting medical devices), which may lead to systemic infection associated with high mortality rates. *Candida* spp. are also the most common cause of mucosal infection of the oral and vaginal sites, where biofilm infection has been increasingly recognized. Despite the ubiquitous nature of the biofilm matrix, we are only beginning to understand the synthesis and composition of this material for a handful of species. This review will discuss select components of the extracellular matrix of fungal biofilms, including their synthesis, structure, and function.

A Complex Assembly of Self, Host, and Neighbor

Two themes are common in the composition of microbial matrix. First, there is a contribution from each of the four macromolecular classes: carbohydrate, protein, lipid, and nucleic acid (Table 1). Second, there are complex interactions among the matrix components. The abundance and specific chemistry of biofilm matrices among these components can be as diverse as the microbes that produce them, in addition to the conditions under which biofilms are formed. The greatest divergence in matrix composition appears at the protein level [9–11]; however, differences have also been observed in carbohydrate content and have been the focus of most studies. For example, *A. fumigatus, C. albicans, Cryptococcus neoformans*, and *Saccharomyces cerevisiae* have each been shown to produce distinct, complex matrix polysaccharides frequently composed of two or more monosaccharide components [11]. Interestingly, many of the matrix components are similar, at least in part, to cell wall constituents. In fact, certain cell wall production enzymes are important for the production of a matrix [12,13]. However, the mature matrix structures most often vary from their cell wall counterparts in size, branching, and sometimes in the combination of monosaccharide components. This suggests that matrix
polysaccharides are either distinct from the cell wall or further modified after release from the cell wall. In the case of *C. albicans*, assembly of the mannan-glucan complex (MGCx) was found to occur in the extracellular space, demonstrating a critical role for the polysaccharide modification enzymes identified in the matrix proteome. There has been minimal structural study of matrix lipid composition and assembly; the observations demonstrated both similarities and differences between matrix components and the cell plasma membrane [11,14].

Under in vivo conditions, the extracellular matrix complexity increases further. *C. albicans* biofilms in three common infection site models each produced a matrix that contained a striking amount of host components—up to 98% of matrix proteins were of host origin [15]. The most abundant host proteins varied based on the host niche: hemoglobin, albumin, and alpha globulins in the venous catheter model; amylase and hemoglobin in the denture model; and fibrinogen, keratin, and hemoglobin in the urinary catheter model. However, there was a conserved group of proteins that included the matricellular proteins fibrinogen, fibronectin, hemoglobin, and vitronectin, suggesting biofilm relevance across infection sites. DNA from the host

Fig 1. Scanning electron micrograph of a *Candida albicans* biofilm grown on a rat central venous catheter. The image demonstrates fungal yeast and hyphal cell morphologies as well as abundant extracellular matrix material. Scale bar represents 10 μm. doi:10.1371/journal.ppat.1005828.g001
is also a common matrix factor that has been linked to biofilm structural integrity and total matrix production by *Aspergillus* biofilms [16].

Biofilm infections are often polymicrobial, with combinations of bacterial–bacterial and bacterial–fungal species described [17,18]. The matrix composition of mixed-species biofilms has not been closely examined. However, recent study of a *C. albicans* and *Staphylococcus aureus* mixed biofilm found the *Candida*-derived extracellular matrix encased the bacterial community [19]. It is plausible that entities from each species might help or hinder the production of matrix by the other, for example, the complex 3D network of matrix-rich *Candida* biofilm may conceivably provide a hypoxic microenvironment that nourishes the growth of anaerobic bacteria [20]. Future experiments in this area will be tasked with identifying changes in the mixed biofilm matrix compared to either biofilm species alone.

### Key Components for a Protective Matrix

One of the medically relevant traits of the biofilm extracellular matrix is its ability to protect cells from extraordinarily high levels of anti-infectives. The initial link between *Candida* matrix and resistance was discovered by the Douglas laboratory when they correlated matrix abundance with biofilm tolerance to the antifungal drugs amphotericin B and fluconazole [21]. The *Candida* matrix polysaccharide first linked to biofilm resistance to multiple drugs was β-1,3 glucan [13,22,23]; through a mechanism of drug sequestration, this matrix polysaccharide prevents drugs from reaching their cellular targets. β-1,3 glucan has also been suggested to prevent neutrophil activation, accounting for *Candida* biofilm resistance to killing by these innate immune cells [24]. The exact nature of the matrix–antifungal drug interaction remains undefined. However, preliminary nuclear magnetic resonance (NMR) interaction studies and the differing physiochemical properties of the antifungal drugs impacted by this resistance mechanism suggest a noncovalent drug–matrix interaction [11]. While most matrix biochemical studies have been undertaken with *C. albicans*, phenotypic studies suggest that the matrix drug sequestration phenomenon is common for other *Candida* species [25].

More recent work found surprisingly low levels of β-1,3 glucan but found larger quantities of β-1,6 glucan and α-mannan, which interact to form an MGCx [11,26]. This polysaccharide interaction was discovered to be key for protection of the biofilm from drug treatment.

Compared to bacterial biofilms, where extracellular DNA (eDNA) is an established mode of horizontal gene transfer, *C. albicans* eDNA is largely noncoding [11]. Autolysis likely

---

*Percent values in parentheses indicate relative abundance for certain components within the extracellular matrix. GAG: galactosaminoalactan; MCCx: mannan-glucan complex; eDNA: extracellular DNA.*

| Species               | Characterized matrix components * | Drug resistance | Immune resistance | Relevant sources |
|-----------------------|----------------------------------|-----------------|-------------------|------------------|
| *Aspergillus fumigatus* | Carbohydrates: GAG, galactomannan, α-1,3 glucan, monosaccharides (43%); proteins: major antigens and hydrophobins (40%); lipids (14%); melanin, polysols, eDNA | GAG, eDNA | GAG | [7,27,32,37] |
| *Candida albicans*     | Glycoproteins and neutral polysaccharides (25%); 458 distinct proteins (55%); lipids, including neutral and polar glycerolipids and sphingolipids (15%); nucleic acids (5%); phosphorus, uronic acid, hexosamine | MGCx (α-mannan, β-1,6 glucan, and β-1,3 glucan), eDNA | β-1,3 glucan | [11,21,38] |
| *Cryptococcus neoformans* | Carbohydrates: glucuronoxylomannan, xyllose, mannose, glucose, galactoxylomannan | — | — | [6] |
| *Saccharomyces cerevisiae* | Carbohydrates including glucose, mannose, and galactose; proteins in lower abundance including Tdh3, Hsp26, and Sod2 | — | — | [8,39] |

*Table 1. Fungal Matrix Polysaccharide Content and Function.*

doi:10.1371/journal.ppat.1005828.t001
contributes to eDNA entering the matrix, as chitinase activity increases DNA release by *Aspergillus* in biofilms [16,27]. The exact mechanism of how eDNA might contribute to drug resistance remains unclear but may also be due to reduced drug penetration.

The role of matrix in multispecies interactions has not been intensively studied. However, it does appear that bacterial biofilm growth with *Candida* is linked to protection of the prokaryotes from antibacterial agents. For example, *S. aureus* grown in a mixed biofilm with *C. albicans* had heightened resistance to vancomycin [28]. Furthermore, recent work with an *S. aureus* and *Candida* mixed model identified a role for the *C. albicans* matrix mannan-glucan complex in antibacterial shielding [29]. *C. albicans* matrix carbohydrate was also found to provide protection to *Escherichia coli* against the antibiotic ofloxacin in a mixed biofilm scenario [30]. These findings further demonstrate the nonspecific nature of protection by the matrix from numerous drugs with widely divergent physiochemical properties. Interestingly, the galactosaminogalactan (GAG) matrix polysaccharide of *Aspergillus* has been similarly linked to antifungal protection [31]. Additionally, GAG affects virulence through the masking of cell wall β-1,3 glucan and modulating host immune responses, including neutrophil apoptosis [31–33].

**Unraveling the Matrix: Exploitation of Biofilm Drug Targets**

During biofilm growth, production of extracellular matrix proceeds rapidly during the first 24 hours of maturation. Attempts to target this biofilm component suggest therapeutic promise for either the prevention or treatment of biofilm infections. It is intriguing that the most effective of the currently available antifungal drug classes for treatment of *Candida* biofilms are the echinocandin antifungals, which inhibit cell wall β-1,3 glucan synthesis [34]. It has been postulated this inhibition of glucan synthesis in the cell wall results in less β-1,3 glucan in the matrix [35]. We posit that either the production or assembly enzymes of importance for the mature MGCx may be promising therapeutic targets. Attempts to hydrolyze these polysaccharide and nucleic acid matrix components have been successful in sensitizing both *Candida* and *Aspergillus* biofilms to available antifungals [26,27]. The combination of existing antifungals with a hydrolyzing enzyme may be useful for mucosal or topical applications. However, disruption of systemic biofilms, such as those on vascular catheters, would be anticipated to enhance cell dispersion and production of disseminated infection, which would consequently require additional pharmacological interventions both locally for biofilm and systematically to eradicate microorganisms migrating to tissues and organs.

Peptides targeting the host and microbe matrix interactions have also demonstrated efficacy in treatment and prevention studies both in vitro and in vivo [36]. Specifically, targeting of host protein binding to the fibronectin binding site in *C. albicans* was modestly effective in inhibiting biofilm formation. The identification and targeting of these additional matrix targets, particularly those that are conserved among pathogens, holds promise for effective prevention and treatment strategies.

The biofilm matrix represents a complex interaction of multiple macromolecular components. A role in cell protection has been identified for several of these constituents. However, the function for the majority of matrix elements remains unexplored. Deciphering the exact composition and roles for these materials should lead to advances in biofilm prevention, therapy, and diagnosis.

**References**

1. Flemming H.C. and Wingender J., The biofilm matrix. Nat Rev Microbiol, 2010. 8(9): p. 623–33. doi: 10.1038/nrmicro2415 PMID: 20676145
2. Chandra J., et al., Biofilm formation by the fungal pathogen Candida albicans: development, architecture, and drug resistance. J Bacteriol, 2001. 183(18): p. 5385–94. PMID: 11514524
3. Uppuluri P., et al., Dispersion as an important step in the Candida albicans biofilm developmental cycle. PLoS Pathog, 2010. 6(3): p. e1000828. doi: 10.1371/journal.ppat.1000828 PMID: 20360962
4. Costerton J.W., Stewart P.S., and Greenberg E.P., Bacterial biofilms: a common cause of persistent infections. Science, 1999. 284(5418): p. 1318–22. PMID: 10334980
5. Baillie G.S. and Douglas L.J., Matrix polymers of Candida biofilms and their possible role in biofilm resistance to antifungal agents. J Antimicrob Chemother, 2000. 46(3): p. 397–403. PMID: 10980166
6. Martinez L.R. and 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(14): p. 4592–601. PMID: 17513597
7. Beauvais A., et al., An extracellular matrix glues together the aerial-grown hyphae of Aspergillus fumigatus. Cell Microbiol, 2007. 9(6): p. 1588–600. PMID: 17371405
8. Beauvais A., et al., Characterization of a biofilm-like extracellular matrix in FLO1-expressing Saccharomyces cerevisiae cells. FEMS Yeast Res, 2009. 9(3): p. 411–9. doi: 10.1111/j.1567-1364.2009.00482.x PMID: 19207290
9. Reichhardt C., et al., Analysis of the Aspergillus fumigatus Biofilm Extracellular Matrix by Solid-State Nuclear Magnetic Resonance Spectroscopy. Eukaryot Cell, 2015. 14(11): p. 1064–72. doi: 10.1128/EC.00050 -15 PMID: 26163318
10. Thomas D.P., Bachmann S.P., and Lopez-Ribot J.L., Proteomics for the analysis of the Candida albicans biofilm lifestyle. Proteomics, 2006. 6(21): p. 5795–804. PMID: 17001605
11. Zarnowski R., et al., Novel entries in a fungal biofilm matrix encyclopedia. MBio, 2014. 5(4): p. e01333–14. doi: 10.1128/mBio.01333-14 PMID: 25096878
12. Nett J.E., et al., Putative role of beta-1,3 glucans in Candida albicans biofilm resistance. Antimicrob Agents Chemother, 2007. 51(2): p. 510–20. PMID: 17130296
13. Peters B.M., et al., Polymicrobial interactions: impact on pathogenesis and human disease. Clin Microbiol Rev, 2012. 25(1): p. 193–213. doi: 10.1128/CMR.00013-11 PMID: 22232376
14. Harriss M.J. and Noverr M.C., Ability of Candida albicans mutants to induce Staphylococcus aureus vancomycin resistance during polymicrobial biofilm formation. Antimicrob Agents Chemother, 2010. 54(9): p. 3746–55. doi: 10.1128/aac.00573-10 PMID: 20566760
15. E.F. Kong, C.T., S. Kucharíková, D. Andes, P. Van Dijck, M. Jabra-Rizk., Protection of Staphylococcus aureus Against Antimicrobials by Candida albicans Biofilm Matrix. 13th ASM Conference on Candida and Candidiasis. 2016 April 13–17; Seattle, Washington, United States of America.
16. Fox E.P., et al., Anaerobic bacteria grow within Candida albicans biofilms and induce biofilm formation in suspension cultures. Curr Biol, 2014. 24(20): p. 2411–6. doi: 10.1016/j.cub.2014.08.057 PMID: 25308076
17. Al-Fattani M.A. and Douglas L.J., Biofilm matrix of Candida albicans and Candida tropicalis: chemical composition and role in drug resistance. J Med Microbiol, 2006. 55(Pt 8): p. 999–1008. PMID: 16849719
18. Vediyappan G., Rossignol T., and d’Enfer C., Interaction of Candida albicans biofilms with antifungals: transcriptional response and binding of antifungals to beta-glucans. Antimicrob Agents Chemother, 2010. 54(5): p. 2096–111. doi: 10.1128/AAC.01638-09 PMID: 20194705
19. Xie Z., et al., Candida albicans biofilms do not trigger reactive oxygen species and evade neutrophil killing. J Infect Dis, 2012. 206(12): p. 1936–45. doi: 10.1093/infdis/jis07 PMID: 23033146
25. Mitchell K., et al., Role of matrix beta-1,3 glucan in antifungal resistance of non-albicans Candida biofilms. Antimicrob Agents Chemother, 2013. 57(4): 1918–1920. doi: 10.1128/AAC.02378-12 PMID: 23318790

26. Mitchell K.F., et al., Community participation in biofilm matrix assembly and function. Proc Natl Acad Sci U S A, 2015. 112(13):4092–7. doi: 10.1073/pnas.1421437112 PMID: 25770218

27. Rajendran R., et al., Extracellular DNA release acts as an antifungal resistance mechanism in mature Aspergillus fumigatus biofilms. Eukaryot Cell, 2013. 12(3): p. 420–9. doi: 10.1128/EC.00287-12 PMID: 23314962

28. Harriott M.M. and Noverr M.C., Candida albicans and Staphylococcus aureus form polymicrobial biofilms: effects on antimicrobial resistance. Antimicrob Agents Chemother, 2009. 53(9): p. 3914–22. doi: 10.1128/AAC.00657-09 PMID: 19564370

29. Kong, E.F., et al. Protection of Staphylococcus aureus against antimicrobials by Candida albicans biofilm matrix. 13th ASM Conference on Candida and Candidiasis. 2016 April 13–17; Seattle, Washington, United States of America.

30. De Brucker K., et al., Fungal beta-1,3-glucan increases ofloxacin tolerance of Escherichia coli in a polymicrobial E. coli/Candida albicans biofilm. Antimicrob Agents Chemother, 2015. 59(6): p. 3052–8. doi: 10.1128/AAC.00650-14 PMID: 25753645

31. Lee M.J., et al., The Fungal Exopolysaccharide Galactosaminogalactan Mediates Virulence by Enhancing Resistance to Neutrophil Extracellular Traps. PLoS Pathog, 2015. 11(10): p. e1005187. doi: 10.1371/journal.ppat.1005187 PMID: 26492565

32. Gravelat F.N., et al., Aspergillus galactosaminogalactan mediates adherence to host constituents and conceals hyphal beta-glucan from the immune system. PLoS Pathog, 2013. 9(8): p. e1003575. doi: 10.1371/journal.ppat.1003575 PMID: 23990787

33. Gresnigt M.S., et al., A polysaccharide virulence factor from Aspergillus fumigatus elicits anti-inflammatory effects through induction of Interleukin-1 receptor antagonist. PLoS Pathog, 2014. 10(3): p. e1003936. doi: 10.1371/journal.ppat.1003936 PMID: 24603878

34. Ghannoum M., et al., The Role of Echinocandins in Candida Biofilm-Related Vascular Catheter Infections: In Vitro and In Vivo Model Systems. Clin Infect Dis, 2015. 61 Suppl 6: p. S618–21. doi: 10.1093/cid/civ815 PMID: 26567279

35. Walsh T.J., Azie N., and Andes D.R., Development of New Strategies for Echinocandins: Progress in Translational Research. Clin Infect Dis, 2015. 61 Suppl 6: p. S601–3. doi: 10.1093/cid/civ676 PMID: 26567276

36. Nett J.E., et al., Targeting Fibronectin To Disrupt In Vivo Candida albicans Biofilms. Antimicrob Agents Chemother, 2016. 60(5): p. 3152–5. doi: 10.1128/AAC.03094-15 PMID: 26902759

37. Reichhardt C., et al., Analysis of the Aspergillus fumigatus Biofilm Extracellular Matrix by Solid-State Nuclear Magnetic Resonance Spectroscopy. Eukaryot Cell, 2015.

38. Martins M., et al., Addition of DNase improves the in vitro activity of antifungal drugs against Candida albicans biofilms. Mycoses, 2012. 55(1): p. 80–5. doi: 10.1111/j.1439-0507.2011.02047.x PMID: 21668524

39. Faria-Oliveira F., et al., Elemental biochemical analysis of the polysaccharides in the extracellular matrix of the yeast Saccharomyces cerevisiae. J Basic Microbiol, 2015. 55(6): p. 685–94. doi: 10.1002/jobm.201400781 PMID: 25589358